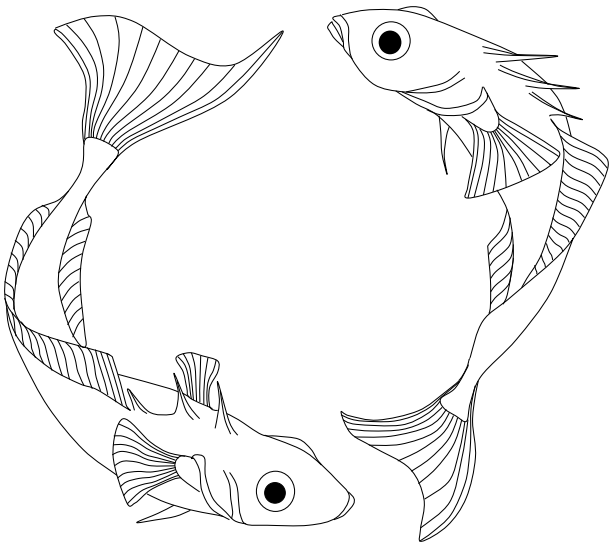
Evolutionary and ecological perspectives on epidemiological traits in helminth infections of sticklebacks



Dissertation

zur Erlangung des akademischen Grades

– Doctor rerum naturalium –

der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von
Agnes Piecyk

Kiel, Februar 2019

"The root of the spirit of biologists is their interest in all that lives, an intensive, honest and selfless interest […]"

Niko Tinbergen

Erste Gutachterin	Dr. Olivia Roth
Zweiter Gutachter	Prof. Dr. Hinrich Schulenburg
Tag der mündlichen Prüfung	04.04.2019
Zum Druck genehmigt	04.04.2019

CONTENTS

SUMMARY

The interaction of an organism with its environment is a hallmark of life and pre-requisite for natural selection. Among the strongest evolutionary processes is the interaction between hosts and parasites that are engaged in a constant arms race of parasite exploitation and host defence. This antagonistic co-evolution is shaped through host and parasite genotypes, their local environmental conditions, and their potential for plastic responses. However, the relative contribution of these effects is often unclear. Here, I aimed to find answers to the questions how and why epidemiological traits vary among populations by using hosts and parasites from geographically distinct and ecologically divergent populations. I used three-spined sticklebacks (*Gasterosteus aculeatus*) as vertebrate model organisms to study defence mechanisms against helminth parasites. Helminth parasites are of exceptional interest because they can have complex immune modulatory effects on their hosts. This phenomenon is already applied in clinical settings, where helminths, their ova, or their products are used to treat autoimmune or inflammatory disorders (*helminth therapy*). Nevertheless, many questions on the specificity of the host-helminth interaction have yet to be answered. For instance: Are there differences between host genotypes or parasite species? What are the effects over time? Are effects localized or systemic?

Using evolutionary and ecological perspectives, I specifically asked: What are the effects of host and parasite genotypes and their interaction? Does the potential for interaction effects differ with geographical scale? Does immune modulation differ over the time course of infection, and if so, is the temporal component dependent on parasite strain and/or host type? Indeed, my colleagues and I found that different strains of the same cestode species (*Schistocephalus solidus*) had profoundly different effects on divergent *G. aculeatus* types. This effect was linked to the co-evolutionary history and ecology of *G. aculeatus* and *S. solidus*. My results demonstrate that the infection outcome was largely determined by effects of host and parasite genotypes, while interaction effects were generally weak and only evident over the scale of continents.

Gene expression profiles that differed between uninfected fish from different populations mostly converged upon infection. Thus, the parasite-induced phenotypic plasticity transcended host genetic differences. This thesis also reveals that *S. solidus* immune modulation is time-, host- and parasite strain-dependent. Sticklebacks that assumingly co-evolved with a highly virulent *S. solidus* strain were more resistant against *S. solidus* and had a well-orchstrated immune response (potentially diminishing immunopathological side effects) compared to hosts without this co-evolutionary background. Late stages of infection with a highly virulent *S. solidus* strain had a systemic effect by increasing the susceptibility towards another helminth species (*Diplostomum pseudospathaceum*).

My data present a snapshot in time and space that provides insights into potential (co-) evolutionary backgrounds. Whether the epidemiological traits of *Gasterosteus aculeatus* and *Schistocephalus solidus* are indeed shaped through co-evolution is one of the challenges for future investigations. However, by revealing the dominant effect of the parasite and the relative importance of induced plasticity, this thesis advances our understanding about the role of each partner in a host-parasite interaction. My results are of significant importance for the investigation of the premises and consequences of helminth therapy. I propose to incorporate evolutionary and ecological perspectives in future research.

ZUSAMMENFASSUNG

Leben zeichnet sich dadurch aus, dass Organismen mit ihrer Umgebung wechselwirken. Diese Wechselwirkung ist eine Voraussetzung für Natürliche Selektion. Einer der relevantesten Prozesse ist das Zusammenspiel von Parasiten mit ihren Wirtsorganismen. Deren Wettlauf von Ausnutzung und Abwehr (Wirt-Parasit-Koevolution) wird durch die jeweiligen Genotypen, Umweltbedingungen und Möglichkeiten einer dynamischen Antwort (*phenotypic plasticity*) geprägt. Allerdings bleibt es unklar, welche jener Effekte für die jeweiligen Infektionen ausschlaggebend sind.

In dieser Arbeit suche ich Antworten auf die Fragen *wie* und *wieso* Charakteristika von Infektionen (*epidemiological traits*) sich zwischen Populationen unterscheiden. Als Modellsystem nutzte ich Dreistachlige Stichlinge (*Gasterosteus aculeatus*) und deren Helminthen (v.a. Bandwürmer der Art *Schistocephalus solidus*) aus verschiedenen Populationen Europas und Nordamerikas. Aufgrund ihrer Fähigkeit, das Immunsystem der Wirte zu beeinflussen, sind Helminthen von besonderer Bedeutung für die Grundlagenforschung in Bereichen der Wirt-Parasit-Koevolution und Immunologie, aber auch für die Medizinische Forschung. Unter dem Begriff der *Helminthentherapie* werden schon heute Würmer, deren Eier oder Produkte eingesetzt, um Autoimmunerkrankungen oder chronische Entzündungen zu behandeln. Dennoch sind viele Fragen offen, wie zum Beispiel: Gibt es Unterschiede zwischen Genotypen der Wirte oder verschiedenen Helminthenarten? Welchen Einfluss hat die Dauer der Infektion? Sind Effekte lokalisiert oder systemisch?

Um jene Fragen zu beantworten, müssen evolutionsökologische Perspektiven miteinbezogen werden. In diesem Zusammenhang fragte ich insbesondere: Gibt es sogar Unterschiede zwischen Wirten und Parasiten derselben Art? Gibt es (ko)evolutionäre oder ökologische Einflüsse, die gegebenenfalls auf einer geographischen Ebene sichtbar wären? Wie wichtig ist eine dynamische Antwort auf Seiten der Wirte, aber auch der Parasiten? Meine Ergebnisse lassen vermuten, dass koevolutionäre und ökologische Faktoren (wie Unterschiede in der Diversität und Prevalenz von Parasiten) zu immunologischer Heterogenität zwischen Populationen führen können.

Jene Heterogenität der Stichlinge verschiedener Populationen wurde in Infektionen durch den Effekt des Parasiten dominiert. Ich zeige (i) dass das Parasitenwachstum mit einem geographischen Muster im Zusammenhang steht; (ii) dass Expressionsmuster von Kandidatengenen der Stichlinge eher vom Parasitentypus abhängig sind als von der Herkunft der Stichlinge; und (iii) dass die Infektion mit einem bestimmten *S. solidus* Typus einen systemischen Effekt haben kann, indem die Wahrscheinlichkeit von Koinfektionen mit einer weiteren Helminthenart ab einem bestimmten Zeitpunkt erhöht wird. Stichlinge mit einer koevolutionären Vergangenheit mit *Schistocephalus solidus* waren resistenter und zeigten eine gut aufeinander abgestimmte Immunantwort, wenn sie mit ihrem sympatrischen *S. solidus* infiziert wurden. Eine gut aufeinander abgestimmte Immunantwort kann immunopathologische Effekte (wie sie auch in Autoimmun- und Entzündungskrankheiten auftreten) abschwächen. Meine Arbeit zeigt außerdem, dass eine differenzierte Auseinandersetzung mit *Resistenz* vor allem in Bezug auf Helmintheninfektionen notwendig ist. Resistenz kann sich zu verschiedenen Zeitpunkten des Infektionsprozesses manifestieren: als Abwehr von Infektion (qualitative Resistenz) oder als Verringerung der Infektionsintensität, hier des Parasitenwachstums (quantitative Resistenz).

Zusammenfassend lässt sich erklären, dass die evolutionären und ökologischen Perspektiven dieser Doktorarbeit unser Verständnis der Wechselwirkung von Helminthen und ihren Wirten enorm verbessern. Ein umfassendes Verständnis ist essentiell, um die Voraussetzungen und die Konsequenzen von Helminthentherapie zu verstehen. Im Rahmen dieser Doktorarbeit stelle ich weitere Untersuchungen vor, die auf meinen Ergebnissen aufbauen. Ich empfehle, den hier gezeigten starken Effekt der Parasiten-induzierten Plastizität in zukünftigen Arbeiten aus Bereichen der Wirt-Parasit Koevolution, Immunologie und Medizin zu berücksichtigen.

INTRODUCTION

Natural variation

The biodiversity of life concerns the human kind since we started to interact with nature, in other words since our species has evolved. We manipulated nature with tools of artificial selection and systematics in order to use and structure our environment. The research field of evolutionary biology now attempts to understand and explain why such an immense variation of life exists. Novel fields like ecological immunology or evolutionary medicine emerged. Researchers have learned that the understanding of the evolutionary (phylogenetic and adaptive) and the proximate (mechanistic and ontogenetic) causes is essential to understand natural variation of biological traits (Williams and Nesse, 1991; Nesse et al., 2010; Nesse, 2013; Graham, 2013; Stearns & Medzhitov, 2016). This idea goes back to Niko Tinbergen who worked on three-spined sticklebacks and published his essay *"On aims and methods in ethology"* half a century ago (Tinbergen, 1963).

The co-evolution of hosts and parasites

Species interactions are of central importance for the diversification of life (Thompson, 1999a). The reciprocal evolutionary change (antagonistic co-evolution) of hosts and parasites generates and maintains diversity within and between species. Host-parasite co-evolution drives ecosystem and population dynamics (Thompson, 1998; Fuhrman, 1999; Brockhurst et al., 2006), the evolution of genetic diversity (Buckling and Rainey, 2002; Paterson et al., 2010), and sexual reproduction (Hamilton et al., 1990; Ebert and Hamilton, 1996; Lively, 1996; Morran et al., 2011). Proposed by Van Valen in the 1970s, an increase of *momentary fitness* of one species comes with a decrease of *momentary fitness* among ecologically interacting species (Van Valen, 1973; Van Valen, 1974). Based on Lewis Carroll's *Through the Looking-Glass*, Van Valen named his hypothesis the *Red Queen Hypothesis*. The Queen's statement that "*it takes all the running you can do, to keep in the same place*" was henceforth applied to the arms race of hosts and parasites.

Parasites rely on their hosts for resources and may significantly decrease host fitness; thus, hosts evolve and reduce the harm of parasites, which results in a high potential for rapid and adaptive divergent evolution (Hamilton, 1980; Paterson et al., 2010; Schmid-Hempel, 2011; Eizaguirre et al., 2012a). Host-parasite co-evolution requires (i) genetic variation in host resistance and parasite infectivity and (ii) host genotype-parasite genotype (GxG) specific interactions (Carius et al., 2001). Thus, some hosts will be susceptible to a certain subset of parasite genotypes whereas other hosts will be infected by another subset and *vice versa* (Lambrechts et al., 2006). Immunological heterogeneity among hosts is a cause and an effect. Reciprocal evolution of host and parasite genotypes has been detected in various systems including bacteria-phage associations (Buckling and Rainey, 2002; Paterson et al., 2010), the nematode *Caenorhabditis elegans* and the bacterial pathogen *Bacillus thuringiensis* (Schulte et al., 2010; Papkou et al., 2019), rodent malaria model systems (Lambrechts et al., 2005; Grech et al., 2006), plant-pathogen interactions (Burdon and Jarosz, 1991; Kaltz and Shykoff, 2002), and immune gene evolution in three-spined sticklebacks (Eizaguirre et al., 2012b, 2012a).

The co-evolutionary dynamics are intertwined with the ecological context (Kawecki and Ebert, 2004; Lazzaro and Little, 2009; Schulenburg et al., 2009; Mostowy and Engelstadter, 2011; Auld and Brand, 2017). Environmental variables such as temperature (Blanford et al., 2003; Studer et al., 2010), resource availability and nutrition levels (Forde et al., 2008; Brunner et al., 2014) and biotic factors such as inter- and intra-specific competition (Fellowes et al., 1998; Jager and Schorring, 2006; Rauch et al., 2008) and the number and type of parasites (Betts et al., 2018; Kalbe et al., 2002; Scharsack et al., 2007) shape the co-evolutionary trajectories of interacting species (giving rise to terms like GxGxG or GxGxE). As a result, variation is further increased by differences in the shape and strength of life history trade-offs of hosts and parasites (Duffy and Forde, 2009; Hansen and Koella, 2003; Schmid-Hempel, 2003).

The differences between environments may drive divergent selection causing local adaptation or (on broader scales) geographic mosaics. *Local adaptation* (Williams, 1966) conceptualizes that in a given habitat, evolutionary fitness of local genotypes is higher than in other habitats and that foreign genotypes, i.e. migrants, have a lower relative fitness (Kawecki and Ebert, 2004). In other words, parasites are more likely to infect sympatric hosts than allopatric hosts or hosts are more resistant against sympatric parasites than against allopatric parasites. While the raw material for co-evolutionary change is largely provided at the local scale, trade-offs and evolutionary constraints shape evolutionary trajectories at a phylogenetic level (Thompson, 1999b). Accordingly, the *Geographic Mosaic Theory of Coevolution* (Thompson, 1994, 1999a) predicts that spatially structured mosaics of traits arise from divergent selection (selection mosaics; selection hot spots and cold spots) and geographic remixing (gene flow, genetic drift, local extinctions) between subpopulations (Lively, 1999; Gomulkiewicz et al., 2000; Thompson and Cunningham, 2002).

What are co-evolving traits of hosts and parasites?

The co-evolutionary nature of host and parasite traits implies that epidemiological traits are under shared physiological and genetic control (Restif and Koella, 2003; Salvaudon et al., 2005; Lambrechts et al., 2006; Salvaudon et al., 2007). "*Just as variation in traits in populations is the raw material for the evolution of species, variation in outcome is the raw material for the evolution of interactions*" (Thompson, 1988). Following up on Thompson's *interaction norm* concept (genotype-by-genotype-by-environment interaction), Agrawal (2001) provides an even more detailed picture of species interactions: "*Reciprocal phenotypic change between individuals of interacting species represents an interaction norm where the response of one species to the other creates the environment to which the other species may then respond.*" (Agrawal, 2001). In this regard, phenotypic plasticity is defined as intra-individual variation including the influence of the genome (influenced by past selection) and the environment (Agrawal, 2001; West-Eberhard, 2003). Genotypes and allele frequencies of hosts and parasites change over evolutionary time scales, while the response of an individual to different environmental conditions (known as *reaction norm*) is plastic.

The relative contribution of environmentally mediated phenotypic plasticity to epidemiological traits can be substantial (Lazzaro and Little, 2009). It has, for example, been shown that even the mechanism of host defence can depend on the environment, such as host nutrition (Cumnock et al., 2018). However, host-parasite research mostly studies genotype and plastic effects separately and the relative importance of phenotypic plasticity and genotypic adaptations still need to be determined.

Excluding the effect of environmental variables for simplicity, infection phenotypes of a host-parasite association rely on host and parasite genotypes. Visualizing an epidemiological trait in dependence of host and parasite genotypes, different scenarios could emerge (Figure 1).

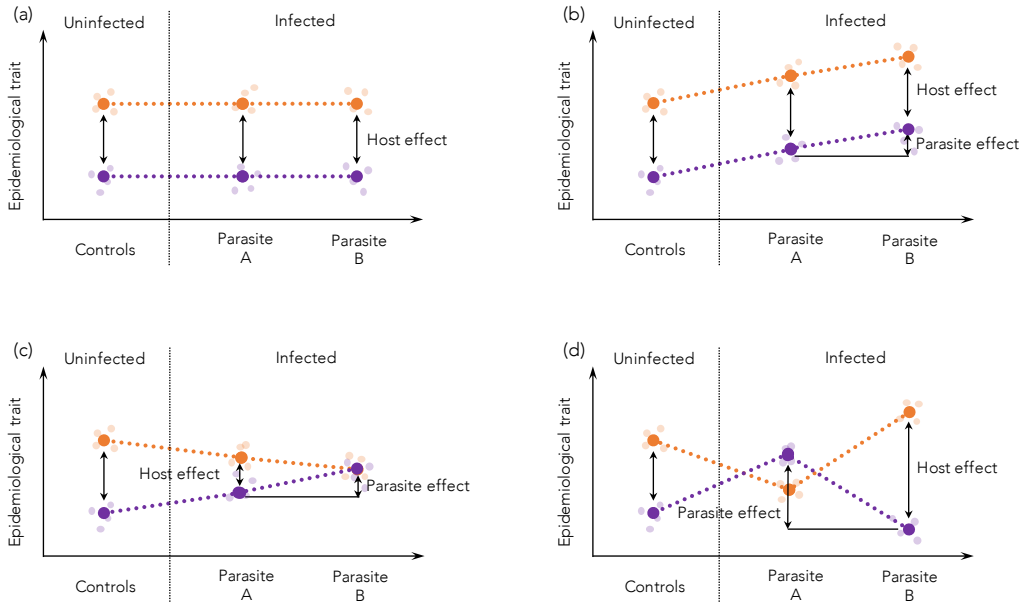


Figure 1. Partitioning of host, parasite, and interaction effects on an epidemiological trait. Two different host genotypes are indicated by violet and orange dots (replicates and means) and lines (reaction norms). Two different parasite genotypes, A and B, are arrangend along the x-axis. The infection phenotype could be any measure of epidemiological trait, such as host health, fitness, resistance, or virulence. **(a)** Host main effect: the vertical spacing between the parallel lines represents genetic differences betwenn the two host types. Parallel horizontal lines indicate absence of a plastic response towards infection. Differences among hosts that are infected with the same parasite (vertical spacing between the means) indicate a phenotypically plastic response of the parasite. **(b)** Host genotype and parasite genotype main effects. The positive slope indicates different effects of the two parasite types (parasite main effect) and thus a phenotypically plastic response of the host and the parasite. **(c,d)** Host genotype by parasite genotype interaction: non-parallel lines indicate interaction effects: the host effect depends on the parasite genotype. **(c)** Main-effect components can cumulate, causing non-crossing reaction norms. **(d)** Host genotype by parasite genotype interaction with crossing reaction norms. The figure is adapted from Chapter 1.

Epidemiological traits encompass various stages of the infection process. Parasite infectivity (the ability to colonize and invade a host; measured as infection rate) is distinct from virulence, which was traditionally defined as host mortality but meanwhile encompasses all detrimental effects of a parasite on host traits related to fitness (Bull, 1994; Read, 1994; Lambrechts et al., 2006). Likewise, host defence strategies encompass avoidance behaviour and herd immunity (Anderson and May, 1985), barrier organs, and immune functions (Schmid-Hempel, 2003; Murphy and Weaver, 2017). These strategies limit (i) the level of infection – thus counteract parasite infectivity – and (ii) the negative outcomes of infection – thus counteract parasite virulence. Processes at work encompass mechanisms of resistance and tolerance. Resistance and tolerance are not mutually exclusive (Sternberg et al., 2013). However, the effects on ecological and evolutionary interactions between hosts and parasites differ greatly. For example, parasite prevalence (the percentage of infected individuals; Bush et al., 1997) is expected to decrease if hosts evolve resistance whereas parasite prevalence is expected to increase if hosts evolve tolerance (Roy and Kirchner, 2000; Boots et al., 2009; Best et al., 2014). Tolerance has been defined as a host trait that limits the damage of a parasite burden (Råberg et al., 2007). Råberg later refined his definition of tolerance in explicitly stating that tolerance acts without preventing infection or reducing infection intensity, i.e. the number or density of parasites (Råberg, 2014). In statistical terms, tolerance explains variation in the relationship between infection intensity and measures of host health. This means that different host types that are infected with a certain parasite type will show different reaction norms (slopes of the relationship between infection intensity and host health or fitness) of these measures of health (Read et al., 2008; Råberg, 2014). Resistance was classically defined as a trait that prevents infection. Nowadays, this term incorporates mechanisms that reduce the risk of infection and/or infection intensity or parasite growth (Råberg et al., 2007; Råberg, 2014; Zeller and Koella, 2017). This differentiated perspective on resistance opened new possibilities to study macroparasite infections. Accordingly, helminth growth suppression was recently defined as an underrated form of resistance (Weber et al., 2017).

Glossary	
Co-evolution	Reciprocal adaptation and counter-adaptation of interacting species
Epidemiological trait	Host and/or parasite life-history trait determining health-related states (and/or their distribution)
Helminths	Ancient metazoa classified as platyhelminths (flatworms; subgroups are cestodes and trematodes) and nematodes (roundworms)
Infectivity	The ability to colonize and invade a host
Infection phenotype	Sum of the epidemiological (life history) traits of infected hosts
Reaction norm	The phenotypes that a genotype can express across a range of environmental conditions
Resistance	Host defence mechanisms preventing infection and/or limiting parasite replication or growth
Tolerance	Host defence mechanisms limiting detrimental effects of parasites without reducing infection risk and/or parasite growth or replication
Th1 cell*	$CD4^+$ effector T cell typically controlling infections by microbes
Th2 cell*	$CD4^+$ effector T cell typically controlling infections by extracellular parasites, particularly helminths
Treg cell*	$CD4^+$ effector T cell with immunosuppressive functions
Virulence	Detrimental effects of parasites on host traits related to fitness

* T helper cell subsets orchestrate certain immune responses. Their classification is based on their production of cytokines and transcription factors. However, these cells are not committed to a certain lineage but show flexible cytokine production and expression of transcription factors.

Helminth-host interactions

Helminths are a group of ancient metazoa encompassing cestodes, nematodes, and trematodes (Anthony et al., 2007). Helminth parasites infect about two billion people worldwide and represent a persistent source of morbidity and mortality (Vos et al., 2016). Many helminth infections are listed as neglected tropical diseases (International Helminth Genomes Consortium, 2018; World Health Organization, 2018). Helminths establish long-lasting, chronic infections; their generation times are similar to those of the host, and disease severity typically correlates with parasite burden. The past and ongoing evolution of complex immune evasive and/or immune modulatory mechanisms is inevitable.

Helminthic parasites interfere with characteristic elements of innate and adaptive immunity (Anthony et al., 2007; McSorley et al., 2013). A prominent observation is the switch of an early T helper 1 (Th1) type response towards a T helper 2 (Th2) type response in chronic helminth infections. The activities of these T helper cell subsets are characterized by distinct functions and cytokines (Maizels et al., 1993; O'Shea and Paul, 2010; Maizels and McSorley, 2016). Th1 type cytokines, such as Interleukin-1β (IL-1β) and Tumour necrosis factor α (TNF-α), act pro-inflammatory; Th2 type cytokines can inhibit Th1 cells and acute-phase cytokines, induce alternatively activated macrophages, and stimulate B-cells and antibody production (Mosmann and Sad, 1996; Rodríguez-Sosa et al., 2002; Liu et al., 2009b; Maerten et al., 2005; Peón et al., 2016). The fact that high parasite burdens can persist despite increased Th2 responses brought another T cell subset, namely immunosuppressive regulatory T (Treg) cells, into focus (Maizels and Yazdanbakhsh, 2003; Maizels, 2005; Nutman, 2015; Maizels and McSorley, 2016). Tregs expand upon long-term helminth infections and are known to be key controllers of immune system homeostasis (Maizels and Yazdanbakhsh, 2003; O'Shea and Paul, 2010). These cells may promote the persistence of the parasite within the host and protect from immunopathology. Accordingly, helminth immune modulatory potentials can actually have detrimental as well as beneficial consequences for the host.

In this respect, the frequency of immunopathological disorders such as autoimmune diseases, allergies and asthma increases dramatically in post-industrial countries since the second half of the 20th century (Stearns and Medzhitov, 2016). A spatio-temporal correlation with the decline of infectious diseases stimulated new perceptions of the aetiology of autoimmune and inflammatory disorders. The vertebrate immune system co-evolved with helminth parasites (Maizels et al., 1993; Anthony et al., 2007; Maizels, 2005; Khan and Fallon, 2013). It has been shown that helminth infections can alter susceptibility to macroparasites (Lello et al., 2004; Pedersen and Antonovics, 2013; Benesh and Kalbe, 2016) and microbes (Graham, 2008; Broadhurst et al., 2012; Reynolds et al., 2015; Giacomin et al., 2015; Gause and Maizels, 2016) and *vice versa*.

Moreover, helminth-mediated down-regulation of immunity has been observed to suppress autoimmune and inflammatory disorders such as celiac disease, asthma, rheumatoid arthritis, type 1 diabetes, multiple sclerosis, and inflammatory bowel diseases (Maizels and Yazdanbakhsh, 2003; McSorley et al., 2013; Helmby, 2015; Maizels and McSorley, 2016; Smallwood et al., 2017). *Helminth therapy* using the intestinal pig whipworm *Trichuris suis* or the human hookworm *Necator americanus* became a promising field of research (Maizels, 2005; Summers et al., 2005a, 2005b; Croese et al., 2006; Liu et al., 2009a; Weinstock and Elliott, 2013). However, clinical trials resulted in mixed results. Some studies reported the absence of therapeutic effects (Bager et al., 2010; Bourke et al., 2012); others emphasized the detrimental consequences of helminth infections, such as reduced vaccine responses, diminished protective immunity to other infectious agents, and potential reduction of tumour immunosurveillance (Liu et al., 2009b; Maizels and McSorley, 2016). It has been suggested that only certain helminth species could have beneficial effects in helminth therapies (Leonardi-Bee et al., 2006; Cooper, 2009; Helmby, 2015). Open questions are: Which species are appropriate? Should infections be localized or systemic and/or acute or chronic? What is the role of host genetics? (Helmby, 2015)

Taking an evolutionary ecologist's perspective, I'd expect consequences of helminth infections to further depend on (i) the ecology of the interacting species including environmentally mediated plasticity and (ii) on the co-evolutionary history. We have just begun to consider and determine the factors influencing helminth infection phenotypes. Thus, within this thesis, I asked whether even different types (or 'strains') of one helminth species could cause different molecular interplays and infection outcomes in different types of the same host species. In order to include ecological and co-evolutionary perspectives as well as temporal components in studies of helminth immune modulation, I chose the three-spined stickleback as a vertebrate model system.

The model system

The three-spined stickleback (*Gasterosteus aculeatus*) is a small teleost that became an important model in biology and subdisciplines such as behaviour, genetics and genomics, evolutionary ecology and parasitology as well as immunology (Colosimo et al., 2005; Gibson, 2005; Barber and Nettleship, 2010; Jones et al., 2012; Feulner et al., 2013; Barber, 2013; Robertson et al., 2015; Lohman et al., 2017; Brunner et al., 2017). This fish is distributed across the Northern Hemisphere where it adapted to a wide range of habitats (Bell and Foster, 1994).

The *G. aculeatus* species complex encompasses thousands of populations that differ in phenotypic and genotypic traits including morphology, behaviour, and immunity (Bell and Foster, 1994). The recent (re-)colonization history of northern populations dates back to the retreat of the ice sheet after the last glacial maximum approximately 12,000 years ago, when numerous freshwater ecotypes repeatedly evolved from marine ancestors (Bell and Foster, 1994; Mäkinen et al., 2006). Divergent evolution in sticklebacks can be rapid and occurs under a variety of geographical and ecological contexts (McKinnon and Rundle, 2002; Lescak et al., 2015). While bottlenecks, founder effects and genetic drift seem to play minor roles, stickleback divergent selection is largely driven by natural and sexual selection (McKinnon and Rundle, 2002). For instance, parasites can drive local adaptation and genomic differentiation in this species (MacColl, 2009; Eizaguirre et al., 2012b; Feulner et al., 2015; Robertson et al., 2015) and habitat specific immunity and immune gene expression have been described (Wegner et al., 2003; Scharsack et al., 2007; Eizaguirre et al., 2011; Lenz et al, 2013; Huang et al., 2016; Lohman et al., 2017). A lot of knowledge on parasite selection of sticklebacks stems from studies involving the macroparasite *Schistocephalus solidus* (Hammerschmidt and Kurtz, 2009; MacColl, 2009; Barber, 2013). The diphyllobothriidean cestode *Schistocephalus solidus* is a trophically transmitted parasite with a three-host life cycle. The first larval stage, a free-living coracidium, infects cyclopoid copepods; the worm develops into a procercoid, i. e. the second larval stage, and becomes infective to *G. aculeatus*. Development of the plerocercoid, which is the third larval stage, occurs in this obligatory second intermediate host.

S. solidus grows massively in the body cavity of the fish, sometimes even exceeding the host's weight (Smyth, 1946; Clarke, 1954; Arme and Owen, 1967). The cestode matures in the definitive host, mostly piscivorous birds, and reproduces via self- or cross-fertilization (Smyth, 1946; Wedekind et al., 1998; Schärer and Wedekind, 1999). The reproductive output is directly related to *S. solidus'* size (Tierney and Crompton, 1992) and the eggs are defecated into the water. The final host can be replaced by an *in vitro* breeding system, facilitating controlled infections under standardized laboratory conditions (Smyth, 1946, 1954; Wedekind, 1997).

S. solidus prevalence, the percentage of infected sticklebacks, differs between populations and can be up to 100% (Smyth, 1946; Arme and Owen, 1967; Hopkins and Smyth, 1951; Barber and Scharsack, 2010). Its detrimental effects on sticklebacks were shown both in nature and in the laboratory (Arme and Owen, 1967; Tierney and Crompton, 1992; Heins et al., 1999; Heins et al., 2014). These effects often correlate with *S. solidus'* size. Thus, the parasite's size can provide information on (i) parasite development and fitness (Tierney and Crompton, 1992), (ii) host exploitation, i.e. virulence, (Arme and Owen, 1967; Heins and Baker, 2003; Bagamian et al., 2004; Heins, 2012) and (iii) the ability of the host to control the parasite's growth, i.e. resistance (Weber et al., 2017). It was suggested that *S. solidus* growth depends on host and/or parasite population-specific traits, which remain to be determined (Scharsack et al., 2016). Studies using hosts and parasites from different populations from Europe (Kalbe et al., 2016), from across continents (Weber et al., 2016) and *in vitro* leukocyte responses (Franke et al., 2014) indicate local adaptation of sticklebacks and *S. solidus*. However, to the best of my knowledge, common garden experiments (accounting for environmental variation and the effects of plasticity and genetics) have not been conducted.

Objectives

The co-evolutionary trajectories of hosts and parasites depend on the ecological context and on the potential for plastic responses. The consideration of the (co-)evolutionary and ecological factors that influence epidemiological traits has important implications for treatment and prevention strategies in human health. The immune modulatory characteristics of helminth infections, for example, can have advantageous and disadvantageous consequences for the host. The aim of this thesis is to advance our understanding of how and why epidemiological traits of host-helmith interactions vary among populations. I specifically asked (i) What are the effects of the host, the parasite and their interaction on epidemiological traits of helminth infections? (ii) What are the relative contributions of the genotypes and their phenotypic plasticity? (Figure 1) I use this framework to infer the consequences of different (co-)evolutionary trajectories of geographically distinct and ecologically divergent populations.

This thesis provides a more comprehensive view of the specificity of vertebrate immune defence by testing the effects (i) of different types (or 'strains') of the same host and parasite species, (ii) over the time course of infection, (iii) on co-infection probability, (iv) on different geographical (and phylogenetic) scales and (v) by investigation of the molecular phenotypes.

I used stickleback-*S. solidus* associations with phenotypically divergent forms to disentangle the host's and the parasite's contribution to infection phenotypes. I determined infection rates, host condition and immunological parameters, parasite size and expression levels of genes that are involved in helminth infections of sticklebacks. The immune genes were chosen from transcriptome and qPCR studies and categorized into innate immune genes, adaptive immune genes, and genes of complement components (Haase et al., 2014; Robertson et al., 2015; Stutz et al., 2015; Huang et al., 2016; Brunner et al., 2017). Subsets of these genes were classified as indicative for a T helper 1 type response, a T helper 2 type response, and T regulatory functions. I eventually included genes with potential regulatory functions (kindly provided by J. Gismann and M. Heckwolf).

OUTLINE

This thesis contains three chapters representing three separate manuscripts. The manuscripts for *Chapter 1* and *Chapter 2* have been submitted and structured according to the journal guidelines. The fond and the format were adjusted for this thesis.

I characterized contrasting stickleback (*Gasterosteus aculeatus*) populations with high resistance and low resistance against *Schistocephalus solidus* in *Chapter 1* (Figure 2). I found that two main effects – the host and the parasite – influenced the infection phenotype without crossing reaction norms. *S. solidus* strains from across the Northern Hemisphere grew generally larger in low resistance (DE) hosts from a population with high parasite diversity and low *S. solidus* prevalence. My results indicate that *G. aculeatus* and *S. solidus* from NO (low parasite diversity and high *S. solidus* prevalence) co-evolved high virulence and high resistance. The condition and immunological parameters of the two host types converged upon infection and *S. solidus* size followed the same geographic pattern in both host types.

I used these two types of hosts (low resistance and high resistance) and *S. solidus* (high growth and low growth) in co-infection experiments in *Chapter 2*. The aim was to study helminth immune modulation and the influence on co-infection probabilities over time and with respect to different co-evolutionary backgrounds. Co-infection probability depended on *S. solidus* type and developmental stage. Stickleback immune gene expression profiles differed remarkably between infected individuals of the two host types. I demonstrate an up-regulation of T regulatory functions when pro-inflammatory genes were up-regulated in high resistance hosts that co-evolved with high growth *S. solidus* (originating from NO).

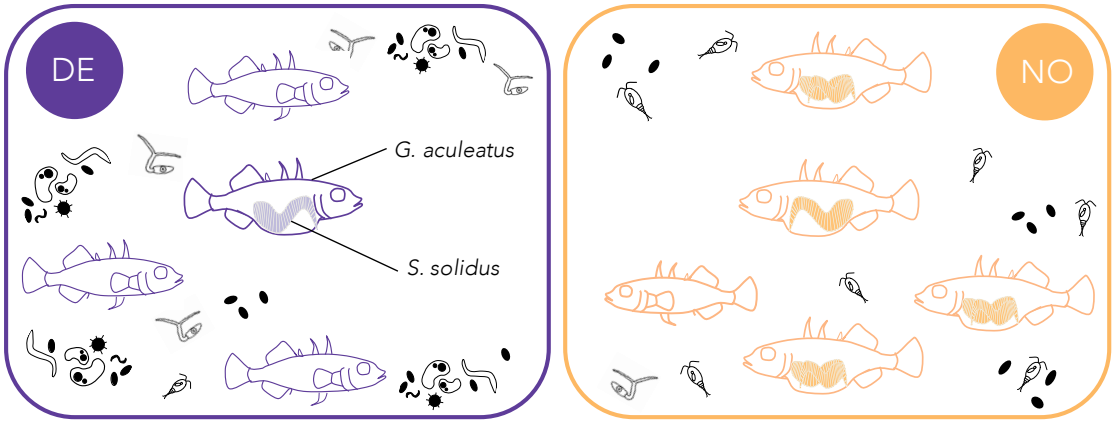


Figure 2. Characteristics of the two reference host populations. I used laboratory-bred first generation offspring from sticklebacks from Lake Großer Plöner See (DE, Germany) and Lake Skogseidvatnet (NO, Norway) in Chapter 1 and Chapter 2. These populations differ remarkably in parasite diversity (Shannon diversity index) and abundance (the mean number of parasites per fish) (Feulner et al., 2015; Huang et al., 2016). Parasite diversity is high and *S. solidus* prevalence (the number of infected individuals) is low (< 1%) in DE, whereas *S. solidus* prevalence is high and parasite diversity is low in NO (20 to > 50%). It was hypothesized that DE *G. aculeatus* and *S. solidus* evolved under de-escalated arms race dynamics, while NO *G. aculeatus* and *S. solidus* co-evolved high resistance (and/or tolerance) and high virulence.

Following up on those projects, I investigated the generalisability of my results and the specificity of distinct defence mechanisms across different geographic scales by incorporating species pairs from another continent in *Chapter 3*. In addition to European hosts and parasites, I used individuals from two Alaskan populations with known differences in phenotypic outcome of *S. solidus* infection. Baseline differences of host parameters again converged upon infection and were irrespective of the continent. Quantitative resistance and tolerance were host population-specific while qualitative resistance only occurred in one combination of an Alaskan host population with a European parasite strain. These results indicate that evolution favours distinct defence mechanisms when assessed on different geographic scales. I also conclude that the relative contribution of the *S. solidus*-induced phenotypic plasticity of *G. aculeatus* might generally be stronger than the genetic underpinnings of the different hosts.

Chapter 3 relates to an ongoing collaboration with colleagues from Stony Brook University, New York. The respective project includes studies of host and parasite microbiomes, which are not presented in this thesis.

CHAPTER 1

Specificity of resistance and geographic patterns of virulence in a vertebrate host-parasite system

Agnes Piecyk[1,2*], Olivia Roth[2], Martin Kalbe[1, ^]

[1] Department of Evolutionary Ecology, Max Planck Institute for Evolutionary Biology, August-Thienemann-Straße 2, 24306 Plön, Germany

[2] Marine Ecology, GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrookerweg 20, 24105 Kiel, Germany

[*] corresponding author: apiecyk@evolbio.mpg.de

[^] deceased

(in revision at *BMC Evolutionary Biology*)

ABSTRACT

Background: Host genotype - parasite genotype co-evolutionary dynamics are influenced by local biotic and abiotic environmental conditions. This results in spatially heterogeneous selection among host populations. How such heterogeneous selection influences host resistance, parasite infectivity and virulence remains largely unknown. We hypothesized that different co-evolutionary trajectories of a vertebrate host-parasite association result in specific virulence patterns when assessed on a large geographic scale. We used two reference host populations of three-spined sticklebacks and nine strains of their specific cestode parasite *Schistocephalus solidus* from across the Northern Hemisphere for controlled infection experiments. Host and parasite effects on infection phenotypes including host immune gene expression were determined.

Results: *S. solidus* strains grew generally larger in hosts coming from a population with high parasite diversity and low *S. solidus* prevalence (DE hosts). Hosts from a population with low parasite diversity and high *S. solidus* prevalence (NO hosts) were better able to control the parasite's growth, regardless of the origin of the parasite. Host condition and immunological parameters converged upon infection and parasite growth showed the same geographic pattern in both host types.

Conclusion: Our results suggest that NO sticklebacks evolved resistance against a variety of *S. solidus* strains, whereas DE sticklebacks are less resistant against *S. solidus*. Our data provide evidence that differences in parasite prevalence can cause immunological heterogeneity and that parasite size, a proxy for virulence and resistance, is, on a geographic scale, determined by main effects of the host and the parasite and less by an interaction of both genotypes.

KEYWORDS

host-parasite interaction, immunological heterogeneity, virulence, stickleback, *Schistocephalus solidus*

BACKGROUND

The interaction of an organism with its environment is a hallmark of life and a pre-requisite for natural selection. Local adaptation is driven by abiotic conditions and biotic interactions within and between species. Among the strongest evolutionary processes is the co-evolution between hosts and parasites (1–5). Parasites rely on host resources and have the potential to drastically reduce host fitness (6). To diminish the harm of parasites, effective defence strategies have evolved on the host side (4,7). However, heterogeneous environments select for different defence strategies among host populations, which results in immunological heterogeneity (8,9). The variation of host defence against parasites can range from mechanisms that decrease the risk of infection to processes that diminish the harm of parasites, such as resistance (i.e. the prevention of infection or the control of parasite growth) and tolerance (i.e. the ability to limit health or fitness effects of a distinct infection intensity) (10,11). Likewise, parasite infectivity and virulence (i.e. the detrimental effects on host traits related to fitness) are spatially structured both by environmental parameters and co-evolutionary processes.

The epidemiological traits are shaped through main effects of the host and the parasite and by interaction effects (Figure 1). The relative contribution of each of the interaction partners may differ along the infection process and depend on the geographic scale and the degree of environmental heterogeneity. Controlled infection experiments can be used to first identify environmental and evolutionary causes shaping the epidemiological traits and, second, to study the mechanisms and the adaptive significance thereof. Experiments revealed rapid and adaptive co-evolution of host and parasite genotypes in various systems, including phage-bacteria associations (4,12), malaria systems (13,14), plant-pathogen interactions (15,16), and immune gene evolution in three-spined sticklebacks (17,18). We chose the association of three-spined sticklebacks and their specific macroparasite *Schistocephalus solidus* to determine host and parasite effects along the infection process and on different geographic scales.

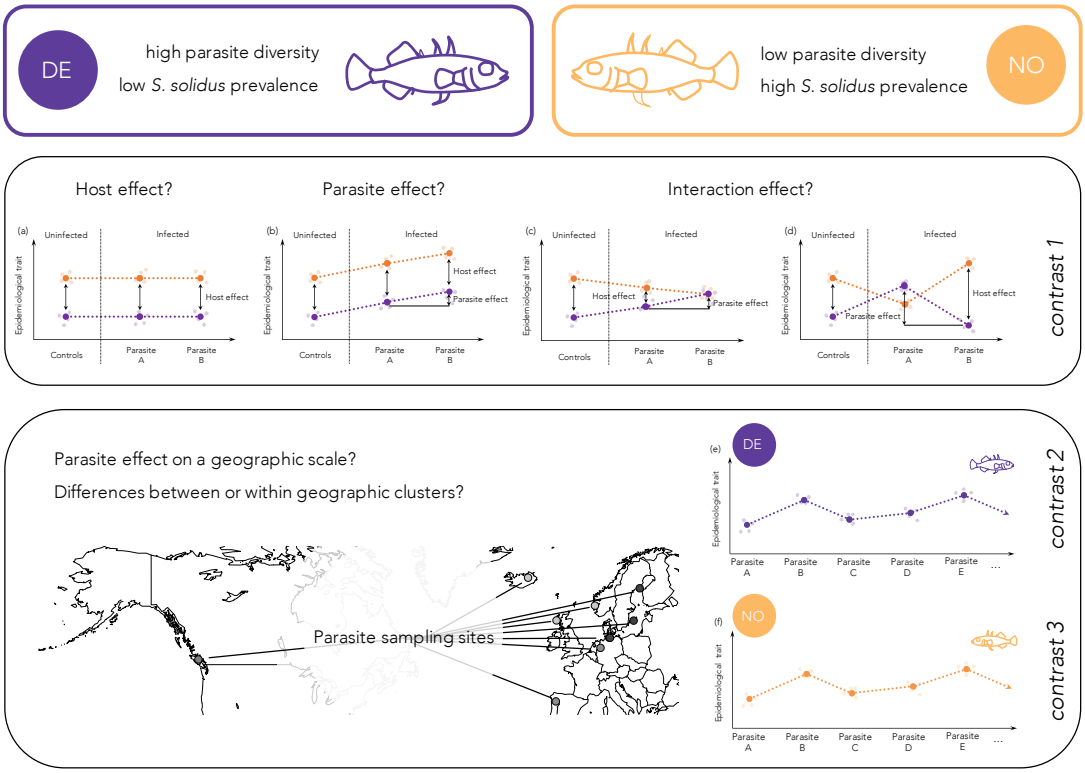


Figure 1. Theoretical framework of the study. Reference hosts came from two contrasting populations, indicated by violet (DE) and orange (NO) dots and lines. For the sake of simplicity, we exemplify possible outcomes with a subset of hypothetical parasites A to E. We asked whether main effects of the host, the parasite, and/or host-parasite interaction effects shaped epidemiological traits (life history traits of the host and/or the parasite). **(a, b, c, d)** Partitioning of host, parasite, and interaction effects on an epidemiological trait. **(a, b)** Host genotype and parasite genotype main effects. The host effect (vertical spacing between the two lines) indicates the genetic difference between the two host types. Parallel horizontal lines in **(a)** indicate absence of a plastic response towards infection. Differences among hosts that are infected with the same parasite (vertical spacing between the dots) indicate a phenotypic plastic response of the parasite. The positive slope in **(b)** indicates different effects of the two parasite types (parasite effect) and thus a phenotypic plastic response of the host and the parasite. **(c)** and **(d)** demonstrate host genotype-parasite genotype interaction effects, because the host effect depends on the parasite type. Crossing reaction norms in **(d)** clearly show the interaction effect; but note in **(c)** that the main-effect components can cumulate, causing non-crossing reaction norms. We tested the predictions with data from *contrast 1*. **(e, f)** To further understand the parasite effect on a larger geographic scale, each of the two host types was exposed to parasites from different geographic clusters across the Northern Hemisphere. We tested these predictions with data from *contrast 2* and *contrast 3*.

Three-spined sticklebacks (*Gasterosteus aculeatus*; hereafter 'sticklebacks') live in numerous freshwater and marine habitats across the Northern Hemisphere. Various studies reported habitat-specific immune responses (19–24). A lot of attention has been paid on the "supermodel" (25) interaction between sticklebacks and the cestode *Schistocephalus solidus*, as both can be bred in the laboratory facilitating controlled infection experiments (25). *S. solidus* has a three-host life cycle with copepods as first intermediate host and *G. aculeatus* as specific second intermediate host. *S. solidus* grows massively in the body cavity of the fish, sometimes even exceeding the host's weight (26,27). Reproduction is confined to the definite host, mostly piscivorous birds. The parasite's reproductive output is directly related to its size (28). *S. solidus'* detrimental effects on sticklebacks were shown both in nature and in the laboratory and have been linked to the size of the parasite (27–30). This cestode is assumed to be a driving force of divergent selection in three-spined sticklebacks (31). Studies using hosts and parasites from different populations from Europe (32), from across continents (33) and *in vitro* leukocyte responses (34) indicate local adaptation of sticklebacks and *S. solidus*. It was suggested that *S. solidus* growth depends on host and/or parasite population-specific traits (35).

We assumed that sticklebacks evolved environment-specific immunological adaptations to *S. solidus* and that *S. solidus* evolved environment-specific virulence. We specifically asked if such divergent evolution could cause different immunological activation in response to a variety of *S. solidus* strains (i.e. *S. solidus* parasites from distinct locations). The following was hypothesized: (i) the infection phenotype differs between sticklebacks from heterogeneous environments (indicating a host effect); (ii) the infection phenotype differs between *S. solidus* strains (indicating a parasite effect); (iii) the infection phenotype differs according to stickleback-*S. solidus* interactions (indicating an interaction effect) (Figure 1). These hypotheses were tested with three distinct analyses. First, hosts from two contrasting reference populations of *G. aculeatus* were experimentally infected with *S. solidus* from four European locations in order to test if host effects, parasite effects and/or interaction effects influenced *S. solidus* infection phenotypes in *G. aculeatus* (the corresponding analyses are referred to as *contrast 1*; Figure 1).

In order to test the parasite effect in further detail, each of these reference host types was infected with *S. solidus* strains from across the Northern Hemisphere (the corresponding analyses are referred to as *contrast 2* and *contrast 3*; Figure 1; Table 1; Table S1). *S. solidus* sampling sites covered four geographic areas (clustered localities) corresponding to *G. aculeatus* phylogeny: the Atlantic region (NU, ISC, SKO), the Baltic region (OBB, NST, GOT), European Inland (SP, IBB), and the Pacific (ECH) (Figure 2; Table 1).

Table 1. Summary table of sample sizes within contrasts of interest.

		Baltic								European Inland				Pacific				Atlantic									
Analysis	Host	OBB		GOT		NST				SP		IBB		ECH				NU		ISC				SKO			control
contrast 1	DE (A)	na		na		(a)	2			na		(a)	5	na				na		(a)	2			(d)	4		20
	DE (B)	na		na		(b)	10			na		(b)	3	na				na		(b)	5			(a)	7		18
	DE (C)	na		na		(c)	8			na		(c)	10	na				na		(c)	5			(c)	3		20
	NO (A)	na		na		(a)	2			na		(a)	3	na				na		(a)	1			(d)	4		20
	NO (B)	na		na		(b)	4			na		(b)	2	na				na		(b)	6			(a)	8		20
	NO (C)	na		na		(c)	2			na		(c)	2	na				na		(c)	5			(c)	2		20
contrast 2	DE (D)	(a)	4	(a)	5	(a)	4			(a)	1		na	(a)	3			(a)	1		na			(a)	7		20
	DE (E)	(b)	2	(b)	0	(b)	3			(b)	0		na	(b)	2			(b)	3		na			(b)	0		20
	DE (F)	(c)	4	(c)	3	(c)	9			(c)	7		na	(c)	2			(c)	6		na			(c)	5		20
contrast 3	NO (A)	(a)	1	(d)	1	(a)	2			(a)	2	(a)	3	(a)	1			(b)	5	(a)	1			(d)	4		20
	NO (B)	(b)	10	(b)	4	(b)	4			(d)	5	(b)	2	(c)	4			(d)	7	(b)	6			(a)	8		20
	NO (C)	(c)	1	(a)	2	(c)	2			(b)	2	(c)	2	(b)	0			(c)	6	(c)	5			(c)	2		20

Naïve laboratory bred first generation offspring from three-spined sticklebacks *Gasterosteus aculeatus* from Lake Großer Plöner See, Germany (DE), and Lake Skogseidvatnet, Norway (NO), were infected with *Schistocephalus solidus* parasites from different geographic locations or sham-exposed as controls. The top row indicates *S. solidus* geographic cluster; abbreviations in the second row refer to *S. solidus* sampling sites (OBB: Obbola, Sweden; GOT: Gotland, Sweden; NST: Neustädter Binnenwasser, Germany; SP: Xinzo de Limia, Spain; IBB: Ibbenbürener Aa, Germany; ECH: Vancouver Island, Canada; NU: North Uist, Scotland; ISC: Lake Myvatn, Iceland; SKO: Lake Skogseidvatnet, Norway; control: sham-exposed control). Capital letters indicate fish families (offspring of one pair of sticklebacks), lower case letters indicate worm sibships (offspring of one pair of worms). Per treatment, i.e. fish family x worm sibship combination, 100 copepods and subsequently 20 fish were exposed to single infective *S. solidus* larvae or sham-exposed; combinations with 'na' were not included in the respective analysis. Numbers in columns of *S. solidus* exposed fish indicate the number of infected individuals. We used *contrast 1* to test for host, parasite and interaction effects; *contrast 2* and *contrast 3* were used to test parasite effects on a broader geographic scale. NO data in *contrast 1* is a data subset of *contrast 3*. We accounted for multiple testing.

Figure 2. Sampling sites. Sticklebacks originated from Lake Großer Plöner See, Germany (DE), ~ 25 km from Neustädter Binnenwasser (NST; one of the sampling sites of *S. solidus*) and Lake Skogseidvatnet, Norway (NO). *S. solidus* were sampled from nine different locations across Europe and the Pacific (more information in Table S1). Colors indicate four geographic clusters (pink: Pacific, orange: Atlantic, violet: Baltic, green: European Inland). The map was drawn with the R package maps (78); colors were chosen from the ColorBrewer palette (77).

The two host populations differ remarkably in parasite diversity (Shannon diversity index) and abundance (the mean number of parasites per fish) (24,36). Parasite diversity is high and *S. solidus* prevalence (the number of infected individuals) is low (< 1%) in the German habitat (DE), whereas *S. solidus* prevalence is high and parasite diversity is low in the Norwegian population (NO) (20 to > 50%). Under the assumption that immune defence is costly and co-evolves with parasite virulence (7,37–40), we hypothesized that sticklebacks from the highly *S. solidus* exposed (NO) population evolved *S. solidus* specific resistance, whereas this might not be the case for the rarely *S. solidus* exposed (DE) population. We suggested that *S. solidus* specific resistance could be effective against sympatric and potentially even allopatric strains. In order to cover numerous important parameters along the infection process, infection rates and the size of the parasite, as well as host condition and immunological parameters were determined (10). The size of the parasite is used a measure of host resistance and parasite virulence (32,11,41). The immunological activation was inferred from the size of the major immune organs and by immune gene expression analyses. We asked whether host population and/or parasite strain, cluster or growth caused distinct gene expression profiles. This study investigates evolutionary and proximate (physiological and molecular) causes of immunological heterogeneity, the specificity of resistance and the contribution of host and parasite on infection phenotypes.

RESULTS

Both intermediate hosts (copepods and sticklebacks) were infected with *S. solidus* from every location (SI.1; Tables S2 and S3). We obtained 227 plerocercoids from 1342 fish (excluding two infected controls and one double infected fish). The average weight of *S. solidus* plerocercoids 55 (+/-2) days post exposure (DPE) was 61.8 mg and varied between 0.6 mg and 151.4 mg. Neither infection rates in copepods nor infection rates in fish influenced *S. solidus* size in the fish (LMMs for average parasite index (PI) per worm sibship as dependent variable; worm origin, infection rates in copepods and in fish as fixed effects, round as random term).

Contrast 1, the comparison of DE and NO hosts infected with four different European *S. solidus* strains, covered 587 fish: 118 controls (excluding two infected DE controls), 105 infected fish, 364 exposed but uninfected fish; 11 fish died. *Contrast 2*, testing the parasite effect in DE hosts, covered 522 fish: 60 controls, 71 infected fish, 335 exposed but uninfected fish; 14 fish died. *Contrast 3*, testing the parasite effect in NO hosts, covered 60 controls, 92 infected fish, 433 exposed but uninfected fish; 15 fish died.

Constitutive differences between the host populations (contrast 1)

Contrast 1, the combination of the two hosts and four *S. solidus* strains, was used to test for host effects, parasite effects and host-parasite interaction effects on infection rates and infection phenotypes (Figure 1; Table 1). *S. solidus* infection rates were consistent among host populations (host effect: $X^2_1 = 2.27$, $p = 0.132$; *S. solidus* effect: $X^2_3 = 0.882$, $p = 0.830$; host-parasite interaction effect: $X^2_3 = 6.42$, $p = 0.093$; Table S4). However, all four *S. solidus* strains were significantly smaller in NO hosts (parasite index, PI, the relative weight of *S. solidus* in the host (27); host effect: $F_{1,95} = 23.48$, $p < 0.0001$). The differences between *S. solidus* strains were independent of the host population (host-parasite interaction effect on PI: $F_{3,95} = 0.995$, $p = 0.399$) (Figure 3; Tables S5-S7).

We detected constitutive differences in condition and immunological parameters of the two stickleback populations (more information in SI.3). DE sticklebacks had a significantly higher condition (CF; an estimate of the overall condition (42)) if they were uninfected (X^2_1 = 44.252, p < 0.0001) or infected with *S. solidus* from the Baltic (NST) (X^2_1 = 10.48, p = 0.001). Hepatosomatic indices (HSI, an estimate of metabolic reserves (43)) were higher in DE controls compared to NO controls (X^2_1 = 26.93, p < 0.0001). Head kidney indices (HKI, the relative weight of the major immune organ in fish) were generally higher in DE fish (X^2_4 = 49.47, p < 0.0001) and DE controls showed higher reactive oxygen species (ROS) production of head kidney leukocytes (X^2_1 = 24.1, p < 0.0001). Splenosomatic indices (SSI, the relative weight of the major secondary immune organ (44)) were significantly higher in DE controls (X^2_1 = 79.38, p < 0.0001) and in DE hosts infected with Baltic (NST) *S. solidus* (X^2_1 = 30.75, p < 0.0001) or European Inland (IBB) *S. solidus* (X^2_1 = 19.02, p < 0.0001). The effects were not directly related to *S. solidus* size but to *S. solidus* strain. We detected no significant differences in these condition and immunological parameters between DE and NO sticklebacks if they were infected with *S. solidus* from two Atlantic populations (SKO, ISC) (Figure S1).

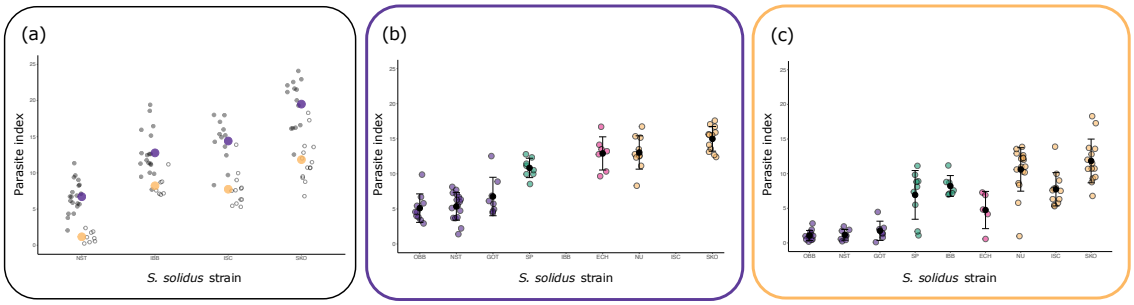


Figure 3. *S. solidus* growth differs significantly between host populations and between geographically clustered parasite strains. Naïve laboratory bred F1 offspring from sticklebacks from lake Großer Plöner See, Germany (DE), and Lake Skogseidvatnet, Norway (NO), were experimentally infected with single *S. solidus* larvae from nine different locations ('strains') across the Northern Hemisphere. Fish were dissected 55 (+/- 2) days after exposure to the parasite. The parasite index (PI) was calculated as the proportion of the parasite's weight from the total weight of infected fish. **(a)** DE and NO hosts were infected with four different European *S. solidus* strains (*contrast 1*). Black and white dots represent individuals; violet: mean parasite indices in DE hosts; orange: mean parasite indices in NO hosts (Table S6). **(b)** Parasite indices in DE hosts (*contrast 2*). Black dots and bars indicate the mean and the standard deviation. Color coding follows Figure 2. **(c)** Parasite indices in NO hosts (*contrast 3*). Black dots and bars indicate the mean and the standard deviation. Color coding follows Figure 2.

Total RNA from spleen was used to determine expression levels of 24 key immune genes. We ran non-parametric permutational multivariate analyses of variance (PERMANOVA) including host and parasite main effects and their interaction. The main effects were significant predictors while the interaction did not influence immune gene expression profiles (host effect: $PERMANOVA_{innate}$: $F_{1,148} = 10.69$, $p < 0.0001$; $PERMANOVA_{adaptive}$: $F_{1,148} = 13.58$, $p < 0.0001$; $PERMANOVA_{complement}$: $F_{1,148} = 7.03$, $p = 0.0001$; *S. solidus* effect: $PERMANOVA_{innate}$: $F_{4,148} = 3.74$, $p = 0.0002$; $PERMANOVA_{adaptive}$: $F_{4,148} = 2.73$, $p = 0.007$; $PERMANOVA_{complement}$: $F_{4,148} = 3.82$, $p = 0.0002$; host-parasite interaction effect: $PERMANOVA_{innate}$: $F_{4,148} = 0.93$, $p = 0.45$; $PERMANOVA_{adaptive}$: $F_{4,148} = 1.01$, $p = 0.41$; $PERMANOVA_{complement}$: $F_{4,148} = 0.40$, $p = 0.94$). Pairwise PERMANOVAs were used *a posteriori* in order to identify significantly different groups (45).

Immune gene expression profiles differed significantly between DE and NO controls ($PERMANOVA_{innate}$: $F_{1,48} = 3.32$, $p < 0.001$; $PERMANOVA_{adaptive}$: $F_{1,48} = 6.76$, $p = 0.002$; $PERMANOVA_{complement}$: $F_{1,48} = 4.78$, $p = 0.004$; Table S11; Figure 4). DE sticklebacks had higher expression levels of genes of innate and adaptive immunity, while complement genes were lower expressed than in NO stickleback (Table S8; Figure S6). ISC *S. solidus* infection caused different innate immune gene expression in DE and NO sticklebacks ($PERMANOVA_{innate}$: $F_{1,22} = 3.58$, $p = 0.004$; Table S9; Figure 4), which was driven by remarkably low expression of Interleukin-1β (*il-1β*) in DE sticklebacks ($F_{1,18} = 20.0$, $p < 0.001$) (Table S9; Figure S6). Expression profiles of NST-, IBB- and SKO-infected fish did not differ significantly between host populations (Figure 4).

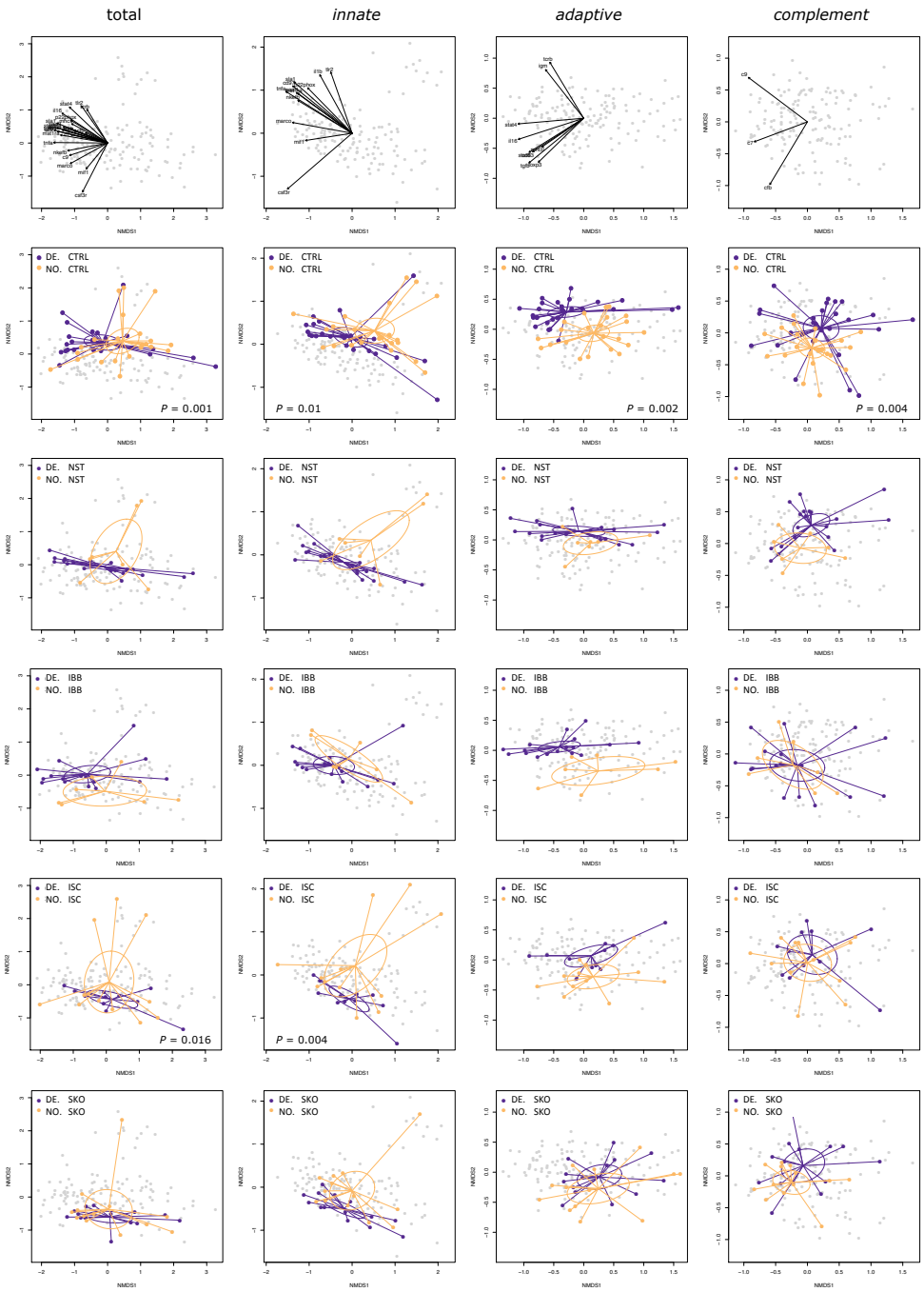


Figure 4. Multivariate gene expression patterns differ between DE and NO sticklebacks. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions comparing data from NO and DE sticklebacks (*contrast 1*). NMDS were based on log10-transformed calibrated normalized relative quantities (CNRQ values) of all 24 immune genes, twelve genes of innate immunity (*marco*, *mst1ra*, *mif*, *il-1β*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), nine genes of adaptive immunity (*stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *tgf-β*, *il-16*, *mhcII*, *tcr-β*), or three genes of the complement system (*cfb*, *c7*, *c9*). Each dot represents one individual; colors refer to the host population. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction. The contribution of each gene is shown in the first row. The second row shows data from sham-exposed (CTRL) sticklebacks. The third to sixth row show data from infected individuals. Function metaMDS() was used to plot the NMDS; the contribution of each gene was plotted by use of the envfit() function (both functions are implemented in R package *vegan* (75)).

Parasite indices show a geographic pattern in both host types

To further understand the effect of the parasite on infection phenotypes, we exposed DE hosts (*contrast 2*) and NO hosts (*contrast 3*) to *S. solidus* strains from across the Northern Hemisphere (Figure 1). The infection rates did not differ significantly between parasite strains in DE sticklebacks (*contrast 2*: $X^2_6 = 7.15$, $p = 0.307$), but did so in NO sticklebacks (*contrast 3*: $X^2_8 = 21.62$, $p = 0.006$) (Tables S3-4). Parasite indices differed between parasite strains (*contrast 2*: $F_{6,62} = 42.39$, $p < 0.0001$; *contrast 3*: $F_{8,81} = 61.09$, $p < 0.0001$). We found a clear pattern with *S. solidus* from the Baltic being significantly smaller than worms from the other origins; Atlantic *S. solidus* were the largest in both host types (Tables S10-S12; Figure 3).

Immune gene expression is parasite strain specific

Building on from the idea that *S. solidus* growth follows a geographic pattern, we asked whether the molecular phenotypes would show the same clustering. We studied the influence of *S. solidus* strain on stickleback immune gene expression by running pairwise PERMANOVAs within host populations (*contrast 2* or *contrast 3*) and tested (i) if gene expression differed within and/or between geographic clusters (Atlantic, Baltic, European Inland, Pacific) and (ii) if immune gene expression differed between sham-exposed controls and *S. solidus* infected sticklebacks for each parasite origin. Gene expression neither differed significantly within the clustered localities, nor between Baltic and Atlantic or European parasites, although the parasite indices differed considerably (Figures 3 and 5). Immune gene expression profiles only differed between clustered localities if sticklebacks were infected with *S. solidus* from the Pacific (ECH) versus the Baltic or Atlantic region (Figure 5).

In DE sticklebacks (*contrast 2*), Pacific *S. solidus* infection was associated with higher expression of innate immune genes ($PERMANOVA_{innate}$: $F_{1,33} = 3.88$, $p = 0.018$), adaptive immune genes ($PERMANOVA_{adaptive}$: $F_{1,33} = 4.16$, $p = 0.013$) and complement components ($PERMANOVA_{complement}$: $F_{1,33} = 8.1$, $p = 0.001$) compared to infection with Baltic *S. solidus* (Table S13). Compared to infection with Atlantic *S. solidus*, Pacific *S. solidus* infection was associated with higher expression of adaptive immune genes

($PERMANOVA_{adaptive}$: $F_{1,26}$ = 5.84, p < 0.001) and complement components ($PERMANOVA_{complement}$: $F_{1,26}$ = 3.66, p = 0.016) in DE sticklebacks; only *mhcII* RNA levels were lower in Pacific *S. solidus* infections ($F_{1,26}$ = 15.71, p = 0.0007; Table S14).

In *contrast 3*, NO sticklebacks infected with Pacific *S. solidus* showed differential expression of genes of innate ($PERMANOVA_{innate}$: $F_{1,29}$ = 3.26, p = 0.006) and adaptive immunity ($PERMANOVA_{adaptive}$: $F_{1,29}$ = 5.8, p = 0.002) in comparison to infection with Baltic *S. solidus*. Seven innate immune genes (*marco*, *mif1*, *tnfr1*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*) and five adaptive immune genes (*stat4*, *cd83*, *foxp3*, *tgf-β*, *il16*) were significantly higher expressed in Pacific *S. solidus* infections; only RNA levels of *mhcII* were significantly lower (Table S15). In comparison to infection with Atlantic *S. solidus*, Pacific *S. solidus* infection was linked to higher expression of innate immune genes ($PERMANOVA_{innate}$: $F_{1,47}$ = 2.95, p = 0.014), adaptive immune genes ($PERMANOVA_{adaptive}$: $F_{1,47}$ = 5.27, p = 0.004) and complement components ($PERMANOVA_{complement}$: $F_{1,47}$ = 5.16, p = 0.008) in NO hosts. Seven genes of innate immunity (*mst1ra*, *il-1β*, *tnfr1*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), seven genes of adaptive immunity (*stat4*, *igm*, *cd83*, *foxp3*, *tgf-β*, *il16*, *mhcII*) and complement *c9* were significantly higher expressed in NO sticklebacks infected with Pacific *S. solidus* in comparison to infection with Atlantic *S. solidus* (Table S16).

We next tested if immune gene expression patterns differed between infected and control fish within *contrast 2* or *contrast 3*. Again, gene expression patterns were not related to parasite indices or size but strain-specific.

In DE hosts (*contrast 2*), expression of genes of all three functional arms of the stickleback's immune system differed significantly between sham-exposed controls and fish infected with Pacific *S. solidus* ($PERMANOVA_{innate}$: $F_{1,32}$ = 7.51, p < 0.0001; $PERMANOVA_{adaptive}$: $F_{1,32}$ = 6.47, p < 0.001; $PERMANOVA_{complement}$: $F_{1,32}$ = 5.57, p = 0.007; Table S17) or Scottish (NU) *S. solidus* ($PERMANOVA_{innate}$: $F_{1,35}$ = 4.89, p = 0.003; $PERMANOVA_{adaptive}$: $F_{1,35}$ = 3.925, p = 0.009; $PERMANOVA_{complement}$: $F_{1,35}$ = 4.75, p = 0.014; Table S18). Infection with Norwegian (SKO) *S. solidus* altered expression of adaptive immune genes ($PERMANOVA_{adaptive}$: $F_{1,35}$ = 8.76, p < 0.0001) and complement genes ($PERMANOVA_{complement}$: $F_{1,35}$ = 3.42, p = 0.028; Table S19).

In NO sticklebacks (*contrast 3*), innate immune genes and complement components were differentially expressed between controls and hosts infected with Pacific *S. solidus* ($PERMANOVA_{innate}$: $F_{1,26} = 5.43$, $p = 0.0118$; $PERMANOVA_{complement}$: $F_{1,26} = 7.61$, $p = 0.008$; Table S20). Adaptive immune genes were differentially expressed between controls and Atlantic (NU) *S. solidus* infections ($PERMANOVA_{adaptive}$: $F_{1,39} = 5.71$, $p = 0.002$; Table S20).

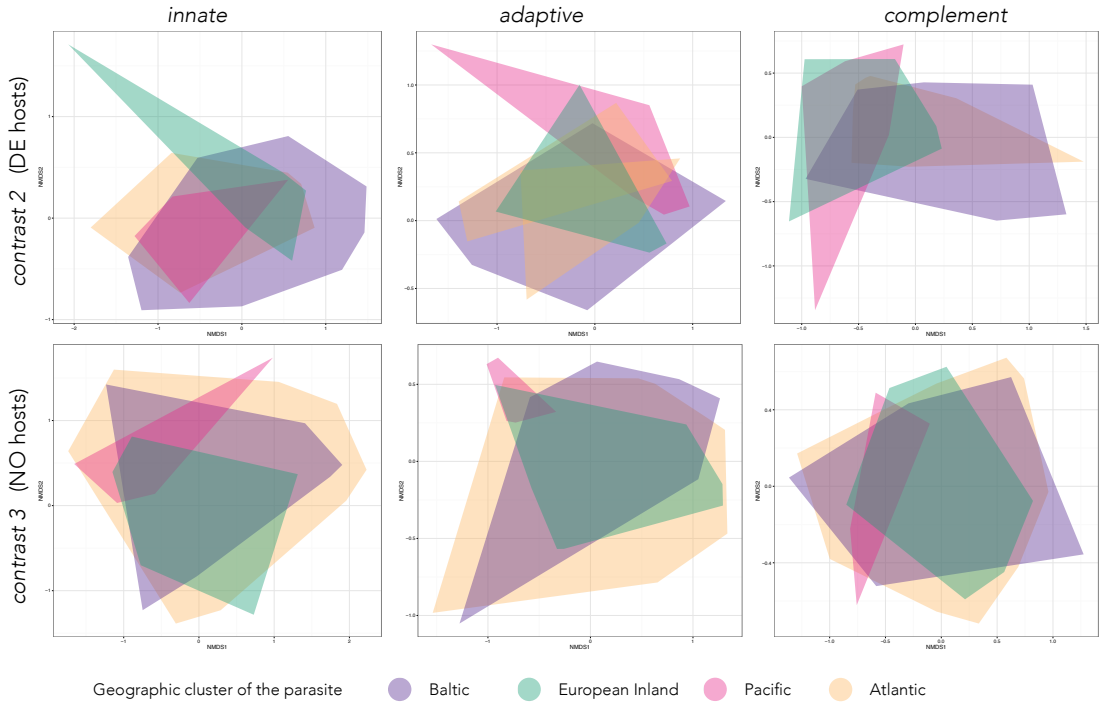


Figure 5. Infection with Pacific *S. solidus* drives significantly different multivariate gene expression patterns. Multivariate patterns in gene expression were visualized by non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions using function metaMDS() from *vegan* (75). Polygons were plotted using *ggplot2* (76). NMDS were based on log10-transformed calibrated normalized relative quantities (CNRQ values) of twelve genes of innate immunity (*marco*, *mst1ra*, *mif*, *il-1β*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), nine genes of adaptive immunity (*stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *tgf-β*, *il-16*, *mhcII*, *tcr-β*), or three genes of the complement system (*cfb*, *c7*, *c9*). Upper panel: data from DE hosts infected with seven different *S. solidus* strains from the four clustered localities (*contrast 2*); lower panel: data from NO hosts infected with nine different *S. solidus* strains from the four clustered localities (*contrast 3*). Color coding follows Figure 1.

DISCUSSION

Parasites are important components of the host's environment and a crucial agent of natural selection (5,7,8,34,35,41). The co-evolution between hosts and parasites entails complex dynamics, influencing host defence and parasite infectivity and virulence. We used controlled infection experiments of three-spined sticklebacks from two contrasting populations with a variety of *Schistocephalus solidus* strains in order to characterize specificity and consequences of divergent co-evolution in a vertebrate host-parasite association. We propose that main effects of the host and the parasite determine *S. solidus* virulence, whereas the interaction might play a minor role.

Immunological differences between host populations

NO sticklebacks come from a population with high *S. solidus* prevalence and low parasite diversity (24,36). Since immune defence is costly and co-evolves with parasite virulence (7,37–40), we hypothesized that NO sticklebacks evolved specific resistance against *S. solidus*. Infection rates did not differ significantly between host populations, but *S. solidus* plerocercoids were consistently smaller in NO hosts. This supports our hypothesis that NO hosts evolved increased resistance against *S. solidus* as inferred from parasite growth suppression (11,41). We found that controls from the DE population had higher immunological activity than NO controls (Figure 4; SI.3-4). This is in line with the natural situation, as DE hosts are constantly challenged through high parasite diversity and abundance. However, the differences in immunological activation between the two host populations mostly converged upon infection: while immune gene expression profiles and respiratory burst activity of head kidney leukocytes differed significantly between controls, those parameters converged when fish were infected with *S. solidus* from most origins (Figure 4; Figure S1). This resembles the results from among-lake reciprocal transplant experiments (46) and comparisons of wild and laboratory-raised fish (47). Consistently, these findings emphasize the importance of environmental effects on immune gene expression relative to genetic adaptation. We infer from our data that phenotypic plasticity in response to parasite infection is a stronger contributor to immunological activation than host genotype.

Parasite strain specific immune gene expression

Host immune gene expression did not depend on *S. solidus* size or geographic cluster, but was parasite strain specific. Immune gene expression profiles differed between NO and DE controls and if fish were infected with Icelandic (ISC) *S. solidus* (*contrast 1*). Notably, Icelandic sticklebacks seem to be genetically distinct from other Atlantic populations (48).

Within DE hosts (*contrast 1*) and within NO hosts (*contrast 2*), expression profiles of infected fish did not differ between or within clustered parasite localities, but only if sticklebacks were infected with *S. solidus* from the Pacific (ECH) (Fig. 3). Those parasites originated from the geographically most distant population, indicating the potential of local adaptation at this scale (33). Infection with Pacific *S. solidus* was consistently associated with high expression of most immune genes but low expression levels of *mhcII*. Major histocompatibility complex (MHC) class II molecules are important components of adaptive immunity and activate T-cell mediated humoral immune responses (49). In our experiments, low expression of *mhcII* was often associated with low expression of the gene of T-cell receptor subunit TCR-β that is involved in MHC ligand binding (SI.4). If a speculative active down-regulation of this arm of the immune system in allopatric combinations results from a direct manipulation by *S. solidus* remains to be answered.

In comparison to sham-exposed controls, Pacific *S. solidus* infection caused high expression of pro-inflammatory and complement genes in hosts of both populations (Tables S17 and S20). Genes of adaptive immunity were highly expressed (*foxp3*) or down-regulated (*tcr-β* and *mhcII*) in DE hosts. A simultaneous up-regulation of *foxp3* is indicative of a T regulatory response (47) that potentially protects the host but may also enable parasite growth through anti-inflammatory activities. Indeed, ECH *S. solidus* were three times bigger in DE sticklebacks (Fig. 2). Pacific and Atlantic *S. solidus* reached similar sizes in DE hosts but, except for a potential involvement of *tcr-β* and/or *mhcII*, distinct genes were differentially expressed between hosts infected with parasites from different populations (SI.4.3).

We infer that (i) the relative parasite size and immune gene expression profiles are similar in infected fish of the two populations (similar parasite effect) but that (ii) complex ecological and co-evolutionary adaptations at different localities caused distinct levels of virulence and resistance.

Geographic pattern of virulence

Parasite indices were strikingly similar between the two host populations with regard to the geographic origin of the parasite. *S. solidus* from Atlantic populations grew consistently larger and Baltic parasites were the smallest in both host types (Figure 3). The geographic pattern of virulence in both host types highlights the parasite main effect. The greatest difference was the suppression of Pacific *S. solidus* growth through Atlantic (NO) sticklebacks relative to Baltic (DE) sticklebacks. Sticklebacks from the Atlantic region likely originate from the Pacific (50), so we suggest a relatively similar genetic background of Pacific and Atlantic *G. aculeatus – S. solidus* species pairs. Such a similarity could explain the higher resistance of Atlantic hosts against Pacific parasites. Baltic stickleback populations, in contrast, form a cluster that is distinct from European Inland populations (48). This, again, is a pattern that we also see in *S. solidus* growth (Fig. 2). Thus, the geographic pattern of virulence corresponds to the host's recolonization history after the last glaciation (48). Based on these data and a previous study (35), we hypothesize that the parasite's phylogeny resembles the phylogeny of its highly specific host. A genetic basis could explain the same clusters of *S. solidus* growth in both host types. Latitude or geographical distance between host and parasite source populations did not explain parasite size. This renders the question of what could have selected for different *S. solidus* types.

We propose that *S. solidus* evolved different life-history strategies in response to distinct selection by their hosts and habitat-specific trade-offs. Baltic *S. solidus* from NST, where *S. solidus* prevalence is extremely low (32), did not reach the proposed minimum weight (50 mg) for sexual reproduction in final hosts (28,51,52). Baltic *S. solidus* from Swedish populations (OBB, GOT), where *S. solidus* prevalence is actually high (T. Henrich; pers. comm.), showed the same growth pattern. Hence, parasite prevalence might be one explanation (32,34,35,41), but is certainly not the only cause for different growth strategies, especially in the light of ecological effects on exposure risk (33). Another possible inference is that *S. solidus* from the Baltic region reach sexual competence at lower weights than those from other populations, which is supported by the fact that smaller worms can reproduce (26). Nevertheless, mapping variation on fitness differences in the natural habitat remains to be investigated.

CONCLUSIONS

We tested the specificity and immunological activation of three-spined sticklebacks *Gasterosteus aculeatus* towards various strains of the cestode *Schistocephalus solidus* at different stages of the infection process. (i) *S. solidus* infection rates were consistent among the two host populations whereas (ii) the growth of the parasite differed significantly among host populations and among parasite strains from different geographic clusters. Parasite indices were determined by main effects of the host and the parasite with insignificant interaction effects. (iii) Immune gene expression profiles were host-parasite combination specific, suggesting stronger interaction effects at this level of the infection process. Our results highlight the differences between mechanisms of distinct stages of the infection process and provide new insights into cestode growth suppression as a form of resistance (41).

We found constitutive immunological population differences but similar responses to infection. Our data provide evidence for (co-)evolutionary and ecological effects on immune functions that favour immunological heterogeneity.

We propose that sticklebacks and *S. solidus* from a population with high *S. solidus* prevalence (NO) co-evolved high virulence and high resistance. The high resistance of NO hosts against *S. solidus* (host main effect) was not strain specific on an intermediate geographic scale (across Western Europe). On a larger geographic scale, parasites from the most distant (Pacific) population triggered elevated immunological parameters. The analogous clustering of parasite growth according to geography in the two host populations highlights the strong contribution of the parasite main effect on infection phenotypes. We suggest that patterns of local adaptation are either weak, absent or might be found at large scales (32–35).

METHODS

Experimental hosts and parasites

Hosts and parasites were laboratory-raised first generation offspring from wild-caught individuals. Sticklebacks originated from lake Großer Plöner See, Germany (DE), and lake Skogseidvatnet, Norway (NO) and were kept in the institute's aquaria system at 18 °C and a light:dark rhythm of 16:8 hours. All fish were approximately nine months old at the start of the respective experiment. Sticklebacks were experimentally infected in 18 different combinations. We two experiments with essentially the same procedures. Each experiment was composed of three rounds using distinct fish families and parasite sibships. 'Fish family' refers to offspring from one pair of sticklebacks; 'parasite sibship' refers to offspring from one pair of worms. Parasite sibships from one origin are here referred to as 'strain'. Sham-exposed controls were included in each round. A total of 1345 fish were analysed (Table 1; Table S1). We tested for host, parasite and host-parasite interaction effects using 'contrast 1'. The respective infection experiments were run simultaneously and involved the exact same parasite sibships for both host populations, which should reduce any confounding factors. Parasite effects were further tested within each host type by using *S. solidus* strains from across the Northern Hemisphere (Table 1; Figure 1 and 2).

Schistocephalus solidus plerocercoids had been sampled from naturally infected sticklebacks from nine different locations (Figure 2; Table S1). The sampling sites cover four geographic areas corresponding to *G. aculeatus* phylogeny: the Atlantic region (NU, ISC, SKO), the Baltic region (OBB, NST, GOT), European Inland (SP, IBB), and the Pacific (ECH). The parasites were bred *in vitro* in the laboratory in 2012 – 2014. The eggs were kept at 4 °C in the dark.

Infection experiments

S. solidus eggs developed at 20 °C for three weeks. A 3:8 hours light:dark cycle and another light stimulus initiated hatching of the first larval stage (coracidia). Single coracidia were immediately fed to *Macrocyclops albidus* copepods (first intermediate hosts) from laboratory cultures. Copepods were kept at 16:8 hours light:dark cycles at 18 °C and fed with *Paramecium* three times a week. Infection success was determined by inspection for procercoids (second larval stage) *in vivo* 7 to 11 DPE. On day 16, sticklebacks were exposed to single infected copepods or uninfected controls. By this time, *S. solidus* is infective to its second intermediate host and differences in infection success are unlikely to be caused by variation in ontogeny (53,54). The fish were starved for two days and isolated in individual tanks. We assigned numbers to each treatment group, i.e. worm sibship and the control, and used a random design for the exposure to avoid any observer bias. The fish were transferred to 16 L aquaria according to their numbers 24 hours after exposure. The water was sieved in order to determine the number of ingested copepods per treatment. Sticklebacks were kept in aerated aquaria connected to a flow-through freshwater system at 18 °C and a light:dark rhythm of 16:8 hours. The density of 20 individuals per aquarium was maintained by replacing dead fish with spine-clipped sticklebacks from the same fish family.

The fish were fed with frozen *Chironomidae* larvae three times a week but starved for two to four days before dissection. We dissected the fish in the laboratory 55 (+/- 2) DPE. Fish of every treatment group per experiment were dissected on each day. Sticklebacks were euthanized with MS222 (1 g/L), weighed and measured (standard length, i.e. without tail fin).

The head kidneys, spleen, liver, gonads, and, if present, worms were weighted to the nearest 0.1 mg. The carcasses were stored on ice upon dissection. Head kidney cells were immediately prepared for flow cytometric analyses. Spleen, liver and worms were transferred to RNAlater® (Sigma R0901; tenfold volume per weight), kept at 4 °C for one day and stored at -20 °C until further use.

Phenotypic parameters

Infection rates were calculated with respect to the number of copepods that had not been ingested and include data from double infected hosts and fish that died before the day of dissection. The parasite index (PI) is a proxy for parasite size and host exploitation (32) and is calculated as the proportion of the total weight of an infected fish accounted for by the parasite (27). The condition factor (42) and the hepatosomatic index (HSI) (43) are estimates of host condition. The splenosomatic index (SSI) (55) and head kidney index (HKI) were used as first proxies of immunological activation. The head kidney is the major immune organ in bony fish (44). Thus, head kidney leukocytes (HKL) were studied in more detail (56) (SI.3). Briefly, total cell numbers were determined by a modified protocol (57) of the Standard cell dilution assay (58). Granulocytes and leukocytes were identified according to their FSC/SSC profiles using a Becton Dickinson FACS Calibur and BD CellQuest™ pro software (Version 6.0). We calculated a granulocyte to lymphocyte ratio (G/L ratio) as a rough activity estimate of the innate versus the adaptive immune system (59), and used a lucigenin-enhanced chemiluminescence assay (60,59) to measure the phagocytic capacity of HKL by quantifying the respiratory burst reaction in relative luminescence units (RLUs). More details can be found in SI.3.

Gene expression analyses

Differential gene expression of *S. solidus* infected fish and sham-exposed controls was studied by quantitative real time reverse transcription PCR (RT-qPCR). Total RNA from spleen was extracted with the NucleoSpin®96 Kit (Macherey-Nagel) according to the manufacturer's manual. Samples were thawed at 4 °C, transferred to new tubes, supplied with ß-mercaptoethanol (1% V/V) containing lysis buffer and homogenized for 2 x 3 min at 30 Hz using Tissue Lyser II (Qiagen).

A DNase digestion step was included. RNA was eluted with 40 µL RNase-free H_2O. RNA concentration and quality were measured spectrophotometrically (NanoDrop; Thermo Scientific). Samples with concentrations below 6 ng/µL or A_{260}/A_{280} ratios < 1.9 were excluded. Reverse transcription was performed on 6.4 ng of total RNA using the Omniscript® RT Kit (Qiagen) with oligo dT priming and RNase inhibition (0.2 µL per reaction) at 37°C for 60 min. 12.8 µL of sample RNA were used if the concentration was below 39 ng/µL. The cDNA was stored at -20 °C and diluted 1:5 with RNase-free H_2O before pre-amplification. Pre-amplification was performed with TaqMan® PreAmp Master Mix (Applied Biosystems) according to the manufacturer's instructions with 14 cycles. The PCR product was diluted 1:5 with low TE buffer. Differences in transcription levels were tested using 96.96 Dynamic Array IFCs on a Biomark™ HD system (Fluidigm) according to the manufacturer's protocol. EvaGreen was used as DNA binding dye. Samples were spread across four IFCs. All targets for a given sample were included in the same run and measured in triplicates (technical replicates). Inter-run calibrators, dilution series, and negative controls were included on each IFC. *Fluidigm Analysis software* was used to assess melting curves of all qPCR assays in order to confirm specific amplification. Samples with suspicious T_m profiles in more than two targets or failed amplifications were excluded. *qbase+ 3.0* (Biogazelle) was used for calculation of calibrated normalized relative quantities (CNRQ values). Replicates with variability (difference in quantification cycle, Cq) > 0.5 and wells with Cq > 28 were excluded, resulting in 94 % pass rate. The average Cq was calculated as arithmetic mean; targets were scaled to average. We determined target and run specific amplification efficiencies. Expression stability of putative reference targets was inferred from geNorm M and Coefficient of Variation (CV) values (61,62). The most stably expressed reference targets *rpl13* and *ubc* (M = 0.133, CV = 0.046) were used for normalization. CNRQs were log10 transformed for analysis. Three missing values from gene *csf3r* and one missing value from *tlr2* were replaced by the mean expression of the respective gene. We analysed gene expression data of a total of 284 individuals from 18 different combinations including controls.

Genes targeted in expression analyses

We used 28 different primer pairs targeting mRNA from immune related genes and putative reference genes (*b2m*, *ef1a*, *rpl13a*, *ubc*; described in (63)). Targets of interest covered genes of innate immunity (*cd97*, *csf3r*, *il-1β*, *marco*, *mif*, *mst1ra*, *nkef-b*, *tnfr1*, *saal1*, *tlr2*, *p22^{phox}*, *sla1*), adaptive immunity (*cd83*, *foxp3*, *igm*, *il-16*, *stat4*, *stat6*, *tgf-β*, *mhcII*, *tcr-β*) and the complement system (*cfb, c7, c9*). Primers are described in (46), (47), (64) and in Piecyk, Ritter & Kalbe (*in review*).

Statistical analyses

Statistical analyses were performed with R v. 3.2.0; (65)). We used (generalized) mixed effects models (GLMMs) from *nlme* (66) and *lme4* (67) to include random terms and fixed effects according to the experimental design. Infection rates were analysed by using the number of infected individuals as proportional data in GLMMs with binomial error structure and logit link function. The interaction of host and parasite was included in *contrast 1* (Table 1). Genotypic variation was generally accounted for by including parasite sibship or 'round', i.e. worm sibship x fish family combination, as random term. Models for fish parameters included the sex of the fish as another random effect to account for sex-specific differences. Model selection was based on the Akaike information criterion (AIC) (68) and log likelihood ratio tests. Whenever needed, we incorporated *heteroscedasticity in the model fit* by definition of the varIdent variance structure for factorial variables. R^2 values of mixed effects models (69,70), were calculated with function sem.model.fits() from *piecewiseSEM* (71). Significantly different groups were identified with glht() post hoc tests from *multcomp* (72) using Tukey's all-pair comparisons or user defined contrasts according to the respective hypothesis. Multiple testing was accounted for by false discovery rate (FDR) correction (73). Gene expression data was derived from infected and control fish from each family. Differential immune gene expression was analysed between groups within contrasts by multivariate statistics on data of all 24 immune genes and, if significant, according to functional groups (*innate*, *adaptive*, *complement*).

Non-parametric permutational multivariate analyses of variance (PERMANOVA (73)) were calculated on Euclidian distance matrices (74) using function adonis() from *vegan* (75). For each test, a random subset of 10,000 permutations was used; permutations were constrained within 'round'. The weight of the fish was included as covariate to account for size related effects. Post hoc pairwise comparisons were FDR-corrected (73). If multivariate statistics indicated significant differences, we used linear mixed models (LMMs) to identify which genes were differentially expressed. Again, we accounted for unequal variances and used FDR correction due to multiple testing. In each case, the raw *p*-values are reported. Data was plotted using *ggplot2* (76); colours for plots and figures were chosen from the ColorBrewer palette (77). Multivariate patterns in gene expression were visualized by non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions (function metaMDS()); the contribution of each gene was plotted by use of the envfit() function (both implemented in *vegan*). The *maps* package (78) was used to draw the map of the sampling sites.

ABBREVIATIONS

CF: condition factor; CNRQ: calibrated normalized relative quantities; DE, NO: sticklebacks from Lake Großer Plöner See (Germany), Lake Skogseidvatnet (Norway); DPE: days post exposure; ECH, GOT, IBB, ISC, NST, NU, OBB, SKO, SP: parasite strains from Echo Lake, (Canada), Gotland (Sweden), Ibbenbürener Aa (Germany), Myvatn (Iceland), Neustädter Binnenwasser (Germany), North Uist (Scotland), Obbola (Sweden), Lake Skogseidvatnet (Norway), Xinzo de Limia (Spain); FDR: false discovery rate; G/L: granulocyte to lymphocyte ratio; (G)LMM: (generalized) linear mixed model; HKI: head kidney index; HKL: head kidney leukocytes; HSI: hepatosomatic index; IFC: integrated fluidic circuits; PERMANOVA: permutational multivariate analysis of variance; PI: parasite index; RLU: relative luminescence units; ROS: reactive oxygen species; SSI: splenosomatic index

DECLARATIONS

Ethics approval and consent to participate

Animal experiments were approved by the Ministry of Energy Transition, Agriculture, Environment and Rural Areas of Schleswig-Holstein under reference number V 312–7224.123-34.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analysed during this study will be available in the Open Access Data Repository of the Max Planck Society EDMOND (https://edmond.mpdl.mpg.de).

Competing interests

The authors do not have competing interests.

Funding

This study was financially supported by the German Science Foundation DFG (Priority program 1399; received by MK). AP received financial and scientific support from the International Max Planck Research School (IMPRS) for Evolutionary Biology, OR was funded by an ERC Starting Grant (MALEPREG) and grants from the German Research Foundation (349393951, 237263721 & 274695381). These funders had no role in study design, data collection, analysis and interpretation, or preparation of the manuscript.

Authors' contributions

AP carried out the experiments and molecular work, analysed the data, participated in design of the study and drafted the manuscript. OR helped to interpret the data and revised the manuscript. MK conceived the study and discussed the data. All authors have read and approved the manuscript.

Acknowledgments

We highly acknowledge and honor Dr. Martin Kalbe, who passed away shortly before submission of this manuscript. We are grateful for his supervision and guidance not only in a scientific context but also in personal relationships. He taught us a lot. G. Augustin, A. Baade, R. Derner, R. Leipnitz, H. Luttmann, D. Martens, I. Moreau, I. Samonte, G. Schmiedeskamp, I. Schultz, M. Schwarz, and N. Wildenhayn helped with experimental procedures, lab work and animal husbandry. Thanks go to N. Erin, M. Hasler, M. Milinski, A. Nolte, M. Ritter, and H. Schulenburg for helpful discussion. Two anonymous reviewers gave useful comments on an earlier version of the manuscript. D. Bolnick, F. Franke, T. Henrich, A. Rahn and J. Scharsack provided *Schistocephalus solidus* eggs.

REFERENCES

1. Ebert D, Hamilton WD. Sex against virulence: the coevolution of parasitic diseases. Trends Ecol Evol. 1996 Feb;11(2):79–82.
2. Bull JJ. Perspective: Virulence. Evolution. 1994 Oct;48(5):1423.
3. Thompson JN. Rapid evolution as an ecological process. Trends Ecol Evol. 1998 Aug 1;13(8):329–32.
4. Buckling A, Rainey PB. Antagonistic coevolution between a bacterium and a bacteriophage. Proc Biol Sci. 2002 May 7;269(1494):931–6.
5. Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT. Introduction. Ecological immunology. Philos Trans R Soc B Biol Sci. 2009 Jan 12;364(1513):3–14.
6. Schmid-Hempel P. Evolutionary Parasitology The Integrated Study of Infections, Immunology, Ecology, and Genetics. Oxford University Press, Inc.: New York, NY, USA; 2011.
7. Jokela J, Schmid-Hempel P, Rigby MC. Dr. Pangloss restrained by the Red Queen – steps towards a unified defence theory. Oikos. 2000 May 1;89(2):267–74.
8. Lazzaro BP, Little TJ. Immunity in a variable world. Philos Trans R Soc B Biol Sci. 2009 Jan 12;364(1513):15–26.
9. Graham AL. Optimal immunity meets natural variation: the evolutionary biology of host defence. Parasite Immunol. 2013 Nov 1;35(11):315–7.
10. Schmid-Hempel P. Variation in immune defence as a question of evolutionary ecology. Proc R Soc Lond B Biol Sci. 2003 Feb 22;270(1513):357–66.
11. Råberg L. How to Live with the Enemy: Understanding Tolerance to Parasites. PLOS Biol. 2014 Nov 4;12(11):e1001989.
12. Paterson S, Vogwill T, Buckling A, Benmayor R, Spiers AJ, Thomson NR, et al. Antagonistic coevolution accelerates molecular evolution. Nature. 2010 März;464(7286):275–8.
13. Lambrechts L, Halbert J, Durand P, Gouagna LC, Koella JC. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to Plasmodium falciparum. Malar J. 2005 Jan 11;4(1):3.
14. Grech K, Watt K, Read AF. Host–parasite interactions for virulence and resistance in a malaria model system. J Evol Biol. 2006 Sep 1;19(5):1620–30.
15. Burdon JJ, Jarosz AM. Host-Pathogen Interactions in Natural Populations of Linum marginale and Melampsora lini: I. Patterns of Resistance and Racial Variation in a Large Host Population. Evolution. 1991 Feb 1;45(1):205–17.
16. Kaltz O, Shykoff JA. Within- and among-population variation in infectivity, latency and spore production in a host–pathogen system. J Evol Biol. 2002 Sep 1;15(5):850–60.
17. Eizaguirre C, Lenz TL, Kalbe M, Milinski M. Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. Nat Commun. 2012 Jan 10;3:621.
18. Eizaguirre C, Lenz TL, Kalbe M, Milinski M. Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. Ecol Lett. 2012 Jul 1;15(7):723–31.

19. Wegner KM, Reusch TBH, Kalbe M. Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J Evol Biol. 2003 Mar 1;16(2):224–32.
20. Kalbe M, Kurtz J. Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke Diplostomum pseudospathaceum. Parasitology. 2006 Jan;132(01):105–116.
21. Scharsack JP, Kalbe M, Harrod C, Rauch G. Habitat-specific adaptation of immune responses of stickleback (Gasterosteus aculeatus) lake and river ecotypes. Proc R Soc B Biol Sci. 2007 Jun 22;274(1617):1523–32.
22. Eizaguirre C, Lenz TL, Sommerfeld RD, Harrod C, Kalbe M, Milinski M. Parasite diversity, patterns of MHC II variation and olfactory based mate choice in diverging three-spined stickleback ecotypes. Evol Ecol. 2011 May 1;25(3):605–22.
23. Lenz TL, Eizaguirre C, Rotter B, Kalbe M, Milinski M. Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-wide digital gene expression analysis. Mol Ecol. 2013 Feb 1;22(3):774–86.
24. Huang Y, Chain FJJ, Panchal M, Eizaguirre C, Kalbe M, Lenz TL, et al. Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. Mol Ecol. 2016 Jan 1;n/a-n/a.
25. Barber I. Sticklebacks as model hosts in ecological and evolutionary parasitology. Trends Parasitol. 2013 Nov;29(11):556–66.
26. Clarke AS. Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. Proc Zool Soc Lond. 1954 Aug 1;124(2):257–302.
27. Arme C, Owen RW. Infections of the three-spined stickleback, Gasterosteus aculeatus L., with the plerocercoid larvae of Schistocephalus solidus (Müller, 1776), with special reference to pathological effects. Parasitology. 1967 Mai;57(02):301–314.
28. Tierney JF, Crompton DWT. Infectivity of Plerocercoids of Schistocephalus solidus (Cestoda: Ligulidae) and Fecundity of the Adults in an Experimental Definitive Host, Gallus gallus. J Parasitol. 1992 Dezember;78(6):1049–54.
29. Heins DC, Singer SS, Baker JA. Virulence of the cestode Schistocephalus solidus and reproduction in infected threespine stickleback, Gasterosteus aculeatus. Can J Zool. 1999 Dezember;77(12):1967–74.
30. Heins DC, Barry KA, Petrauskas LA. Consistency of host responses to parasitic infection in the three-spined stickleback fish infected by the diphyllobothriidean cestode Schistocephalus solidus. Biol J Linn Soc. 2014 Dec 1;113(4):958–68.
31. MacColl ADC. Parasite burdens differ between sympatric three-spined stickleback species. Ecography. 2009 Feb 1;32(1):153–60.
32. Kalbe M, Eizaguirre C, Scharsack JP, Jakobsen PJ. Reciprocal cross infection of sticklebacks with the diphyllobothriidean cestode Schistocephalus solidus reveals consistent population differences in parasite growth and host resistance. Parasit Vectors. 2016;9:130.
33. Weber JN, Kalbe M, Shim KC, Erin NI, Steinel NC, Ma L, et al. Resist Globally, Infect Locally: A Transcontinental Test of Adaptation by Stickleback and Their Tapeworm Parasite. Am Nat. 2016 Nov 18;E000–E000.

34. Franke F, Rahn AK, Dittmar J, Erin N, Rieger JK, Haase D, et al. In vitro leukocyte response of three-spined sticklebacks (Gasterosteus aculeatus) to helminth parasite antigens. Fish Shellfish Immunol. 2014 Jan;36(1):130–40.
35. Scharsack JP, Franke F, Erin NI, Kuske A, Büscher J, Stolz H, et al. Effects of environmental variation on host–parasite interaction in three-spined sticklebacks (Gasterosteus aculeatus). Zoology. 2016 Aug;119(4):375–83.
36. Feulner PGD, Chain FJJ, Panchal M, Huang Y, Eizaguirre C, Kalbe M, et al. Genomics of Divergence along a Continuum of Parapatric Population Differentiation. PLOS Genet. 2015 Feb 13;11(2):e1004966.
37. Sheldon BC, Verhulst S. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol. 1996 Aug 1;11(8):317–21.
38. Duffy MA, Ochs JH, Penczykowski RM, Civitello DJ, Klausmeier CA, Hall SR. Ecological Context Influences Epidemic Size and Parasite-Driven Evolution. Science. 2012 Mar 30;335(6076):1636–8.
39. Hasu T, Benesh DP, Valtonen ET. Differences in parasite susceptibility and costs of resistance between naturally exposed and unexposed host populations. J Evol Biol. 2009 Apr 1;22(4):699–707.
40. Duncan AB, Fellous S, Kaltz O. Reverse Evolution: Selection Against Costly Resistance in Disease-Free Microcosm Populations of Paramecium Caudatum. Evolution. 2011 Dec 1;65(12):3462–74.
41. Weber JN, Steinel NC, Shim KC, Bolnick DI. Recent evolution of extreme cestode growth suppression by a vertebrate host. Proc Natl Acad Sci U S A. 2017 Jun 20;114(25):6575–80.
42. Frischknecht M. The breeding colouration of male three-spined sticklebacks (Gasterosteus aculeatus) as an indicator of energy investment in vigour. Evol Ecol. 1993 Sep 1;7(5):439–50.
43. Chellappa S, Huntingford FA, Strang RHC, Thomson RY. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J Fish Biol. 1995 Nov 1;47(5):775–87.
44. Rauta PR, Nayak B, Das S. Immune system and immune responses in fish and their role in comparative immunity study: A model for higher organisms. Immunol Lett. 2012 Nov;148(1):23–33.
45. Anderson MJ. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 2001 Feb 1;26(1):32–46.
46. Stutz WE, Schmerer M, Coates JL, Bolnick DI. Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. Mol Ecol. 2015 Sep 1;24(18):4629–46.
47. Robertson S, Bradley JE, MacColl ADC. Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. Mol Ecol Resour. 2015 Dec 1;n/a-n/a.
48. Fang B, Merilä J, Ribeiro F, Alexandre CM, Momigliano P. Worldwide phylogeny of three-spined sticklebacks. Mol Phylogenet Evol [Internet]. 2018 Jun 12 [cited 2018 Jul 17]; Available from: http://www.sciencedirect.com/science/article/pii/S1055790317308527

49. Zhu L, Nie L, Zhu G, Xiang L, Shao J. Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. Dev Comp Immunol. 2013 Jan;39(1–2):39–62.
50. Ortí G, Bell MA, Reimchen TE, Meyer A. Global Survey of Mitochondrial Dna Sequences in the Threespine Stickleback: Evidence for Recent Migrations. Evolution. 1994 Jun 1;48(3):608–22.
51. Scharsack JP, Koch K, Hammerschmidt K. Who is in control of the stickleback immune system: interactions between Schistocephalus solidus and its specific vertebrate host. Proc R Soc B Biol Sci. 2007 Dec 22;274(1629):3151–8.
52. Hammerschmidt K, Kurtz J. Chapter 5 Ecological Immunology of a Tapeworms' Interaction with its Two Consecutive Hosts. In: Joanne P. Webster, editor. Advances in Parasitology [Internet]. Academic Press; 2009 [cited 2013 Dec 8]. p. 111–37. (Natural History of Host-Parasite Interactions; vol. Volume 68). Available from: http://www.sciencedirect.com/science/article/pii/S0065308X08006052
53. Hammerschmidt K, Koch K, Milinski M, Chubb JC, Parker GA. When to Go: Optimization of Host Switching in Parasites with Complex Life Cycles. Evolution. 2009 Aug 1;63(8):1976–86.
54. Benesh DP, Hafer N. Growth and ontogeny of the tapeworm Schistocephalus solidus in its copepod first host affects performance in its stickleback second intermediate host. Parasit Vectors. 2012;5:90.
55. Seppänen E, Kuukka H, Voutilainen A, Huuskonen H, Peuhkuri N. Metabolic depression and spleen and liver enlargement in juvenile Arctic charr Salvelinus alpinus exposed to chronic parasite infection. J Fish Biol. 2009 Feb 1;74(3):553–61.
56. Scharsack JP, Kalbe M. Differences in susceptibility and immune responses of three-spined sticklebacks (Gasterosteus aculeatus) from lake and river ecotypes to sequential infections with the eye fluke Diplostomum pseudospathaceum. Parasit Vectors. 2014;7(1):109.
57. Scharsack JP, Kalbe M, Derner R, Kurtz J, Milinski M. Modulation of granulocyte responses in three-spined sticklebacks Gasterosteus aculeatus infected with the tapeworm Schistocephalus solidus. Dis Aquat Organ. 2004;59(2):141–150.
58. Pechhold K, Pohl T, Kabelitz D. Rapid quantification of lymphocyte subsets in heterogeneous cell populations by flow cytometry. Cytometry. 1994 Jun 1;16(2):152–9.
59. Kurtz J, Kalbe M, Aeschlimann PB, Häberli MA, Wegner KM, Reusch TBH, et al. Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proc R Soc Lond B Biol Sci. 2004 Jan 22;271(1535):197–204.
60. Scott AL, Klesius PH. Chemiluminescence: A novel analysis of phagocytosis in fish. Dev Biol Stand. 1981;49:243–54.
61. Vandesompele J, Preter KD, Pattyn F, Poppe B, Roy NV, Paepe AD, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun 18;3(7):1–12.
62. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007;8:R19.

63. Hibbeler S, Scharsack JP, Becker S. Housekeeping genes for quantitative expression studies in the three-spined stickleback Gasterosteus aculeatus. BMC Mol Biol. 2008 Jan 29;9(1):18.
64. Brunner FS, Anaya-Rojas JM, Matthews B, Eizaguirre C. Experimental evidence that parasites drive eco-evolutionary feedbacks. Proc Natl Acad Sci. 2017 Mar 16;201619147.
65. R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Available online at: h ttp. Www R-Proj Org. 2015;
66. Pinheiro J, Bates D, DebRoy S, Sarkar D. R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1-117. Available http CRAN R-Proj Orgpackage Nlme. 2014;
67. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models using lme4. ArXiv14065823 Stat [Internet]. 2014 Jun 23 [cited 2015 Jun 23]; Available from: http://arxiv.org/abs/1406.5823
68. Akaike H. Information theory and an extension of the maximum likelihood principle. In: Second international symposium on information theory [Internet]. 1973 [cited 2013 Sep 20]. p. 267–281. Available from: http://ci.nii.ac.jp/naid/10011153980/
69. Nakagawa S, Schielzeth H. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol. 2013 Feb 1;4(2):133–42.
70. Johnson PCD. Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods Ecol Evol. 2014 Sep 1;5(9):944–6.
71. Lefcheck JS. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol Evol. 2016 May 1;7(5):573–9.
72. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. Biom J. 2008;50(3):346–363.
73. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B Methodol. 1995;57(1):289–300.
74. D'haeseleer P. How does gene expression clustering work? Nat Biotechnol. 2005;23(12):1499–1502.
75. Oksanen JF, Blanchet G, Kindt R, Legendre P, Minchin PR, O'Hara RB, et al. vegan: community ecology package. 2.0-0. R package version 2.3-0. 2015.
76. Wickham H. Elegant graphics for data analysis (ggplot2). New York, NY: Springer-Verlag; 2009.
77. Neuwirth E. ColorBrewer Palettes. R package version 1.1-2. 2014. 2015.
78. Brownrigg R. Maps: Draw Geographical Maps. R package version 2.3-9. Enhancements by Thomas P Minka. Original S code by Richard A. Becker and Allan R. Wilks. 2014.

CHAPTER 2

The right response at the right time: Exploring helminth immune modulation in sticklebacks by experimental co-infection

Agnes Piecyk[1,2*], Marc Ritter[1*°] & Martin Kalbe[1(^)]

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

[2] Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany

* shared first authors

° corresponding author: ritter@evolbio.mpg.de

^ deceased

(in review at *Molecular Ecology*)

Short running title: Helminth immune modulation in sticklebacks

ABSTRACT

Parasites are one of the strongest selective agents in nature. They select for hosts that evolve counter-adaptive strategies to cope with infection. Helminth parasites are special because they can influence their hosts' immune responses. This phenomenon is important in epidemiological contexts, where co-infections may be affected. How different types of hosts and helminths interact with each other is insufficiently investigated. We used the three-spined stickleback (*Gasterosteus aculeatus*) – *Schistocephalus solidus* model to study the involved mechanisms and temporal components of helminth immune modulation. Sticklebacks from two contrasting populations with either high resistance (HR) or low resistance (LR) against *S. solidus* were individually exposed to *S. solidus* strains with characteristically high growth (HG) or low growth (LG) in *G. aculeatus*. We determined the susceptibility to another parasite, the eye fluke *Diplostomum pseudospathaceum*, and the expression of 23 key immune genes at three time points after *S. solidus* infection.

D. pseudospathaceum infection rates and the gene expression responses depended on host and *S. solidus* type and changed over time. Whereas the effect of *S. solidus* type was not significant after three weeks, T regulatory responses and complement components were up-regulated at later time points if hosts were infected with HG *S. solidus*. HR hosts showed a well-orchestrated immune response, which was absent in LR hosts. Our results emphasize the role of regulatory T cells and the timing of specific immune responses during helminth infections. This study elucidates the importance to consider different co-evolutionary trajectories and ecologies when studying helminth immune modulation.

KEYWORDS

host-parasite interaction, helminth immune modulation, gene expression, *Gasterosteus aculeatus*, *Schistocephalus solidus*, *Diplostomum pseudospathaceum*

INTRODUCTION

The evolution of species and species interactions are shaped through a complex web of abiotic and biotic factors (Sheldon & Verhulst, 1996; Schulenburg et al., 2009; Maizels & Nussey, 2013; Betts et al., 2016). One of the key forces is the co-evolution between hosts and parasites. Parasites shape their host's immune function and in response undergo rapid evolution of virulence, which results in ongoing antagonistic co-evolution (Buckling & Rainey, 2002; Paterson et al., 2010; Eizaguirre et al., 2012; Dargent et al., 2013). However, the underlying evolutionary trajectories of this co-evolution have mostly been studied in species pairs. Such an approach neglects the complexity of natural systems and the consequences of infection. Indeed, parasite species can influence one another (Benesh & Kalbe, 2016), especially if multiple parasites infect one host. In such a case, co-infecting parasites interact directly or indirectly, for example through resource competition or effects on host immunity (Betts et al., 2016).

The vertebrate immune system co-evolved with helminth parasites (ancient metazoans classified as cestodes, nematodes and trematodes) that are exceptional immune modulators (Maizels, 2005; Anthony et al., 2007; Khan & Fallon, 2013). It has been shown that helminth infections can alter susceptibility to macroparasites (Lello et al., 2004; Pedersen & Antonovics, 2013; Benesh & Kalbe, 2016) and microbes (Graham, 2008; Giacomin et al., 2015). Moreover, helminth-mediated downregulation of host immunity is observed to suppress autoimmune or inflammatory disorders such as asthma, rheumatoid arthritis, type 1 diabetes, multiple sclerosis, and inflammatory bowel diseases (Maizels & Yazdanbakhsh, 2003; Maizels & McSorley, 2016). Helminths typically interfere with characteristic elements of innate and adaptive immunity (Anthony et al., 2007; McSorley et al., 2013). Most knowledge stems from clinical and experimental work involving human patients or murine systems. A prominent observation is the switch between activities of distinct T helper cell subsets over time. Characteristically, an early T helper 1 (Th1) type response is skewed towards a T helper 2 (Th2) type response in chronic helminth infections.

Th1 and Th2 responses are defined by distinct functions and cytokines (Maizels et al., 1993; Maizels & McSorley, 2016). Th1 type cytokines, such as Interleukin-1β (IL-1β) and Tumor necrosis factor α (TNF-α), are pro-inflammatory; Th2 type cytokines can inhibit Th1 cells and acute-phase cytokines, induce alternatively activated macrophages, and stimulate B-cells and antibody production (Liu et al., 2009; Mosmann & Sad, 1996). Nevertheless, high parasite burdens were described despite increased Th2 responses, which brought another T cell subset into focus, namely immuno-suppressive regulatory T (Treg) cells (Maizels & Yazdanbakhsh, 2003; Maizels, 2005; Nutman, 2015; Maizels & McSorley, 2016). Tregs are considered to be key controllers of immune system homeostasis and expand upon longstanding helminth infections. Modulation of these cells may protect from immunopathology and ensure the persistence of the parasite within the host. Helminths are also known to interact with the host's complement system (Heath et al., 1994; Mulcahy et al., 2004) which is considered to link innate and adaptive immunity (Carroll, 2004).

It has recently been suggested that those characteristic elements of innate and adaptive immunity, namely Th1, Th2, Treg cells, and complement components, are of central importance in helminth infections of the three-spined stickleback *Gasterosteus aculeatus* (hereafter 'stickleback') (Haase et al., 2014, 2016; Robertson et al., 2015). Sticklebacks are widely distributed across the Northern Hemisphere and are naturally infected with a wide diversity of parasites (Kalbe et al., 2002; MacColl, 2009; Feulner et al., 2015). Parasites seem to drive local adaptation and genomic differentiation in this species (Eizaguirre et al., 2012; Feulner et al., 2015; Robertson et al., 2015). Habitat specific immunity and immune gene expression have been described (Wegner et al., 2003; Lenz et al., 2013; Huang et al., 2016; Lohman et al., 2017), but little is known about temporal changes, ecological consequences, and the underlying mechanisms of the host's response to infection (Benesh & Kalbe, 2016).

Here, we used controlled infection experiments with sticklebacks and their specific cestode parasite *Schistocephalus solidus* for a thorough investigation of helminth immune modulation in a model vertebrate system. We tested our predictions by using stickleback and *S. solidus* types with different co-evolutionary backgrounds.

Our study addressed the ecological significance by exploring the influence on co-infection probability with a naturally co-occurring parasite, the trematode *Diplostomum pseudospathaceum*. *D. pseudospathaceum* migrates to the immunologically protected eye lens of the fish within 24 hours and evades adaptive immune responses (Chappell et al., 1994). The potentially inflicted cataract formation within the eyes has the potential to impair *G. aculeatus* predator avoidance (Meakins & Walkey, 1975; Karvonen et al., 2004; Seppälä et al., 2004). Both parasite species have a complex life cycle with *G. aculeatus* as intermediate and piscivorous birds as final hosts. We studied the temporal dynamics by sampling at different time points of *S. solidus* development in the stickleback and determined corresponding host immune gene expression patterns.

S. solidus has a three-host life cycle with copepods, *G. aculeatus*, and fish-eating birds as three consecutive hosts (Smyth, 1946; Clarke, 1954; Barber & Scharsack, 2010). The cestode becomes infective for the final host and is able to reproduce above a weight of 50 mg (Tierney & Crompton, 1992; Hammerschmidt & Kurtz, 2009). *S. solidus* is a common parasite of *G. aculeatus* in freshwater and brackish habitats. The outcome of their co-evolution seems to differ greatly between populations (Barber & Scharsack, 2010; Kalbe et al., 2016; Weber et al., 2017). While some sticklebacks evolved high resistance against *S. solidus*, measured as the limitation of cestode growth, the resistance of others is less effective (Kalbe et al., 2016; Weber et al., 2017; Piecyk et al., *in revision*). Likewise, some *S. solidus* types grow consistently fast and reach enormous weights, whereas other strains grow characteristically slow (Benesh & Kalbe, 2016; Kalbe et al., 2016; Ritter et al., 2017; Piecyk et al., *in revision*). We chose hosts and parasites from (i) populations with low *S. solidus* prevalence (< 1%) and high parasite diversity, and (ii) populations with high *S. solidus* prevalence (20 to > 50%) and low parasite diversity.

The former host and parasite types supposedly evolved under de-escalated arms-race dynamics causing slow parasite growth (low growth, LG *S. solidus*) and low resistance (LR sticklebacks). The latter host and parasite types supposedly selected for increased resistance (high resistance, HR sticklebacks) and virulence (high growth, HG *S. solidus*) in their habitat.

We hypothesized that *S. solidus* modulates immune responses in *G. aculeatus* and that this effect differs between contrasting stickleback and *S. solidus* types, as well as over time. More specifically, we expected modulatory effects when *S. solidus* is able to reproduce upon transmission to the final hosts, which should be earlier in fast growing (HG) than in slow growing (LG) types. We further hypothesized an effective immune response in the co-evolved high growth – high resistance (HG-HR) combination, but not in the un-adapted high growth – low resistance (HG-LR) combination.

Expression levels of 23 *G. aculeatus* immune genes that may play key roles in *S. solidus* and *D. pseudospathaceum* infection were analysed to characterize the molecular infection phenotypes. We chose genes that had been identified using transcriptome data (Haase et al., 2014; Huang et al., 2016) and quantitative real-time PCR studies (Brunner et al., 2017; Robertson et al., 2015; Stutz et al., 2015). Our set includes targets from innate and adaptive immunity as well as complement components. We used subsets of these genes to study Th1, Th2 and Treg responses in further detail. The stickleback's immune system is principally able to eliminate *S. solidus* up to 17 days post infection, adaptive immune responses might be active after two to three weeks, and head kidney leukocyte respiratory burst potential (an estimate for innate immune activation) peaks after seven to nine weeks (Barber & Scharsack, 2010). Following those findings, we exposed *S. solidus* infected and sham-exposed control fish to a defined number of *Diplostomum pseudospathaceum* cercariae three, six and nine weeks post *S. solidus* infection. The susceptibility to *D. pseudospathaceum* was used as an indicator for the potential systemic modulatory effect of *S. solidus* and inter-parasitic interactions (Benesh & Kalbe, 2016). *S. solidus'* effect on stickleback immune gene expression was studied in *S. solidus* infected and co-infected hosts (Fig. 1).

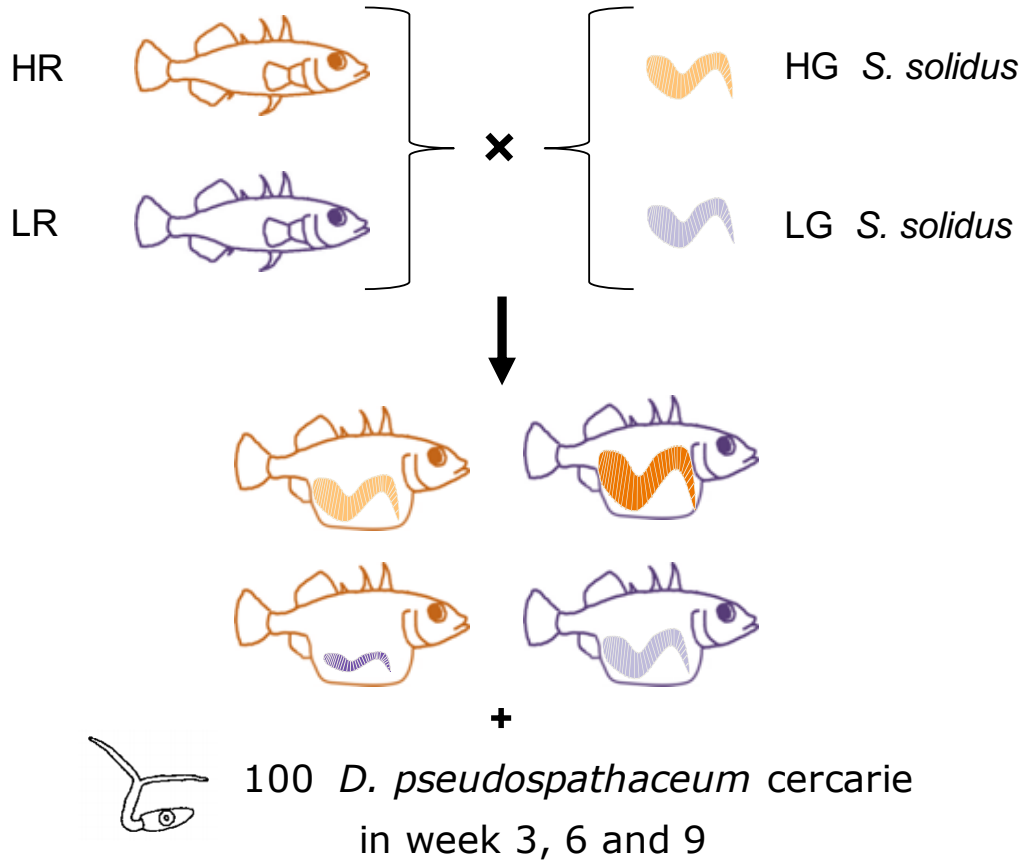


Fig. 1. Experimental design. Two stickleback populations of low resistance (LR) and high resistance (HR) were exposed to *Schistocephalus solidus* of high growth (HG) or low growth (LG). Subsets of *S. solidus* exposed sticklebacks were exposed to 100 cercariae of the eye fluke *Diplostomum pseudospathaceum* at distinct time points (after three, six or nine weeks).

MATERIAL AND METHODS

Experimental design

We performed a fully reciprocal co-infection experiment using two pairs of hosts (HR and LR) and *S. solidus* parasites (HG and LG) with contrasting resistance and growth. The infection success of another parasite species, the eye fluke *D. pseudospathaceum*, and stickleback immune gene expression levels were used as quantitative proxies for *S. solidus* immune modulation. We chose three distinct time points after *S. solidus* infection (week 3, week 6, and week 9) to describe the temporal component of the interaction (Fig. 1).

Study system

We used naïve lab-bred first generation progeny of three breeding pairs of each of the two stickleback populations (Table S1). The fish were kept in the institute's aquaria facilities at 18°C, with 16 hours of light per day, and fed a diet of frozen chironomids, copepods and daphnids three times a week. We chose two populations of cestodes (Table S1). *S. solidus* from lake Skogseidvatnet grow consistently faster than *S. solidus* from Neustädter Binnenwasser (Benesh & Kalbe, 2016; Kalbe et al., 2016; Ritter et al., 2017), thus justifying the conceptual names for the two types: HG (high growth) and LG (low growth) *S. solidus*. Two *S. solidus* sibships were used per population. A parasite sibship refers to offspring from one *S. solidus* pair that was bred *in vitro* (Smyth, 1946; Wedekind et al., 1998). All breeding pairs were weight matched to maximize outcrossing rates (Lüscher & Milinski, 2003). *S. solidus* eggs were stored at 4°C in the dark; hatching was initiated following Dubinina (1980). *Macrocyclops albidus* copepods from laboratory cultures were exposed to single coracidia as the first intermediate host (van der Veen & Kurtz, 2002). The copepods were kept at 18 °C with 16 hours of light per day, and microscopically checked for *S. solidus* infection one week after exposure. Singly infected copepods were used for stickleback exposure 16 days post exposure. Susceptibility to the eye fluke *Diplostomum pseudospathaceum* was used as an ecologically relevant proxy for *S. solidus* immune modulation. We established a pool of *D. pseudospathaceum* shedding snails (intermediate hosts) in the laboratory. The snail species *Limnea stagnalis* exclusively hosts *D. pseudospathaceum* in our sampling area (Faltýnková et al., 2007). *L. stagnalis* were collected in shallow water at different sampling sites of two water bodies connected to the Plöner See lake district (SI.1) in September and October 2015. All snails were screened for parasites in the laboratory on the day of sampling and trematodes were identified according to Faltýnková et al. (Faltýnková et al., 2007). Exclusively *D. pseudospathaceum* positive snails shedding no cercariae of other species were transferred to 16 L tanks in groups of five and fed *ad libitum* with green lettuce.

Infection experiment and fish dissection

Fish were individually isolated in 2 L tanks and starved for 24 hours before exposure to single *S. solidus* infected copepods. Control fish were exposed to uninfected copepods. We transferred the fish to treatment (fish family x worm sibship combination) specific 16 L tanks after 48 hours, in order to give enough time for copepod ingestion. The water of the single tanks was filtered to quantify uningested copepods. Each 16 L tank housed 18 individuals at the beginning of the experiment. To avoid any density-dependent influence on growth (Backiel & Le Cren, 1978), fish numbers were maintained by replacing fish that died before exposure to *D. pseudospathaceum* by spine-clipped naïve individuals from the same stickleback families. Three, six and nine weeks after exposure to *S. solidus,* four fish from every treatment were individually exposed to 100 *D. pseudospathaceum* cercariae. The sticklebacks were isolated in 2 L tanks and starved for 24 hours. *D. pseudospathaceum* cercariae came from a pool of at least 10 snails (Kalbe & Kurtz, 2006; SI.1) to overcome *D. pseudospathaceum* genotype-specific effects. Fish were euthanized two days post *D. pseudospathaceum* exposure by an incision to the brain and weighed to the nearest 0.1 mg. The standard length (without fin) was measured to the nearest mm. Head kidneys, liver and spleen were weighed to the nearest 0.1 mg; head kidneys were immediately transferred to RNAlater (Sigma-Aldrich) and stored at room temperature for 24 hours before freezing at -20°C. The sex was determined for each fish, and body cavities were screened for *S. solidus* infection. If present, plerocercoids were weighed and a parasite index (PI) was calculated as 100 x cestode weight / fish weight (Arme & Owen, 1967). Host condition was estimated via the condition factor (CF; 100 x fish weight/fish length^{b} with HR- and LR-population specific exponents *b*; Frischknecht, 1993) and the hepatosomatic index (HSI; Chellappa et al., 1995). The splenosomatic index (SSI) and a head kidney index (HKI) were calculated as 100 x organ weight/fish weight (Bolger & Connolly, 1989; Kurtz et al., 2004) to estimate immunological activation. *D. pseudospathaceum* infection rates were determined by microscopically counting metacercariae completely within the eye lenses in fish-isotonic NaCl-solution.

RNA extraction and cDNA synthesis

Head kidney RNA was extracted with a NucleoSpin 96 kit according to the manufacturer's protocol (Macherey-Nagel), including on column DNA digestion. Samples were homogenized in lysis buffer with 1% β-Mercaptoethanol using a Tissue Lyser II (Qiagen) for 2 x 3 min at 30 Hz. RNA purity was verified by ensuring all A260/A280 ratios were > 1.95 using a NanoDrop 1000 (Thermo Scientific) spectrophotometer. Reverse transcription reactions to cDNA were performed using the Qiagen Omniscript RT kit, following the manufacturer's protocol (SI.2). The samples were adjusted to 1000 ng RNA per reaction. Five samples with concentrations between 500 and 1000 ng were used in the highest possible concentration and showed comparable results to the remaining dataset. The cDNA was stored at -20°C until use for quantitative real-time PCR (qPCR).

qPCR primer selection and establishment

We chose 32 key targets that had either been published before (Hibbeler et al., 2008; Robertson et al., 2015; Stutz et al., 2015; Brunner et al., 2017) or were designed for this study. We designed intron-spanning primers for *p22phox, mst1ra* and *marco* using Primer 3 (version 4.0.0, http://primer3.ut.ee). All primers were tested on gDNA and cDNA pools of both stickleback populations on a Light cycler II (ABI) with three technical replicates and a negative control using an annealing temperature of 60°C to ensure protocol compatibility. Amplicon specificity was confirmed by melt curve analysis and gel electrophoresis on a 1.5 % agarose gel stained with SybrSafe. Exclusively primers with one unambiguous product and negative gDNA amplification or gDNA product of distinct melting temperature were selected for use. PCR products of all primers were sequenced (SI.3) and confirmed by querying the ENSEMBL stickleback reference genome using blastn (Altschul et al., 1997; Aken et al., 2016; ENSEMBL version 86). Five targets were excluded during establishment (SI.4).

We used four reference genes (*b2m*, *ef1a*, *rpl13a* and *ubc*) (Hibbeler et al., 2008) and 23 immune genes categorized by their functionality in the stickleback's immune system: innate immunity (*cd97*, *csf3r*, *il-1β*, *marco*, *mif1*, *mst1ra*, *nkef-β*, *p22^{phox}*, *saal1*, *sla1*, *tnfr1)*, adaptive immunity (*stat4*, *cd83*, *igm*, *stat6*, *foxp3b*, *il-16*, *tgf-β*, *mhcII, tcr-β),* and complement system (*c7*, *c9*, *cfb*) (SI.5 and Table S3). We further defined gene sets characteristic for a Th1 response (*stat4*, *tnfr1*), Th2 response (*stat6*, *cd83*, *igm*) and Treg response (*il16*, *foxp3*, *tgf-β*).

Gene expression data acquisition

Relative gene expression was measured with Fluidigm 96.96 Dynamic Array integrated fluidic circuits (IFCs) and Biomark HD system using EvaGreen as DNA binding dye. The initial primer concentration was 100 µM (SI.6 and SI.7). In total, 210 samples were analysed on four different IFCs. Samples of all treatment groups and time-points were randomly distributed across IFCs. Each IFC included two inter-run calibrators (IRCs) and a gDNA contamination control. Amplification efficiencies were calculated from serial dilutions of HR and LR cDNA pools in a dilution range from 1:10 to 1:10^4. Primer efficiencies were in the range of 95 % to 112 %, with an R^2 average value of 0.96 SE ± 0.013 (Table S3). Assessment of data quality, reference gene stability, inter-run calibration and calculation of relative expression values was completed using *qBase+* 3.0 (Biogazelle) (Hellemans et al., 2007). We set the negative cutoff to the technical sensitivity limit at cycle 28 and allowed a variation of 0.5 cycles for maximum triplicate variability. Expression stability of reference targets was inferred from geNorm M and Coefficient of Variation (CV) values (Vandesompele et al., 2002; Hellemans et al., 2007). The most stably expressed reference genes *rpl13* and *ubc* (M = 0.139, CV = 0.049) were used for normalization. Relative expression values were calculated using the ΔΔCt method (Pfaffl, 2001) and exported as log10 transformed CNRQ (calibrated normalized relative quantities). We excluded unreliable data from eight samples. Two missing values for gene *cfb* were replaced by the average *cfb* expression. Accordingly, gene expression analyses were based on 202 infected and control sticklebacks.

Data analyses

Host condition and immunological parameters from 501 sticklebacks were analysed. All statistical analyses were performed in R (version 3.2.0, R Core Team, 2015). We distinguished between time points (T: week 3, week 6, week 9) and host types (H: HR, LR), and defined the following treatment groups (P) for the main analyses: (i) sham-exposed controls, (ii) fish infected with LG *S. solidus*, and (iii) fish infected with HG *S. solidus*. We further distinguished between (iv) fish infected with *D. pseudospathaceum*, (v) fish co-infected with LG *S. solidus* and *D. pseudospathaceum*, and (vi) fish co-infected with HG *S. solidus* and *D. pseudospathaceum*, to analyse host parameters, i.e. condition and immunological parameters as well as immune gene expression profiles. Linear mixed effect models (LMMs) and generalized linear mixed effect models (GLMMs) were fit using functions lme() from *nlme* (Pinheiro et al., 2015) and lmer() and glmer() from *lme4* (Bates et al., 2015). Best fitting models were selected with likelihood ratio tests and the Akaike information criterion (AIC) (Akaike, 1973). R^2 values of mixed effects models (Johnson, 2014; Nakagawa & Schielzeth, 2013) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Significantly different groups were identified with glht() post hoc tests from the *multcomp* package (Hothorn et al., 2008) with user defined contrasts according to the respective hypothesis. Apart from that, *p*-values were obtained with Anova() from *car* (Fox & Weisberg, 2011) using Type III Wald chisquare tests or anova() from *stats* (R Core Team, 2015) computing Type III sum of squares for fixed effects of LMMs. We accounted for multiple testing by using the false discovery rate (FDR; Benjamini & Hochberg, 1995). Infection rates were compared using GLMMs with binomial error structure and logit link function. *S. solidus* infection rates were analysed with regard to the number of ingested copepods, and included the origin of the fish, the origin of *S. solidus* and their interaction as a fixed structure.Fish origin, *S. solidus* origin, time, and all interactions were tested as fixed effects to analyse *D. pseudospathaceum* infection rates. We additionally tested effects of fish sex, *S. solidus* sibship and fish family, and ultimately incorporated fish family as a random term in the models.

To test whether the growth of the worm per se affected *D. pseudospathaceum* infection rates, we used data from *S. solidus* infected fish from each week and added the weight of the worm as a covariate in the statistical models (Benesh & Kalbe, 2016). We included the interaction between worm weight and *S. solidus* origin in the model fit in order to test if the relationship between *S. solidus* growth and susceptibility to *D. pseudospathaceum* was population-specific. *Schistocephalus* exposed but uninfected fish were excluded from further analyses, because it is not possible to determine the time point and stage of the infection process in which fish resisted infection. LMMs to study *S. solidus* growth, host condition and immunological parameters were fit with fish family as a random term, and heteroscedasticity was accounted for by defining the respective factorial variables as varIdent variance structure. We used parasite indices, the relative weight of the parasite in an infected fish (Arme & Owen, 1967) of all *S. solidus* infected fish (n = 140) to study parasite growth over time. The model included the origins of host and parasite, as well as sampling time, and all interactions as fixed effects. Host condition and immunological parameters were analysed with GLMMs using host origin, treatment group (defined above), and sampling time, as well as all interactions as fixed effects.

Stickleback immune gene expression was evaluated by non-parametric permutational multivariate analyses of variance (PERMANOVA; Anderson, 2001) on log10 transformed CNRQ values. We first tested if the expression of all 23 immune genes differed between groups within contrasts and, if significant, ran PERMANOVAs according to functional groups (*innate, adaptive, complement; Th1, Th2, Treg*). The analyses were based on Euclidian distances (D'haeseleer, 2005) using function adonis() from the *vegan* package (Oksanen et al., 2015). The main effects were host type (H), time (T), and depending on the comparison of interest, either treatment group or *S. solidus* type (P). The weight of the fish was included as a covariate to account for size related effects. Each test was based on 10,000 permutations. Permutations were constrained within fish family. Post hoc pairwise comparisons were calculated between contrasts of interest within time points.

Experimental treatment effects on single genes of differentially expressed functional groups were tested with LMMs using treatment and fish origin as fixed structure and fish family as random term. Again, we accounted for heteroscedasticity whenever needed and all tests were FDR-corrected (Benjamini & Hochberg, 1995). Data was plotted with *ggplot2* (Wickham, 2009) and *plyr* (Wickham, 2011) using colour schemes from *RColorBrewer* (Neuwirth, 2014). Gene expression was visualized with function aheatmap() from *NMF* (Gaujoux & Seoighe, 2010).

RESULTS

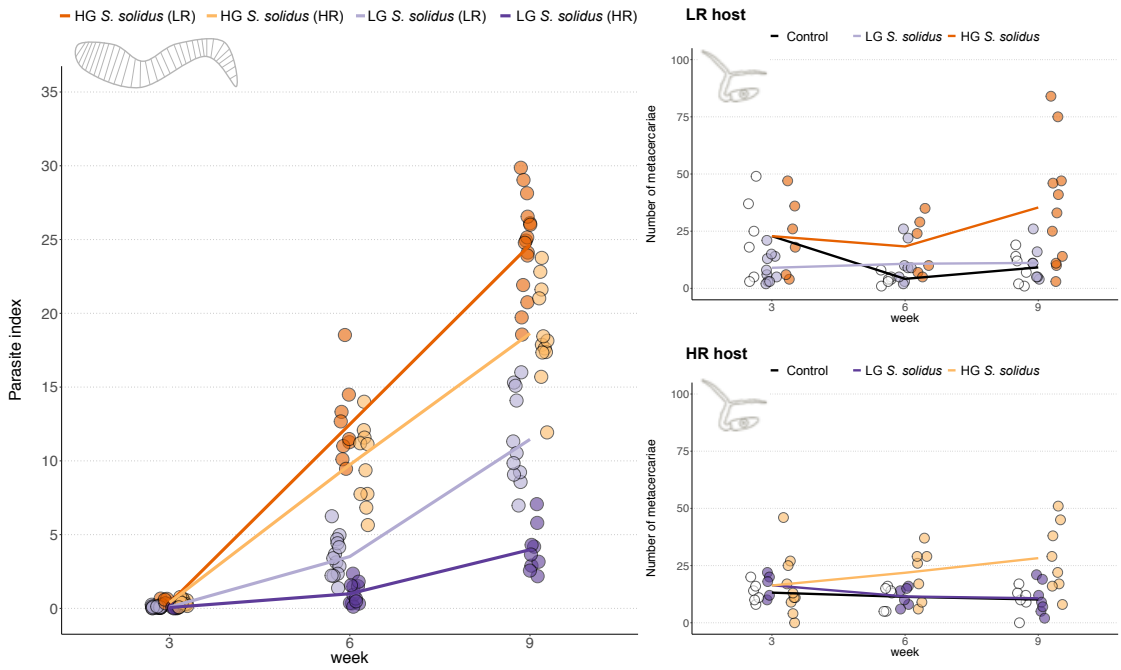


Fig. 2. Effect of *S. solidus* growth on susceptibility to *D. pseudospathaceum*. Sticklebacks with either high resistance (HR) or low resistance (LR) were experimentally infected with single *S. solidus* larvae. Parasite indices (parasite weight corrected for host weight) and susceptibility to the eye fluke *Diplostomum pseudospathaceum* (number of metacercariae in the eye lenses one day after exposure to 100 cercariae) were determined in week 3, 6, and 9 post *S. solidus* infection. Color coding follows Fig. 1.

S. solidus growth and effect on stickleback physiology and susceptibility

S. solidus infection rates did not differ significantly between host or parasite populations (SI.8). The growth of the cestode was significantly affected by *S. solidus* type (Fig. 2; SI.9): high growth (HG) *S. solidus* grew consistently faster than low growth (LG) *S. solidus*. The number of *D. pseudospathaceum* in the eye lenses of sham-exposed and *S. solidus* infected sticklebacks differed according to a three-way interaction between time and host and parasite type (X^2_4 = 24.8413; $p < 0.0001$). Overall, the differences between host populations were not significant (Table S7) and susceptibility to *D. pseudospathaceum* increased over time (Table S8) if sticklebacks were infected with HG *S. solidus*, but not if they were infected with LG *S. solidus* (Fig. 2; SI.10). Post hoc comparisons of the effects of parasite type over time and with regard to host type showed that three weeks after *S. solidus* infection, LR hosts had more *D. pseudospathaceum* metacercariae in their eyes if infected with HG *S. solidus* or sham-exposed, than those infected with LG *S. solidus*; in week 6, *D. pseudospathaceum* numbers in LR fish were highest if hosts were infected with HG *S. solidus* and lowest in controls; in HR hosts, *D. pseudospathaceum* infection rates were significantly higher in HG infected hosts than in controls; nine weeks after *S. solidus* infection, the number of *D. pseudospathaceum* metacercariae was significantly increased if sticklebacks were infected with HG *S. solidus* (Table S9). We tested if this result was weight- rather than population-specific by fitting GLMMs with *S. solidus* weight as covariate (SI.11). At each time point, the number of *D. pseudospathaceum* was not correlated to *S. solidus* weight, and the origin of *S. solidus* remained a significant predictor in week 3 (P effect: $X^2_1 = 6.65$, $p = 0.0099$), week 9 (P effect: $X^2_1 = 53.27$, $p < 0.0001$), and in LR hosts in week 6 (P effect: $X^2_1 = 4.22$, $p = 0.0401$).

Analyses of host condition and immunological parameters are presented in the Supplementary Information (SI.12). Briefly, the condition was higher in HR sticklebacks, regardless of the treatment.

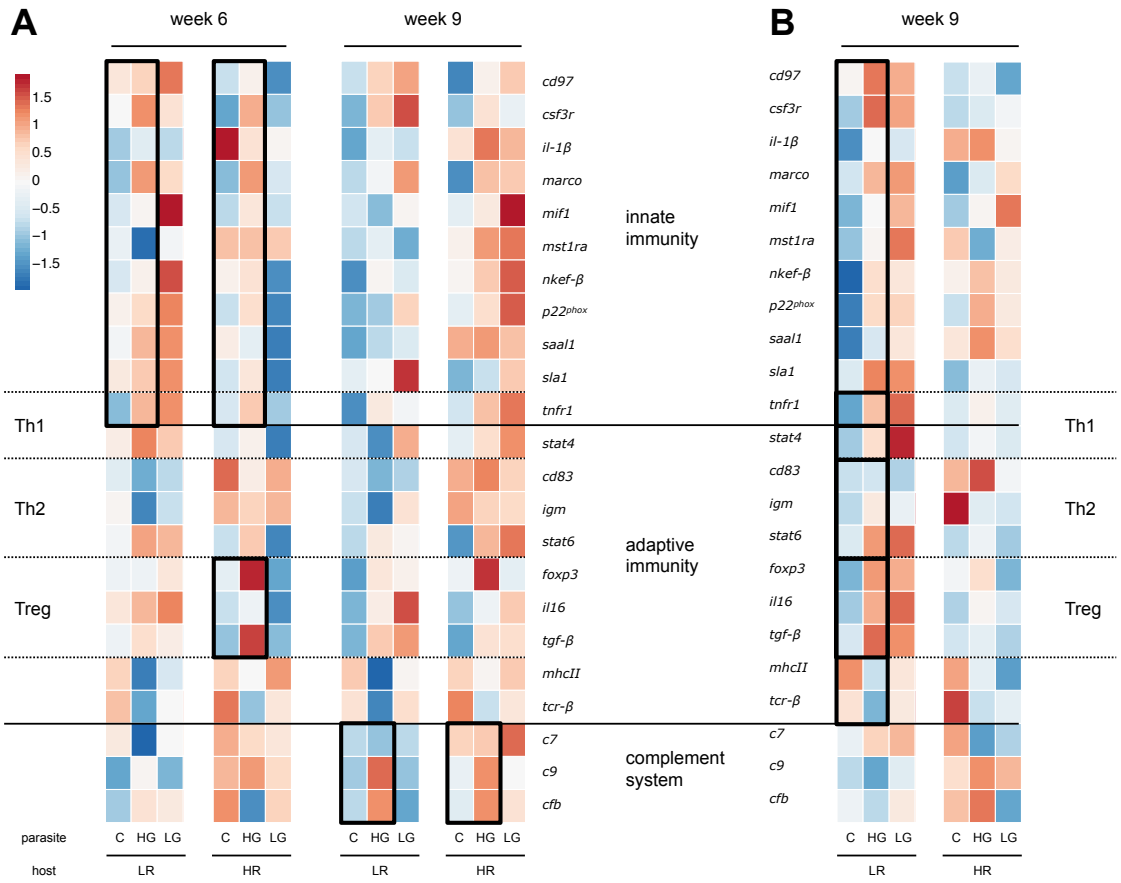


Fig. 3. Effects of infection on immune gene expression in sticklebacks over time. Sticklebacks with low resistance (LR) or high resistance (HR) against *S. solidus* were infected with low growth (LG) or high growth (HG) *S. solidus*; controls (C) were sham-exposed. Heatmaps are based on Euclidian distances of average values of log10-transformed calibrated normalized relative quantities (CNRQ). Rows are centered and scaled to row z-scores across both host types within weeks. Significantly different groups are highlighted by black outlines. **(A)** Expression responses in *S. solidus* infected fish after six and nine weeks. **(B)** Expression responses in *S. solidus* – *D. pseudospathaceum* co-infected fish.

Gene expression profiles

Expression profiles of 23 stickleback immune genes were used to characterize the molecular pathways of the host's immune response to *S. solidus* infection over time. We additionally tested for the effects of *D. pseudospathaceum* infection and *D. pseudospathaceum* infection intensity. Multivariate analyses of variance (PERMANOVAs; (Anderson, 2001; Brunner et al., 2017) revealed significantly different gene expression profiles of treatment groups over time (Fig. 3; SI.13). Three weeks after infection, the profiles did not yet differ significantly between *S. solidus* infected and control fish (Table S10). After six weeks, HG *S. solidus* infected fish up-regulated genes of innate immunity (P effect; $PERMANOVA_{innate}$: $F_{1,17} = 4.9997$, $p = 0.0023$), whereas expression profiles of LG-infected fish did not differ significantly from controls. T regulatory genes were up-regulated in HG infected HR hosts relative to controls (P effect; $PERMANOVA_{Treg}$: $F_{1,8} = 20.14$, $p = 0.0105$) (Fig. 3A; Table S10). In week 9, genes of complement components were significantly up-regulated in HG infected hosts (P effect; $PERMANOVA_{complement}$: $F_{1,17} = 9.899$, $p = 0.0082$) (Fig. 3A; Table S10). FDR correction of quantitative changes in mRNA levels of single genes indicated significant differential expression of *tgf-β* in week 6 and *cfb* in week 9 (Tables S11, S12, S13). Multivariate gene expression did not differ significantly between controls and *D. pseudospathaceum* infected fish (Table S14). The profiles differed significantly between controls and LR hosts that were co-infected with *D. pseudospathaceum* and HG *S. solidus*: genes of innate immunity (co-infection effect; $PERMANOVA_{innate}$: $F_{1,14} = 5.43$, $p = 0.0195$), adaptive immunity (co-infection effect; $PERMANOVA_{adaptive}$: $F_{1,14} = 5.2$, $p = 0.0122$), Th1 (co-infection effect; $PERMANOVA_{Th1}$: $F_{1,14} = 4.8$, $p = 0.0232$), Th2 (co-infection effect; ; $PERMANOVA_{Th2}$: $F_{1,14} = 4.96$, $p = 0.0226$) and T regulatory components (co-infection effect; $PERMANOVA_{Treg}$: $F_{1,14} = 11.68$, $p = 0.0074$) were up-regulated nine weeks after *S. solidus* infection (Table S15). Primarily, *il-1β*, *foxp3*, *tgf-β*, and *il-16* were higher expressed than in controls (Fig. 3B; Table S16). Multivariate gene expression did not differ between co-infected HR fish and the respective controls.

DISCUSSION

Using controlled experimental helminth infections of three-spined sticklebacks, we found that pro-inflammatory, complement and T regulatory pathways are up-regulated in chronic infections with a high growth (HG) *Schistocephalus solidus* type after the cestode reached its reproductive weight. Infection rates of another helminth species, the eye fluke *Diplostomum pseudospathaceum* were time- and *S. solidus* type-dependent.

S. solidus growth and immune modulation is host and parasite type specific

In a community context, host immunity and parasite virulence are shaped by co-occurring species such as predators, prey, pathogens and parasites (Schulenburg et al., 2009). We chose hosts and parasites from contrasting environments, where differences in parasite prevalence and diversity potentially selected for host and parasite types with different resistance and virulence (Feulner et al., 2015; Huang et al., 2016; Kalbe et al., 2016). Consistent with previous data (Kalbe et al., 2016), high resistance (HR) host types suppressed parasite growth more than low resistance (LR) host types and high growth (HG) *S. solidus* grew faster than low growth (LG) *S. solidus* in both host types.

Target immune genes were not significantly differentially expressed after three weeks, when HG and LG *S. solidus* were small (< 3 mg) in both host types. In line with our expectations, LG *S. solidus* were the smallest in every combination and infection rates of *D. pseudospathaceum* were not affected (Fig. 2; SI.9); gene expression profiles of LG-infected sticklebacks did not differ from controls over the course of the experiment (Fig. 3). HG infected sticklebacks increased innate immune responses significantly in week 6, when HG *S. solidus* had reached an average weight of 87 mg in LR hosts and 61 mg in HR hosts (Fig. S1; SI.9). The proposed minimal weight for sexual reproduction in the final host is 50 mg, and modulatory effects of *S. solidus* are expected above this threshold (Hammerschmidt & Kurtz, 2009; Tierney & Crompton, 1992). HR hosts simultaneously up-regulated expression of Treg associated genes, while this regulatory response was absent in LR hosts (Fig. 3). We conclude that HG *S. solidus* evolved fast growth in the context of efficient immune modulatory mechanisms in HR hosts, and that HR hosts evolved a well-orchestrated immune response to infection.

Later stages of chronic helminth infections are suspected to be accompanied by an activation of the complement system (Haase et al., 2016). Here we found that genes of complement components, especially *cfb*, were only up-regulated in HG *S. solidus* infections (Fig. 3A), which indicates that the involvement of complement components is *S. solidus* type specific. Helminth genotype-dependent complement activation was previously proposed for *D. pseudospathaceum* (Haase et al., 2014; Rauch et al., 2008). It is also tempting to speculate that the parasite's ability to change its surface composition could involve complement components and leads to evolutionary relevant variation in infectivity and virulence (Hammerschmidt & Kurtz, 2005).

The role of a T regulatory response in HR hosts

A T regulatory response may be beneficial for both host and parasite at late stages of infection as it facilitates survival of the parasite within the stickleback by preventing pathological inflammatory responses (Liu et al., 2009). We monitored expression levels of the Treg related genes *foxp3*, *tgf-β* and *il-16* in all treatments over time. FoxpP3 (Forkhead Box P3) is a characteristic transcription factor of regulatory T cells; TGF-β (Transforming growth factor β) is linked to development of Treg and Th17 cells (Weaver, et al., 2006; Robertson et al., 2015). TGF-β is often classified as a pro-inflammatory agent despite having regulatory functions (Liu et al., 2009; Zhu et al., 2012; Fischer et al., 2013;). RNA levels of *foxp3* and *tgf-β* were increased in HR stickleback after six weeks. Thus, HG *S. solidus* infected HR hosts up-regulated Tregs when the HG parasite initially triggered innate immunity. We conclude that HR hosts, coming from a population with high prevalence of fast growing *S. solidus*, evolved effective resistance and simultaneous up-regulation of pro-inflammatory innate immune genes and T regulatory components, which diminishes negative effects of the cestode or unspecific side effects such as immunopathology. This result is in line with the good condition of HR hosts and in agreement with the recent emphasis on T regulatory functions in helminth infections (Maizels & Yazdanbakhsh, 2003; Maizels, 2005; Nutman, 2015; Maizels & McSorley, 2016).

Immune gene expression profiles in LR hosts

In stark contrast to the well-orchestrated immune response in HG-infected HR hosts, LR hosts did not up-regulate expression of Treg genes upon infection with HG *S. solidus*. Their gene expression response was inefficient: HG and LG *S. solidus* grew faster and condition was lower in LR than in HR hosts. HG *S. solidus* – *D. pseudospathaceum* co-infected LR sticklebacks showed simultaneous significant up-regulation of Th1 and Th2 effectors, innate immunity, adaptive immunity and Tregs in week 9. Especially expression levels of *il-1β*, *foxp3*, *tgf-β* and *il-16* were significantly higher than in controls. IL-16 (Interleukin 16) is a chemoattractant for monocytes and eosinophils, inducing Th1 cell migration and supposedly contributes to Treg cell expansion, for example through the induction of FoxP3 (McFadden et al., 2007; Murphy & Weaver, 2017). Thus, in low resistant LR hosts, two pleiotropic cytokines were highly expressed in combination with pro-inflammatory molecules during chronic helminth infection. This points towards an ineffective and escalating immune response. We conclude that LR hosts, coming from a population with low *S. solidus* prevalence, cannot mount a concerted and effective immune response when infected with a (HG) *S. solidus* type that evolved fast growth along with strong immune modulation strategies.

S. solidus type-dependent interaction with D. pseudospathaceum

Immune gene expression profiles did not differ significantly between *D. pseudospathaceum* infected and control fish, suggesting an effective immune evasion strategy of *D. pseudospathaceum*. The eye fluke migrates to the immune privileged eye lens within 24 hours, thus evades adaptive immunity, and interacts with innate immunity only within this relatively short timeframe (Chappell et al., 1994; Scharsack & Kalbe, 2014). *D. pseudospathaceum* infection rates are therefore determined by the level of immune activation at the moment of infection. Interestingly, *D. pseudospathaceum* infection rates increased over time if hosts were co-infected with HG *S. solidus*. Thus, the *S. solidus* type affects *D. pseudospathaceum* infection success, which could directly or indirectly be mediated through effects on host metabolism or immunity.

We expect such effects to be influenced by additional naturally co-infecting parasite species with antagonistic or beneficial effects on the interaction with the host (Telfer et al., 2010; Benesh & Kalbe, 2016). Future laboratory and field experiments (such as those from Benesh & Kalbe, 2016) should thus incorporate additional parasite species in order to study situations closer to the natural setting.

D. pseudospathaceum infection rates were not affected by host immune gene expression if fish had only been infected with this species. Immune gene expression profiles did not differ significantly between host types or between co-infected and control fish until week 9 when HG-infected LR stickleback simultaneously up-regulated genes of most functional groups (Fig. 3B). We cannot conclude whether increased *D. pseudospathaceum* infection rates in HG co-infected hosts were the result of a stress response, cooperation, opportunistic exploitation, or correlation between resistance mechanisms against the two helminth species (Betts et al., 2016; Kalbe et al., 2016). Notably, infection with *D. pseudospathaceum* impairs the vision of infected fish and can cause pathological effects such as increased cataract formation (Meakins & Walkey, 1975; Karvonen et al., 2004). These effects could promote transmission to the final host (fish-eating birds) of both parasite species through reduction or interference with predator avoidance (Seppälä et al., 2004). *D. pseudospathaceum* infection rates increased after *S. solidus* size was above the expected minimal weight (50 mg) for sexual reproduction (Fig. S1; Hammerschmidt and Kurtz, 2009; Tierney and Crompton, 1992). Since fitness of both parasite species relies on transmission to the final host, our data point towards a possible cooperation, or at least indirect interaction between *S. solidus* and *D. pseudospathaceum*.

CONCLUSION

Helminth immune modulation is generally expected to change over the time course of infection (Maizels & Yazdanbakhsh, 2003). Nevertheless, immunological heterogeneity between host populations is often neglected and key molecules are under investigation (Benesh & Kalbe, 2016; Sitjà-Bobadilla, 2008). We addressed this knowledge gap by using different naturally co-occurring helminth species (*S. solidus* and *D. pseudospathaceum*) and types (high growth, HG, and low growth, LG, *S. solidus*) to analyze the immune status of host types from different ecologies and co-evolutionary backgrounds with *S. solidus* (high resistance, HR, and low resistance, LR, sticklebacks) over the course of infection. Our results are consistent with the assumption that a well-orchestrated host response mediates high resistance, namely inhibition of parasite growth (Lohman et al., 2017), and includes mechanisms that protect from immunopathological side effects. We demonstrated that expression profiles can differ between host and parasite types and are strongly influenced by co-infection with other parasite species. Understanding the premises and mechanisms of host-helminth interactions will advance our knowledge about co-evolutionary implications, with potential significance for treatment and prevention strategies in human health and other systems.

ACKNOWLEDGEMENTS

First authorship in this paper is shared between AP and MR. We are grateful to Anja Baade, Roswithe Derner, Isabel Moreau, Gisela Schmiedeskamp, Ines Schulz, Michael Schwarz, Nina Wildenhayn, Christoph Gahr, Gerhard Augustin and Daniel Martens for technical support and animal husbandry. We thank Manfred Milinski, Tobias Lenz and Olivia Roth for helpful discussions. AP was financially supported by the International Max Planck Research School (IMPRS) for Evolutionary Biology. MR and MK were funded by a DFG grant (SPP 1399: *Host-Parasite Coevolution*). Animal experiments were approved by the Ministry of Energy Transition, Agriculture, the Environment and Rural Areas of the state Schleswig-Holstein, Germany (reference number: V 312–7224.123-34).

DATA ACCESSIBILITY

All data generated and analysed in this study will be accessible on EDMOND by attachment of the respective doi (https://edmond.mpdl.mpg.de/imeji/) after acceptance of this manuscript.

AUTHOR CONTRIBUTIONS

AP, MR, MK designed the research and experimental approach. MK conceived the study and established and maintained laboratory cultures of hosts and parasites. AP and MR collected parasite specimens, performed the experiment, analysed the data and wrote the manuscript.

REFERENCES

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In *Second international symposium on information theory* (pp. 267–281). Retrieved from http://ci.nii.ac.jp/naid/10011153980/

Aken, B. L., Ayling, S., Barrell, D., Clarke, L., Curwen, V., Fairley, S., Hourlier, T. (2016). The Ensembl gene annotation system. *Database*, *2016*.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389–3402.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anthony, R. M., Rutitzky, L. I., Urban, J. F., Stadecker, M. J., & Gause, W. C. (2007). Protective immune mechanisms in helminth infection. *Nature Reviews Immunology*, *7*(12), 975–987. https://doi.org/10.1038/nri2199

Arme, C., & Owen, R. W. (1967). Infections of the three-spined stickleback, Gasterosteus aculeatus L., with the plerocercoid larvae of Schistocephalus solidus (Müller, 1776), with special reference to pathological effects. *Parasitology*, *57*(02), 301–314. https://doi.org/10.1017/S0031182000072103

Backiel, T., & Le Cren, E. D. (1978). Some density relationships for fish population parameters. *Ecology of Freshwater Fish Production*, 279–302.

Barber, I., & Scharsack, J. P. (2010). The three-spined stickleback-Schistocephalus solidus system: an experimental model for investigating host-parasite interactions in fish. *Parasitology*, *137*(3), 411.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models using lme4. *ArXiv:1406.5823*. Retrieved from http://arxiv.org/abs/1406.5823

Benesh, D. P., & Kalbe, M. (2016). Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly. *Journal of Animal Ecology*, *85*(4), 1004–1013. https://doi.org/10.1111/1365-2656.12527

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300.

Betts, A., Rafaluk, C., & King, K. C. (2016). Host and Parasite Evolution in a Tangled Bank. *Trends in Parasitology*, *32*(11), 863–873. https://doi.org/10.1016/ j.pt.2016.08.003

Bolger, T., & Connolly, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, *34*(2), 171–182. https://doi.org/ 10.1111/j.1095-8649.1989.tb03300.x

Brunner, F. S., Anaya-Rojas, J. M., Matthews, B., & Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proceedings of the National Academy of Sciences*, 201619147. https://doi.org/10.1073/ pnas.1619147114

Buckling, A., & Rainey, P. B. (2002). Antagonistic coevolution between a bacterium and a bacteriophage. *Proceedings. Biological Sciences / The Royal Society*, *269*(1494), 931–936. https://doi.org/10.1098/rspb.2001.1945

Carroll, M. C. (2004). The complement system in regulation of adaptive immunity. *Nature Immunology*, *5*(10), 981–986. https://doi.org/10.1038/ni1113

Chappell, L. H., Hardie, L. J., & Secombes, C. J. (1994). Diplostomiasis: the disease and host-parasite interactions. *Parasitic Diseases of Fish*, 59–86.

Clarke, A. S. (1954). Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. *Proceedings of the Zoological Society of London*, *124*(2), 257–302. https://doi.org/10.1111/j.1469-7998.1954.tb07782.x

Dargent, F., Scott, M. E., Hendry, A. P., & Fussmann, G. F. (2013). Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1773), 20132371. https://doi.org/10.1098/rspb.2013.2371

D'haeseleer, P. (2005). How does gene expression clustering work? *Nature Biotechnology*, *23*(12), 1499–1502.

Dubinina, M. N. (1980). Tapeworms (Cestoda, Ligulidae) of the fauna of the USSR. *Amerind P.* New Dehli, India: Amerind Publishing Co. Pvt. Ltd.

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, *3*, 621. https://doi.org/10.1038/ ncomms1632

Faltýnková, A., Našincová, V., & Kablásková, L. (2007). Larval trematodes (Digenea) of the great pond snail, Lymnaea stagnalis (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. *Parasite*, *14*(1), 39–51. https://doi.org/10.1051/parasite/2007141039

Feulner, P. G. D., Chain, F. J. J., Panchal, M., Huang, Y., Eizaguirre, C., Kalbe, M., Milinski, M. (2015). Genomics of Divergence along a Continuum of Parapatric Population Differentiation. *PLOS Genetics*, *11*(2), e1004966. https://doi.org/ 10.1371/ journal.pgen.1004966

Fischer, U., Koppang, E. O., & Nakanishi, T. (2013). Teleost T and NK cell immunity. *Fish & Shellfish Immunology*, *35*(2), 197–206. https://doi.org/ 10.1016/j.fsi.2013.04.018

Fox, J., & Weisberg, S. (2011). An {R} Companion to Applied Regression. *Second Edition. Thousand Oaks CA: Sage*. Retrieved from http://socserv.socsci. mcmaster.ca/jfox/Books/Companion

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

Gaujoux, R., & Seoighe, C. (2010). A flexible R package for nonnegative matrix factorization. *BMC Bioinformatics*, *11*(1), 367.

Giacomin, P., Croese, J., Krause, L., Loukas, A., & Cantacessi, C. (2015). Suppression of inflammation by helminths: a role for the gut microbiota? *Phil. Trans. R. Soc. B*, *370*(1675), 20140296. https://doi.org/10.1098/rstb.2014.0296

Graham, A. L. (2008). Ecological rules governing helminth–microparasite coinfection. *Proceedings of the National Academy of Sciences*, *105*(2), 566–570. https://doi.org/10.1073/pnas.0707221105

Haase, D., Rieger, J. K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., & Reusch, T. B. H. (2014). Specific Gene Expression Responses to Parasite Genotypes Reveal Redundancy of Innate Immunity in Vertebrates. *PLoS ONE*, *9*(9), e108001. https://doi.org/10.1371/journal.pone.0108001

Haase, D., Rieger, J. K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., Reusch, T. B. H. (2016). Comparative transcriptomics of stickleback immune gene responses upon infection by two helminth parasites, Diplostomum pseudospathaceum and Schistocephalus solidus. *Zoology*, *119*(4), 307–313. https://doi.org/ 10.1016/j.zool.2016.05.005

Hammerschmidt, K., & Kurtz, J. (2005). Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: Individual variation and fitness consequences. *International Journal for Parasitology*, *35*(14), 1499–1507. https://doi.org/10.1016/j.ijpara.2005.08.011

Hammerschmidt, K., & Kurtz, J. (2009). Chapter 5 Ecological Immunology of a Tapeworms' Interaction with its Two Consecutive Hosts. In Joanne P. Webster (Ed.), *Advances in Parasitology* (Vol. Volume 68, pp. 111–137). Academic Press. Retrieved from http://www.sciencedirect.com/science/ article/pii/S0065308X08006052

Heath, D. D., Holcman, B., & Shaw, R. J. (1994). Echinococcus granulosus: the mechanism of oncosphere lysis by sheep complement and antibody. *International Journal for Parasitology*, *24*(7), 929–935. https://doi.org/10.1016/0020-7519(94)90156-2

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, *8*, R19. https://doi.org/10.1186/gb-2007-8-2-r19

Hibbeler, S., Scharsack, J. P., & Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback Gasterosteus aculeatus. *BMC Molecular Biology*, *9*(1), 18. https://doi.org/10.1186/1471-2199-9-18

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363.

Huang, Y., Chain, F. J. J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T. L., … Feulner, P. G. D. (2016). Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. *Molecular Ecology*, https://doi.org/10.1111/mec.13520

Johnson, P. C. D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution*, *5*(9), 944–946. https://doi.org/ 10.1111/2041-210X.12225

Kalbe, M., & Kurtz, J. (2006). Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke Diplostomum pseudospathaceum. *Parasitology*, *132*(01), 105–116. https://doi.org/ 10.1017/S0031182005008681

Kalbe, M., Wegner, K. M., & Reusch, T. B. H. (2002). Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*, *60*(6), 1529–1542. https://doi.org/10.1111/j.1095-8649.2002.tb02445.x

Kalbe, M., Eizaguirre, C., Scharsack, J. P., & Jakobsen, P. J. (2016). Reciprocal cross infection of sticklebacks with the diphyllobothriidean cestode Schistocephalus solidus reveals consistent population differences in parasite growth and host resistance. *Parasites & Vectors*, *9*, 130. https://doi.org/ 10.1186/s13071-016-1419-3

Karvonen, A., Seppälä, O., & Valtonen, E. T. (2004). Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology*, *129*(4), 473–478. https://doi.org/10.1017/S0031182004006006

Khan, A. R., & Fallon, P. G. (2013). Helminth therapies: Translating the unknown unknowns to known knowns. *International Journal for Parasitology*, *43*(3–4), 293–299. https://doi.org/10.1016/j.ijpara.2012.12.002

Kurtz, J., Kalbe, M., Aeschlimann, P. B., Häberli, M. A., Wegner, K. M., Reusch, T. B. H., & Milinski, M. (2004). Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1535), 197–204. https://doi.org/ 10.1098/rspb.2003.2567

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. https://doi.org/10.1111/2041-210X.12512

Lello, J., Boag, B., Fenton, A., Stevenson, I. R., & Hudson, P. J. (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature*, *428*(6985), 840–844. https://doi.org/10.1038/nature02490

Lenz, T. L., Eizaguirre, C., Rotter, B., Kalbe, M., & Milinski, M. (2013). Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-wide digital gene expression analysis. *Molecular Ecology*, *22*(3), 774–786. https://doi.org/10.1111/j.1365-294X.2012.05756.x

Liu, Z., Liu, Q., Bleich, D., Salgame, P., & Gause, W. C. (2009). Regulation of type 1 diabetes, tuberculosis, and asthma by parasites. *Journal of Molecular Medicine*, *88*(1), 27–38. https://doi.org/10.1007/s00109-009-0546-0

Lohman, B. K., Steinel, N. C., Weber, J. N., & Bolnick, D. I. (2017). Gene Expression Contributes to the Recent Evolution of Host Resistance in a Model Host Parasite System. *Frontiers in Immunology*, *8*. https://doi.org/ 10.3389/fimmu.2017.01071

Lüscher, A., & Milinski, M. (2003). Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: an experimental test of predictions of mixed-mating and Hermaphrodite's Dilemma theory. *Journal of Evolutionary Biology*, *16*(5), 1030–1037. https://doi.org/10.1046/j.1420-9101.2003.00552.x

MacColl, A. D. C. (2009). Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, *32*(1), 153–160. https://doi.org/ 10.1111/j.1600-0587.2008.05486.x

Maizels, R. M. (2005). Infections and allergy — helminths, hygiene and host immune regulation. *Current Opinion in Immunology*, *17*(6), 656–661. https://doi.org/ 10.1016/j.coi.2005.09.001

Maizels, R. M., Bundy, D. A., Selkirk, M. E., Smith, D. F., & Anderson, R. M. (1993). Immunological modulation and evasion by helminth parasites in human populations. *Nature*, *365*(6449), 797–805.

Maizels, R. M., & McSorley, H. J. (2016). Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology*, *138*(3), 666–675. https://doi.org/10.1016/j.jaci.2016.07.007

Maizels, R. M., & Nussey, D. H. (2013). Into the wild: digging at immunology's evolutionary roots. *Nature Immunology*, *14*(9), ni.2643. https://doi.org/ 10.1038/ni.2643

Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, *3*(9), 733–744. https://doi.org/10.1038/nri1183

McFadden, C., Morgan, R., Rahangdale, S., Green, D., Yamasaki, H., Center, D., & Cruikshank, W. (2007). Preferential Migration of T Regulatory Cells Induced by IL-16. *The Journal of Immunology*, *179*(10), 6439–6445. https:// doi.org/10.4049/ jimmunol.179.10.6439

McSorley, H. J., Hewitson, J. P., & Maizels, R. M. (2013). Immunomodulation by helminth parasites: Defining mechanisms and mediators. *International Journal for Parasitology*, *43*(3), 301–310. https://doi.org/10.1016/j.ijpara. 2012.11.011

Meakins, R.H., and Walkey, M. (1975). The effects of parasitism by the plerocercoid of Schistocephalus solidus Muller 1776 (Pseudophyllidea) on the respiration of the three-spined stickleback Gasterosteus aculeatus L. J. Fish Biol. *7*, 817–824.

Mosmann, T. R., & Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today*, *17*(3), 138–146. https://doi.org/10.1016/0167-5699(96)80606-2

Mulcahy, G., O'Neill, S., Donnelly, S., & Dalton, J. P. (2004). Helminths at mucosal barriers—interaction with the immune system. *Advanced Drug Delivery Reviews*, *56*(6), 853–868. https://doi.org/10.1016/j.addr.2003.10.033

Murphy, K., & Weaver, C. (2017). *Janeway's immunobiology*. Garland Science.

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Neuwirth, E. (2014). *RColorBrewer Palettes. R package version 1.1-2.* Retrieved from http://CRAN.R-project.org/package=RColorBrewer

Nutman, T. B. (2015). Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. *Parasite Immunology*, *37*(6), 304–313. https://doi.org/10.1111/pim.12194

Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Wagner, H. (2015). *vegan: community ecology package. 2.0-0. R package version 2.3-0.*

Paterson, S., Vogwill, T., Buckling, A., Benmayor, R., Spiers, A. J., Thomson, N. R., Brockhurst, M. A. (2010). Antagonistic coevolution accelerates molecular evolution. *Nature*, *464*(7286), 275–278. https://doi.org/10.1038/nature08798

Pedersen, A. B., & Antonovics, J. (2013). Anthelmintic treatment alters the parasite community in a wild mouse host. *Biology Letters*, *9*(4), 20130205. https://doi.org/ 10.1098/rsbl.2013.0205

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), e45. https://doi.org/10.1093/ nar/29.9.e45

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2015). R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1-121. *Available at http://CRAN. R-Project. Org/Package= Nlme*.

Rauch, G., Kalbe, M., & B.H. Reusch, T. (2008). Partitioning average competition and extreme-genotype effects in genetically diverse infections. *Oikos*, *117*(3), 399–405. https://doi.org/10.1111/j.2007.0030-1299.16301.x

Ritter, M., Kalbe, M., & Henrich, T. (2017). Virulence in the three-spined stickleback specific parasite Schistocephalus solidus is inherited additively. *Experimental Parasitology*, *180*, 133–140. https://doi.org/10.1016/j.exppara.2017.02.016

Robertson, S., Bradley, J. E., & MacColl, A. D. C. (2015). Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. *Molecular Ecology Resources*, https://doi.org/10.1111/1755-0998.12497

Scharsack, J. P., & Kalbe, M. (2014). Differences in susceptibility and immune responses of three-spined sticklebacks (Gasterosteus aculeatus) from lake and river ecotypes to sequential infections with the eye fluke Diplostomum pseudospathaceum. *Parasites & Vectors*, *7*(1), 109.

Schulenburg, H., Kurtz, J., Moret, Y., & Siva-Jothy, M. T. (2009). Introduction. Ecological immunology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 3–14. https://doi.org/10.1098/rstb.2008.0249

Seppälä, O., Karvonen, A., & Tellervo Valtonen, E. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behaviour*, *68*(2), 257–263. https://doi.org/10.1016/ j.anbehav.2003.10.021

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*(8), 317–321. https://doi.org/10.1016/0169-5347(96)10039-2

Sitjà-Bobadilla, A. (2008). Living off a fish: a trade-off between parasites and the immune system. *Fish & Shellfish Immunology*, *25*(4), 358–372. https://doi.org/ 10.1016/j.fsi.2008.03.018

Smyth, J. D. (1946). Studies on Tapeworm Physiology I. The Cultivation of Schistocephalus Solidus In Vitro. *Journal of Experimental Biology*, *23*(1), 47–70.

Stutz, W. E., Schmerer, M., Coates, J. L., & Bolnick, D. I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. *Molecular Ecology*, *24*(18), 4629–4646. https://doi.org/ 10.1111/mec.13295

Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S., & Begon, M. (2010). Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. *Science*, *330*(6001), 243–246. https://doi.org/10.1126/ science.1190333

Tierney, J. F., & Crompton, D. W. T. (1992). Infectivity of Plerocercoids of Schistocephalus solidus (Cestoda: Ligulidae) and Fecundity of the Adults in an Experimental Definitive Host, Gallus gallus. *The Journal of Parasitology*, *78*(6), 1049–1054. https://doi.org/10.2307/3283228

van der Veen, I. T., & Kurtz, J. (2002). To avoid or eliminate: cestode infections in copepods. *Parasitology*, *124*(4), 465–474. https://doi.org/10.1017/ S0031182001001275

Vandesompele, J., Preter, K. D., Pattyn, F., Poppe, B., Roy, N. V., Paepe, A. D., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), 1–12. https://doi.org/10.1186/gb-2002-3-7-research0034

Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M., & Murphy, K. M. (2006). Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties. *Immunity*, *24*(6), 677–688. https://doi.org/10.1016/j.immuni.2006.06.002

Weber, J. N., Steinel, N. C., Shim, K. C., & Bolnick, D. I. (2017). Recent evolution of extreme cestode growth suppression by a vertebrate host. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(25), 6575–6580. https://doi.org/10.1073/pnas.1620095114

Wedekind, C., Strahm, D., & Schärer, L. (1998). Evidence for strategic egg production in a hermaphroditic cestode. *Parasitology*, *117*(04), 373–382.

Wegner, K. M., Reusch, T. B. H., & Kalbe, M. (2003). Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of Evolutionary Biology*, *16*(2), 224–232. https://doi.org/10.1046/j.1420-9101.2003.00519.x

Wickham, H. (2009). *Elegant graphics for data analysis (ggplot2)*. New York, NY: Springer-Verlag.

Wickham, Hadley. (2011). The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, *40*(1), 1–29.

Zhu, L., Nie, L., Zhu, G., Xiang, L., & Shao, J. (2012). Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. *Developmental & Comparative Immunology*, *39*(1–2), 39–62. https://doi.org/10.1016/j.dci.2012.04.001

CHAPTER 3

Experimental evidence that divergent evolution is linked to distinct defence mechanisms

Agnes Piecyk[1,2,°], Megan Hahn[3], Michael A. Bell[4], David C. Heins[5], Tina Henrich[1], Olivia Roth[2], Nolwenn Deilly[3,*], Martin Kalbe[1,*]

[1]Evolutionary Ecology, Max Planck Institute for Evolutionary Biology, Plön, Germany; [2]Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany; [3]School of Marine and Atmospheric Sciences, Stony Brook University, New York, USA; [4]University of California Museum of Paleontology, Berkeley, CA, USA; [5]Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA

° corresponding author: apiecyk@evolbio.mpg.de

* shared senior authorship

(manuscript in preparation)

ABSTRACT

Epidemiological traits of host-parasite associations depend on host effects, parasite effects and interaction effects. While parasites evolve mechanisms to detect, infect and exploit their hosts, hosts evolve mechanisms to prevent infection (qualitative resistance), limit parasite burden (quantitative resistance) and/or decrease the detrimental effects of infection (tolerance). The interaction between hosts and parasites is further shaped by the environment and geographic variation in natural selection. We aimed to determine host and parasite as well as interaction effects over different geographical scales. Therefore, three-spined sticklebacks and their cestode parasites from Alaskan and European populations were used for experimental cross-infections. We hypothesized that host and parasite main effects would dominate both within and across continents. Due to the geographic variation of natural selection, we further expected that the geographical distance (within versus across continents) would alter the potential for interaction effects.

Qualitative resistance only occurred in a combination of hosts and parasites from different continents (implying interaction effects). Quantitative resistance and tolerance were host population-specific. We show that one stickleback population from south-central Alaska (Wolf Lake) prevented infection of European parasites despite having higher tolerance in comparison to the other populations. Molecular phenotypes (host regulatory and immune gene expression) differed between uninfected fish populations but mostly converged upon infection.

Our results indicate that evolution favours distinct defence mechanisms when assessed on different geographic scales. Tolerance did not preclude resistance within a population. We conclude that selection for distinct defence mechanisms is imposed by host, parasite and interaction effects

KEYWORDS

host-parasite interaction, *Gasterosteus aculeatus*, *Schistocephalus solidus*, resistance, tolerance

BACKGROUND

Parasites rely on their hosts for resources and evolve mechanisms that increase their ability to infect and exploit their hosts (Poulin and Morand, 2000; Schmid-Hempel, 2011). Natural selection favours parasite traits that increase their fitness through trade offs of infectivity, growth and transmission. However, hosts evolve defence mechanisms that prevent infection and/or limit parasite growth or the detrimental effects of infections. These defence strategies can be divided into resistance and tolerance. Resistance reduces the likelihood of infection and/or limits parasite replication or growth; tolerance limits the damage of a certain parasite burden without limiting parasite replication or growth (Råberg et al., 2007; Read et al., 2008; Råberg, 2014; Zeller and Koella, 2017). In statistical terms, tolerance explains variation in the relationship between infection intensity and measures of host health or fitness. Different slopes of this relationship indicate variance in tolerance (Read et al., 2008; Råberg, 2014). Resistance and tolerance are not mutually exclusive (Sternberg et al., 2013). However, the effects on ecological and evolutionary interactions between hosts and parasites differ greatly. For example, parasite prevalence is expected to decrease if hosts evolve resistance, whereas parasite prevalence is expected to increase if hosts evolve tolerance (Best et al., 2014; Roy and Kirchner, 2000).

The epidemiological traits of hosts and parasites, such as infectivity, resistance, tolerance, or virulence, depend on the interacting genotypes and on the biotic and abiotic environment (Carius et al., 2001; Lambrechts et al., 2006; Schulenburg et al., 2009; Zeller and Koella, 2017). Host and parasite genotypes and allele frequencies change over evolutionary time scales, while the response of an individual to different environmental conditions (known as 'reaction norm') is plastic. The relative contribution of environmentally mediated phenotypic plasticity to infection phenotypes can be substantial because heterogeneous environments can decouple genotype and phenotype (Lazzaro and Little, 2009).

It has, for example, been shown that whether the host develops resistance or tolerance can depend on the type of the pathogen and is altered by the environment, such as the host energy source (Ayres and Schneider, 2008; Cumnock et al., 2018).

Understanding the variation in host and parasite genetic and plastic effects on infection outcome is crucial for a number of fields, including basic science and explicitly clinical settings. Only recently, evolutionary dynamics have been integrated into medical research laying a foundation for 'evolutionary medicine' (Stearns and Medzhitov, 2016). In this study, we were particularly interested in defence mechanisms of hosts against helminth parasites. Helminths are parasitic worms that can establish long-lasting infections and cause substantial morbidity. The suppression of the parasite's growth is a particularly important form of resistance once the infection is established (Weber et al., 2017). We measured two types of resistance: the ability to prevent infection as a qualitative measurement and the ability to reduce the parasite's growth as a quantitative measurement. We also determined parasite strain and host population specific tolerance by studying the relationship between infection intensity and a measure of host health (body condition) (Råberg et al., 2009). Body condition accurately predicts mate quality, mate choice and fitness in our system (Milinski and Bakker, 1990; Kaufmann et al., 2014). To further understand the molecular phenotypes, we studied regulatory and immune gene expression.

Using a teleost-helminth system, we previously showed that gene expression profiles that differed between uninfected hosts from divergent European populations converged upon infection. We inferred that the effect of parasite-induced phenotypic plasticity might be stronger than the effect of host genotype or host genotype by parasite genotype interaction (Piecyk et al., *in revision*). Here, we aimed to test the generalisability of these results by using hosts and parasites from North-Western America (Alaska) and Central Europe in cross-infection experiments.

We hypothesized (i) baseline differences between host populations within and across continents (indicating host genotype effects) (ii) parasite-strain specific responses to infection within and across continents (indicating parasite genotype effects), and (iii) different potentials for interaction effects on different geographic scales (here, within Alaska and across continents).

Study system

The three-spined stickleback *Gasterosteus aculeatus* (hereafter 'stickleback') is an important model species for evolutionary ecology, evolutionary parasitology, genomics and immunology (Colosimo et al., 2005; Gibson, 2005; Barber and Nettleship, 2010; Barber, 2013; Robertson et al., 2015; Lohman et al., 2017; Brunner et al., 2017). This fish is distributed across the Northern Hemisphere where it adapted to a wide range of habitats (Bell and Foster, 1994). Stickleback populations differ in phenotypic and genotypic traits including morphology, behaviour, and immunity. This might largely be driven by their abiotic environment, as exemplified by marine – freshwater divergence (Jones et al., 2012). However, local adaptation, divergent selection and genomic differentiation have also been linked to parasites (MacColl, 2009; Eizaguirre et al., 2012; Feulner et al., 2015; Nagar and MacColl, 2016). It has been shown that immune gene frequencies and levels of immunological activation differ between environments (Wegner et al., 2003; Huang et al., 2016; Lohman et al., 2017). Thus, in addition to genetic adaptation, phenotypic plasticity seems to contribute significantly to differential immune gene expression (Stutz et al., 2015; Lenz, 2015; Robertson et al., 2015; Piecyk et al., *in revision*).

We aimed to test the effects of host and parasite genotypes and phenotypically plastic immune responses with controlled infection experiments involving sticklebacks and *Schistocephalus solidus* from diverse populations. The trophically transmitted cestode *S. solidus* has a complex life cycle involving two intermediate hosts: the first larval stage (coracidium) infects cyclopoid copepods and develops into the second larval stage (procercoid). *S. solidus* develops into the third larval stage (plerocercoid) when a three-spined stickleback (*Gasterosteus aculeatus*) feeds on an infected copepod.

The parasite penetrates the intestinal wall and enters the body cavity of the fish where it continues to grow for several weeks or months (Smyth, 1946; Clarke, 1954; Hammerschmidt and Kurtz, 2009). The definite host, mostly fish-eating birds, can be replaced by an *in vitro* breeding system (Smyth, 1946, 1954; Wedekind, 1997). *S. solidus* is suggested to be infective for the definite host and able to reproduce above a weight of 50 mg (Tierney and Crompton, 1992). The parasite's size depends on the stickleback's size (Barber, 2005), and *vice versa*. The relative weight of *S. solidus* in the fish, the parasite index (PI; Arme and Owen, 1967), is a measure for parasite fitness (Wedekind et al., 1998; Lüscher and Wedekind, 2002), fecundity reduction (i.e. virulence) (Arme and Owen, 1967; Heins and Baker, 2003; Bagamian et al., 2004; Heins, 2012), and host resistance (Weber et al., 2017).

Approach and aim

Here we used hosts and parasites from geographically distinct populations of the same species in order to test whether divergent host-parasite co-evolution caused different host, parasite and/ or interaction effects when tested on different geographic scales (within Alaska and across continents). We studied whether geographic distances between host and parasite populations could be linked to different defence mechanisms. Building on from the idea that parasite-induced phenotypic plasticity largely determines the infection phenotype of *S. solidus* infected sticklebacks, we hypothesized that gene expression patterns of geographically distinct stickleback populations (even across continents) would converge in response to *S. solidus* infection.

Table 1. Host and parasite origins.

ID		Sampling site		
ALO	Alaskan	Walby Lake	Alaska	61°62'N, -149°22
ALX	Alaskan	Wolf Lake	Alaska	61°65'N, -149°28
GPS	European stickleback	Großer Plöner See	Germany	54°08'N, 10°24'E
SKO	European *S. solidus*	Lake Skogseidvatnet	Norway	60°13'N, 05°53'E

Three-spined sticklebacks (*Gasterosteus aculeatus*) and their specific cestode parasite *Schistocephalus solidus* from two European and two Alaskan populations were bred in the laboratory (Table 1). Stickleback and *S. solidus* from the European populations are characterised by low resistance against *S. solidus* (Großer Plöner See, GPS, Germany; *S. solidus* prevalence is < 1%) and fast growth in *G. aculeatus* (Lake Skogseidvatnet, SKO, Norway; *S. solidus* prevalence ranges between 20% and > 50%) (Benesh and Kalbe, 2016; Kalbe et al., 2016; Weber et al., 2016; Piecyk et al., *in revision*; Piecyk, Ritter & Kalbe, *in review*). Stickleback-*S. solidus* pairs from the Matanuska–Susitna Valley in south-central Alaska are known for their diverse infection phenotypes. Whereas *S. solidus* infected stickleback from Wolf (ALX) are known for their strongly demelanized integument and darkening of the eyes (LoBue and Bell, 1993), those phenotypic changes have never been reported for *S. solidus* infected stickleback from Walby (ALO), even though infection prevalence is much higher (~40 – 76%, rarely down to 15%; Heins et al., 1999, 2018). *S. solidus* infected female stickleback from Walby consistently experience fecundity reduction through nutrient depletion (Heins et al., 2010, 2014). This study is the first to use stickleback and *S. solidus* from Wolf Lake and Walby Lake in controlled experimental infections.

We expected constitutive differences between the three host populations and, according to a previously reported dominant effect of parasite-induced phenotypic plasticity (Piecyk et al., *in revision*), hypothesized that these differences would converge upon infection (parasite main effect). We also hypothesized a host main effect such that the low resistance European hosts would be less resistant against all tested *S. solidus* strains than the Alaskan hosts. A common garden approach was used to disentangle individual variation from confounding factors.

MATERIAL AND METHODS

Experimental design and common garden setup

We tested for host genotype and parasite genotype contribution to infection phenotypes by running a cross-infection experiment involving different Alaskan and European populations of sticklebacks and *S. solidus* strains ('strain' refers to *S. solidus* from a distinct location). We determined (i) the infection rates as a measure of parasite infectivity and host qualitative resistance, (ii) parasite size as a measure for virulence, transmission potential and host quantitative resistance, (iii) proxies of host body condition as measures of tolerance and costs of resistance, and (iv) host immunological parameters including regulatory and immune gene expression as measures of the molecular host-parasite interaction.

The experiment was composed of three rounds. In each round, hosts from three populations were exposed to three parasite strains or sham-exposed (Figure 1). Parasite sibships (n = 4 per *S. solidus* strain) were the same in every round; fish families differed between rounds. Fish from all populations (controls and *S. solidus* exposed) were housed in the same tanks; controls had their own compartment (Figure 1). Each tank housed 16 individuals in Round 1 and 17 individuals in Round 2 and 3.

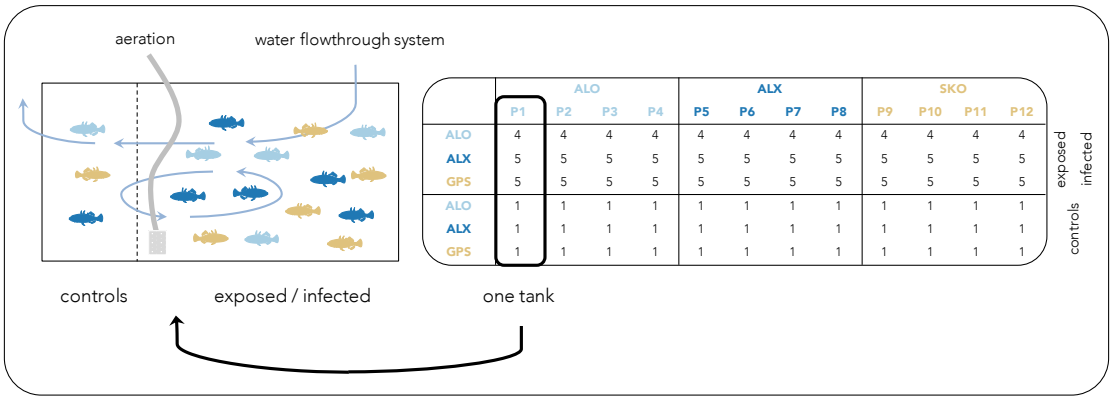


Figure 1. Schematic illustration of the experimental setup. Alaskan (ALO and ALX) and European (SKO and GPS) sticklebacks (*Gasterosteus aculeatus*) and cestodes (*Schistocephalus solidus*) were used for experimental cross infections. The experiment was composed of three rounds with the same parasite sibships (P1 to P12) and different fish families from the respective populations. The table shows sample sizes from one round. Fish from all populations (controls and *S. solidus* exposed; the latter would ultimately be exposed and uninfected or infected) were housed in the same tanks. Blue arrows illustrate the water current. Colours refer to the two Alaskan (ALO = light blue; ALX = dark blue) and European populations (yellow = GPS hosts or SKO parasites).

Hosts and parasites

First generation offspring from wild-caught sticklebacks and *S. solidus* was bred in the laboratory. *G. aculeatus* eggs were fertilized *in vitro*. Eggs from Alaskan fish were rinsed with acriflavine (50 µL/L; 30 sec), methylene blue (500 µL per L from stock: 1 g/L methylene blue; 30 sec) and 3 ppt artificial seawater and shipped on 4 °C to the Max Planck Institute for Evolutionary Biology (MPI), Plön, Germany. Stickleback eggs from the German population were treated in the same way. The progeny was kept at the institute's aquaria system at 18°C and a light:dark rhythm of 16:8 hours. The fish were eight months old at the start of the infection experiment.

S. solidus plerocercoids came from infected Alaskan fish that were shipped to the MPI and dissected immediately upon arrival in June 2016. Pairs of *S. solidus* plerocercoids were weight-matched and bred *in vitro* (Smyth, 1946; Wedekind et al., 1998). European *S. solidus* were bred in December 2015. The eggs were kept at 4 °C in the dark.

Infection experiment

S. solidus eggs were incubated at 18°C for three weeks and hatch was stimulated by light exposure according to Dubinia (1980). Copepods (*Macrocyclops albidus*) from a laboratory culture were exposed to single coracidia the next day and screened for presence of procercoids after one week. Individually housed sticklebacks were starved for one day and exposed to single infected *M. albidus* on day 16. The fish were transferred to 16 L aquaria two days later. Using a common garden approach, fish from all populations and all treatments (sham-exposed, exposed and uninfected, infected) were housed in the same tanks (n = 36; each housing 16 to 17 fish; 45 ALO fish, 60 ALX fish, and 59 GPS fish were exposed to ALO *S. solidus*; 48 ALO fish, 59 ALX fish, and 59 GPS fish were exposed to ALX *S. solidus*; 46 ALO fish, 57 ALX fish, and 57 GPS fish were exposed to SKO *S. solidus*). Water of single tanks was sieved and screened for leftover copepods. All individuals were fed with frozen chironomid larvae three times a week. The number of fish per tank was kept constant by replacing individuals that died before the end of the experiment with naïve fish from the same genetic background. Six controls and one exposed fish died before the end of the experiment. Sticklebacks were euthanized with MS222 and dissected nine weeks post exposure.

The standard length (without fin; +/- 1 mm) and weight (+/- 0.1 mg) were recorded. Head kidneys, liver and spleen were weighted to the nearest 0.1 mg. Head kidneys were immediately transferred to RNAlater (Sigma-Aldrich) and stored at -20°C for RNA extraction. Plerocercoids were removed from the body cavity, weighted, transferred to liquid nitrogen and stored at -80°C. We determined the fish's condition (condition factor, CF, according to Frischknecht, 1993) and hepatosomatic index, HSI, (Chellappa et al., 1995) and immunological activation (splenosomatic index, SSI, head kidney index, HKI). The parasite index (PI, the relative weight of the parasite in the host) was calculated according to Arme and Owen (1967).

RNA extraction and reverse transcription

Head kidney RNA was extracted with a NucleoSpin® 96 kit (Macherey-Nagel). Procedures followed the manufacturer's protocol, including 1% ß-mercaptoethanol for tissue lysis (2 x 3 min at 30 Hz; Tissue Lyser II; Qiagen) and on column DNA digestion. RNA concentration and purity were determined spectrophotometrically (NanoDrop1000; Thermo Scientific). All A_{260}/A_{280} ratios were at least 1.98 and RNA concentrations were adjusted at 500 ng for reverse transcription. We used the Omniscript RT kit (Qiagen) according to the manual but used 0.2 µl of a 4 unit RNase inhibitor (Qiagen) per reaction. The cDNA was stored at -80°C.

Quantitative real-time PCR (RT-qPCR)

Differences in transcription levels of 32 genes were tested using 96.96 Dynamic Array IFCs on a Biomark™ HD system (Fluidigm) with EvaGreen as DNA intercalating dye. We pre-amplified the cDNA samples by using TaqMan PreAmp Master Mix (Applied Biosystems) according to the manufacturer's protocol (14 cycles). The product was diluted 1:5 in low TE buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA). Samples of all treatments were spread across three IFCs. All targets for a given sample were included in the same run and measured in technical triplicates. Inter-run calibrators and negative controls were included on each IFC.

Targets of interest covered four putative reference genes (*b2m, ubc, rpl13a, ef1a*; (Hibbeler et al., 2008)), four regulatory genes (*abtb1, ascl1b, kat2a, mapk13*) and 24 immune related genes from innate immunity (*marco*, *mst1ra*, *mif*, *il-1β*, *tnfr1*, *saal1*, *tlr2, csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), adaptive immunity (*stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *tgf-β*, *il-16, mhcII, tcr-β*) and the complement system (*cfb, c7, c9*) (Brunner et al., 2017; Robertson et al., 2015; Stutz et al., 2015; Piecyk, Ritter & Kalbe (*in review*)).

Melting curves were analysed with the *Fluidigm Analysis software v.4.5.1*. Three targets (*il-1β*, *tgf-β*, and *ascl1b*) were excluded from further analyses due to ambiguous melting curves. The raw data was imported into *qbase+ 3.0* (Biogazelle) (Hellemans et al., 2007) to assess data quality and calculate calibrated normalized relative quantities (CNRQ), which are based on the ΔΔCt method (Pfaffl, 2001). The negative cutoff for technical sensitivity limit was set at cycle 28 and a 0.5 cycle variation was accepted for maximum triplicate variability. Reference targets *rpl13* and *ubc* were used for normalization as inferred from geNorm (M = 0.236) and the Coefficient of Variation (CV = 0.082) (Hellemans et al., 2007; Vandesompele et al., 2002). Target specific amplification efficiencies (1.85 to 2.24) were calculated from a serial dilution. The data was log10 transformed.

Statistical analyses

All statistical analyses were performed in R (version 3.2.0, R Core Team, 2015). Infection rates were analysed with binomial generalized linear mixed effects models (GLMMs) using the function glmer() from *lme4* (Bates et al., 2014). Response variables were proportional data from infected versus uninfected individuals. Infection rates in copepods were analysed with parasite population and round (which is confounded with fish family) and their interaction as fixed effects and parasite sibship as random intercept. Infection rates in fish were analysed with host and parasite populations and their interaction as fixed effects and round and parasite sibship as crossed random effects. We accounted for the number of copepods that were not ingested. Significantly different groups and *p*-values were determined with glht() from *multcomp* (Hothorn et al., 2008) with individually defined contrasts of interest or Type III Wald chisquare tests using Anova() from *car* (Fox and Weisberg, 2016).

Further analyses distinguished between (i) sham-exposed controls, (ii) *S. solidus* exposed but uninfected fish ('exposed'), and (iii) *S. solidus* infected fish. Linear mixed effects models (using lmer() from *lme4* (Bates et al., 2014)) were used to test for differences between parasite growth (PI), fish condition (CF, HSI) and immunological parameters (SSI, HKI). We had to separate the data according to host and parasite origins because SKO parasites did not infect ALX hosts. Including the interaction of host and parasite origin in the model fit would have caused rank deficient fixed-effect model matrices. Accordingly, models on data from infected fish included host or parasite origin as fixed effect as well as sex of the fish and tank, which is confounded with fish family and parasite sibship, as crossed random effects (random intercepts). We accounted for multiple testing by using the false discovery rate (FDR) according to Benjamini and Hochberg (1995) with an α of 0.05. To test for variation in tolerance, we fitted parasite-strain specific linear mixed effect models (lmer() from *lmerTest* (Kuznetsova et al., 2017) with body condition (CF, HSI) as dependent variable, host population and parasite index and their interaction as fixed effects as well as fish sex and tank as random effects. The corresponding degrees of freedom were approximated with Satterthwaite's method.

Differences between gene expression profiles were tested with a multivariate approach grouping data from all 25 targets (*total*), from eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2, csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive*: *stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16, mhcII, tcr-β*), three complement component genes (*complement*: *c7, c9, cfb*), or three regulatory genes (*regulatory*: *abtb1, kat2a, mapk13*). Non-parametric permutational multivariate analyses of variance (PERMANOVA, (Anderson, 2001)) were based on Euclidian distances and 10,000 permutations that were constrained within tank. Size related effects were accounted for by using the weight of the fish as a covariate. Pairwise PERMANOVAs were used *a posteriori* to identify significantly different groups (Anderson, 2001). We tested for differences in baseline gene expression by using data from sham-exposed controls of the three populations.

We tested whether the host, the parasite and/or their interaction had an effect on gene expression of *S. solidus* exposed stickleback. Since SKO parasites did not infect ALX hosts, host and parasite effects on gene expression profiles of infected individuals were tested by (i) grouping data from each parasite population and (ii) grouping data from each host population (SI.4.2).

Finally, gene expression profiles of infected, exposed, and control fish were compared within each combination of hosts and parasites. Local adaptation of Alaskan hosts and parasites was tested on a data subset of the Alaskan populations. Using the false discovery rate (Benjamini and Hochberg, 1995) we accounted for multiple testing within each combination. Linear mixed effect models (using lmer() from *lme4* (Bates et al., 2014)) with tank as random intercept were used *a posteriori* to identify the genes that were differentially expressed. Plots were created with *ggplot2* (Wickham, 2009) and *aheatmap*() from NMF (Gaujoux & Seoighe, 2010) with colour schemes from *RColorBrewer* (Neuwirth, 2014).

RESULTS

Infectivity and parasite size

We determined the infection probability as a measure for parasite infectivity and host qualitative resistance. *S. solidus* infection rates in copepods (first intermediate hosts) neither differed significantly between rounds nor between parasite populations (SI.1). The interaction between host and parasite populations significantly affected infection rates in fish (GLMM; $p = 0.006$): European high growth (SKO) parasites did not infect sticklebacks from Wolf (ALX) but from Walby (ALO). Alaskan parasites from both populations infected European (GPS) sticklebacks. At the end of the experiment 82 fish were infected, 409 fish were exposed but uninfected and 102 fish were sham-exposed. One designated control fish was infected.

The relative size of the parasite in the host, the parasite index, was used as a measure for virulence, transmission potential and host quantitative resistance. Parasite indices did not differ significantly between parasites within host populations but between host populations (SI.2; Figure 2): Parasite indices were generally higher in European (GPS) sticklebacks. Alaskan *S. solidus* grew larger in GPS hosts than in Alaskan sticklebacks (SI.2). European (SKO) *S. solidus* were significantly smaller in Alaskan sticklebacks from Walby (ALO) than in European (GPS) sticklebacks (LMM; $p < 0.001$).

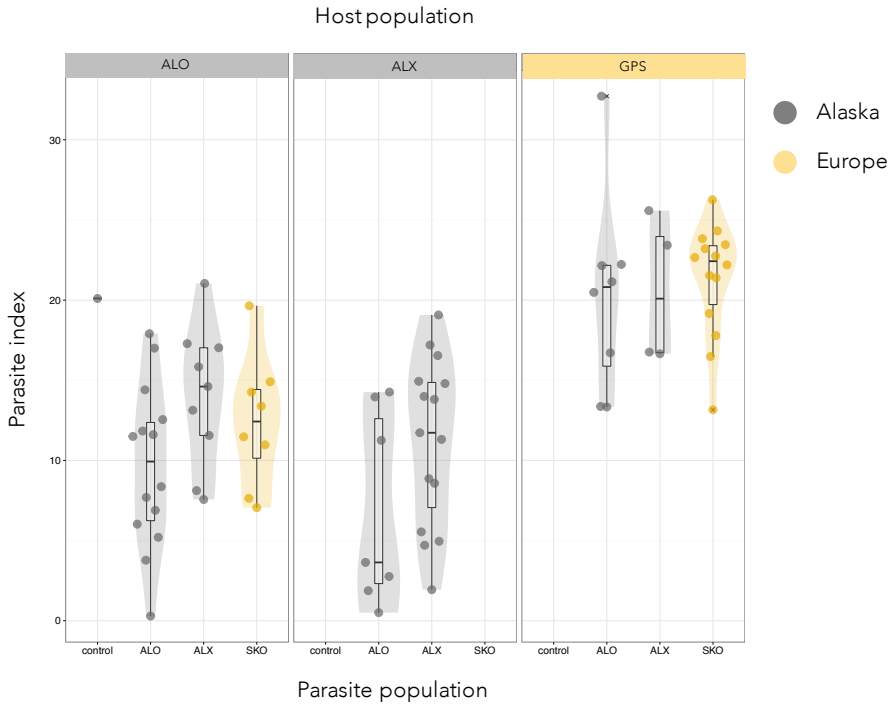


Figure 2. *S. solidus* parasite indices in three different stickleback hosts. Alaskan (ALO and ALX) and European (GPS) stickleback were exposed to Alaskan (ALO and ALX) and European (SKO) *S. solidus*. The infection success and the parasite index (relative weight of the parasite in the host according to (Arme and Owen, 1967)) were determined after nine weeks.

Host condition and immunological parameters

Stickleback body condition was assessed through the condition factor (the ratio between the observed weight and the expected weight at a given length (Frischknecht, 1993)) and the relative weight of the liver (hepatosomatic index, HSI, which is a measure for medium term energy reserves (Chellappa et al., 1995)).

The condition (with the exception of the HSI between GPS and ALX) differed significantly between controls from the different populations. ALX sticklebacks had the lowest condition and GPS sticklebacks had the highest condition (Table S3; Figure S2). GPS sticklebacks had generally larger head kidneys and spleens than sticklebacks from the Alaskan populations (GLMMs; each $p < 0.001$), but spleen size did not differ significantly between GPS and ALO controls (Table S3; Figure S3). Differences between the populations remained if fish were exposed but uninfected (Table S4). The condition factor (CF) of GPS sticklebacks and the HSI of ALO sticklebacks differed significantly between controls and exposed individuals, potentially indicating an effect of exposure to *S. solidus* (Tables S6 and S7).

Infection with ALO and SKO *S. solidus* caused a significant decrease of the overall condition (CF) of European (GPS) hosts; ALX infection was linked to a condition decrease in ALO sticklebacks (Table S6). We detected a host-population specific relation between host condition factor and infection intensity (i.e. tolerance) in ALO and ALX infections (host population-parasite index interaction in ALO infections: $F_{2,21.7} = 9.37$, $p = 0.0012$; host population-parasite index interaction in ALX infections: $F_{2,17.5} = 4.02$, $p = 0.037$) (Figure 3). Medium term energy reserves (HSI) were significantly lower in all infected fish, regardless of host and parasite origin and not affected by the parasite index; the effects did not differ between parasite origins within host populations (Table S7; Figure S2).

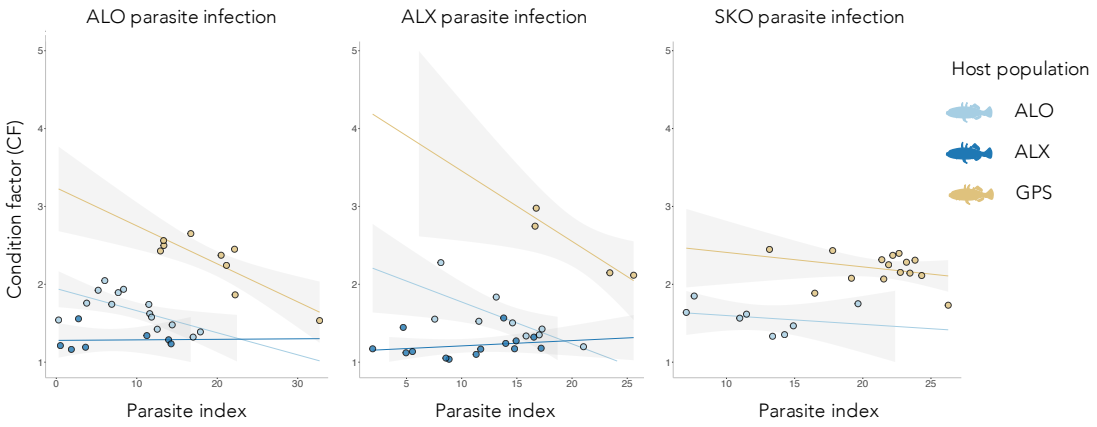


Figure 3. Host population-specific relation between body condition and infection intensity (i.e. tolerance). Each dot represents one individual; lines and shaded areas represent linear regression fits with 95% confidence intervals. Colours indicate the host population.

S. solidus infection resulted in similar immunological parameters (SSI and HKI) in all host populations (Table S5; Figure S3). Splenosomatic indices (SSI) generally increased upon infection; the effects were pronounced in specific combinations: ALX and SKO *S. solidus* in GPS hosts, ALO and ALX *S. solidus* in ALX hosts, and ALO *S. solidus* in ALO hosts (Table S8; Figure S3). Head kidneys were larger in infected Alaskan sticklebacks; head kidneys of GPS hosts were not affected (Table S9; Figure S3).

Stickleback regulatory and immune gene expression

Total RNA from head kidneys was extracted from 84 controls, 101 exposed but uninfected fish ('exposed') and 80 infected sticklebacks. Stickleback population ($PERMANOVA_{total}$: $F_{2,264} = 5.96$, $p < 0.001$) and infection status ($PERMANOVA_{total}$: $F_{2,264} = 3.41$, $p < 0.001$) significantly affected the expression of 25 immune and regulatory genes; interactions were not significant (Table S10).

Baseline gene expression differs between stickleback populations

Gene expression profiles of sham-exposed controls differed between the two Alaskan populations ($PERMANOVA_{total}$: $F_{1,52} = 2.60$, $p = 0.003$; $PERMANOVA_{complement}$: $F_{1,52} = 4.81$, $p = 0.007$) and between European (GPS) sticklebacks and sticklebacks from ALX ($PERMANOVA_{total}$: $F_{1,54} = 3.57$, $p = 0.007$; $PERMANOVA_{innate}$: $F_{1,54} = 2.72$, $p = 0.026$; $PERMANOVA_{complement}$: $F_{1,54} = 2.77$, $p = 0.023$; $PERMANOVA_{regulatory}$: $F_{1,54} = 5.77$, $p = 0.013$). In the multivariate analyses, only regulatory gene expression differed between GPS and ALO controls ($PERMANOVA_{regulatory}$: $F_{1,59} = 2.57$, $p = 0.012$) (SI.4.1: Figure S4; Tables S12-S14). Hierarchical clustering on Euclidian distances indicated highest divergence of ALX profiles (Figure 4A).

A posteriori analyses identified differential expression of *mapk13* (GPS vs ALO: $z = 3.06$, $p = 0.006$; GPS vs ALX: $z = 3.4$, $p = 0.002$), *p22*^{phox} (GPS vs ALX: $z = 3.56$, $p = 0.001$), *saal1* (GPS vs ALX: $z = -3.06$, $p = 0.006$), *tlr2* (GPS vs ALO: $z = 2.56$, $p = 0.028$; GPS vs ALX: $z = 3.09$, $p = 0.006$), *cd83* (GPS vs ALO: $z = -2.94$, $p = 0.009$), *igm* (ALX vs ALO: $z = -2.7$, $p = 0.19$; GPS vs ALX: $z = 3.23$, $p = 0.004$), and *c9* (ALX vs ALO: $z = -2.77$, $p = 0.015$; GPS vs ALX: $z = 3.12$, $p = 0.005$) (Table S15).

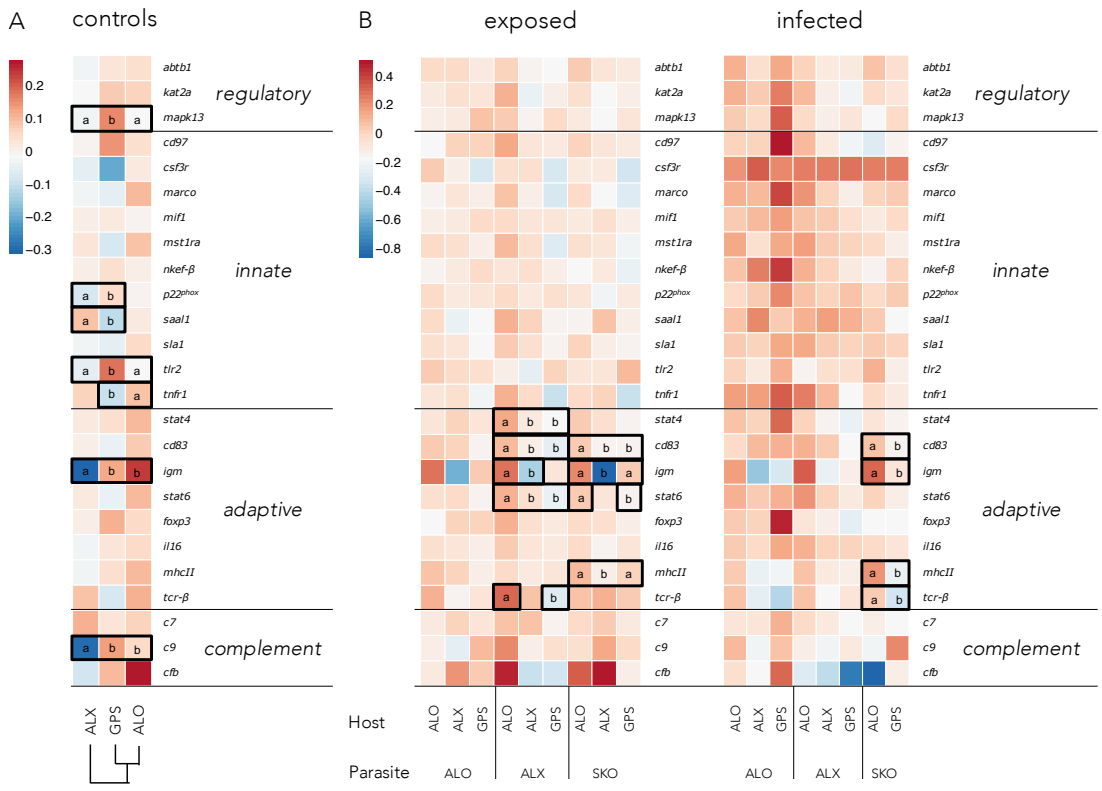


Figure 4. Stickleback gene expression profiles. Alaskan (ALO and ALX) and European (GPS) stickleback were sham-exposed (controls) or exposed to Alaskan (ALO and ALX) or European (SKO) *S. solidus*. Total RNA was extracted from head kidneys after nine weeks. The heatmaps are based on log10 transformed CNRQ values that were averaged across treatments. Lower case letters indicate significantly different expression of single genes. (A) Gene expression profiles of sham-exposed controls. The columns were hierarchically clustered on Euclidian distances. (B) Gene expression profiles of exposed but uninfected (exposed) and infected sticklebacks. Columns were ordered according to treatment.

Overall, host population differences remained if fish were exposed to *S. solidus* but uninfected (host effect: $PERMANOVA_{total}$: $F_{2,101} = 2.75$, $p = 0.0002$; parasite effect: $PERMANOVA_{total}$: $F_{2,101} = 0.5$, $p = 0.096$; host-parasite interaction effect: $PERMANOVA_{total}$: $F_{2,101} = 1.05$, $p = 0.428$) (more information in SI.4.2: Figure S5; Tables S16-S21). In order to understand the host effect in more detail, we used host population as explanatory and found that gene expression profiles differed especially upon exposure to ALX and SKO *S. solidus* (host effect to ALX *S. solidus* exposure: $PERMANOVA_{total}$: $F_{2,31} = 2.1$, $p = 0.004$; $PERMANOVA_{adaptive}$: $F_{2,31} = 3.42$, $p < 0.001$; host effect to SKO *S. solidus* exposure: $PERMANOVA_{adaptive}$: $F_{2,36} = 4.75$, $p < 0.001$; Tables S16-S18). Using parasite strain as explanatory (testing for parasite effects within host populations), gene expression profiles were not significantly affected by *S. solidus* strain (Tables S19-S21).

ALO sticklebacks that were exposed to ALX *S. solidus* showed higher expression of five adaptive immune genes in comparison to ALX or GPS sticklebacks. *Stat4* (ALX vs ALO: z = -3.05, p = 0.007; GPS vs ALO: z = -3.27, p = 0.003), *cd83* (ALX vs ALO: z = -3.37, p = 0.002; GPS vs ALO: z = -5.37, $p < 0.001$), *igm* (ALX vs ALO: z = -3.0, p = 0.008), *stat6* (ALX vs ALO: z = -2.46, p = 0.038; GPS vs ALO: z = -3.93, $p < 0.001$), and *tcr-β* (GPS vs ALO: z = -3.7, $p < 0.001$) were differentially expressed (Table S22) (Figure 4). Sticklebacks that were exposed to SKO *S. solidus* showed differential expression of four adaptive immune genes of which three genes were higher expressed in ALO than in ALX: *cd83* (ALX vs ALO: z = -3.55, p = 0.002; GPS vs ALO: z = -3.53, p = 0.001), *igm* (ALX vs ALO: z = -4.75, $p < 0.001$; GPS vs ALX: z = 3.39, p = 0.002), *stat6* (GPS vs ALO: z = -2.79, p = 0.015), and *mhcII* (ALX vs ALO: z = -3.03, p = 0.007; GPS vs ALX: z = 2.43, p = 0.04) (Figure 4).

Gene expression profiles converge upon infection

Studying data of all infected individuals (n=80), we found that gene expression profiles converged upon *S. solidus* infection (SI.4.3: Figure S6; Tables S24-S29). Only SKO infection caused different adaptive immune gene expression profiles in Alaskan (ALO) versus European GPS stickleback ($PERMANOVA_{adaptive}$: $F_{1,21}$ = 6.64, $p < 0.001$; Table S26). *A posteriori* analyses showed that these differences were driven by higher expression of *cd83* (z = -5.09, $p < 0.0001$), *igm* (z = -4.16, $p < 0.0001$), *mhcII* (z = -2.71, p = 0.007), and *tcr-β* (z = -4.43, $p < 0.0001$) in ALO hosts in comparison to GPS hosts (Table S30).

We next tested whether the infection status (infected, exposed, control) affected regulatory and immune gene expression within each combination of hosts and parasites (SI.4.4). Pairwise comparisons were used to test for differences between (i) infected and control fish, (ii) infected and exposed fish, and (iii) control and exposed fish (SI.4.5 - SI.4.7.).

We detected an ALX parasite effect on innate immune gene expression in ALO and ALX hosts in comparison to the respective controls (ALX infection effect in ALO: $PERMANOVA_{innate}$: $F_{1,38} = 1.38$, $p = 0.009$; ALX infection effect in ALX: $PERMANOVA_{innate}$: $F_{1,38} = 1.57$, $p = 0.007$; Figures S12 and S13; Tables S41 and S44). ALO infection of GPS stickleback was linked to up-regulation of *total*, *innate*, and *regulatory* genes compared to controls ($PERMANOVA_{total}$: $F_{1,38} = 5.71$, $p = 0.02$; $PERMANOVA_{innate}$: $F_{1,38} = 9.92$, $p = 0.004$; $PERMANOVA_{regulatory}$: $F_{1,38} = 7.12$, $p = 0.009$; Figure S14; Tables S45-S47). *Total*, *innate*, *adaptive*, and *regulatory* profiles differed between ALO exposed and ALO infected GPS stickleback ($PERMANOVA_{total}$: $F_{1,21} = 5.8$, $p = 0.007$; $PERMANOVA_{innate}$: $F_{1,21} = 8.85$, $p = 0.003$; $PERMANOVA_{adaptive}$: $F_{1,21} = 5.16$, $p = 0.006$; $PERMANOVA_{regulatory}$: $F_{1,21} = 7.43$, $p = 0.02$; Figure S15; Table S53). We further detected significant differences between SKO exposed and SKO infected GPS stickleback ($PERMANOVA_{total}$: $F_{1,26} = 2.54$, $p = 0.02$; $PERMANOVA_{innate}$: $F_{1,61} = 5.12$, $p < 0.001$; $PERMANOVA_{adaptive}$: $F_{1,26} = 4.33$, $p < 0.001$; Table S55).

Confirming our finding that host population differences remained if fish were exposed to *S. solidus* but uninfected, gene expression profiles did not differ significantly between control and exposed fish (SI.4.7: Tables S56-S64). Moreover, whether Alaskan sticklebacks were infected with sympatric or allopatric *S. solidus* did not affect their gene expression profiles (SI.4.7: Tables S65 and S66).

DISCUSSION

Host defence strategies can be divided into resistance and tolerance. Resistance is defined as the inverse of a parasite burden in a two-step infection process: preventing infection (qualitative) and limiting parasite growth (quantitative); tolerance is defined as the ability to limit detrimental effects caused by a given parasite burden (Schneider and Ayres, 2008; Råberg et al., 2009; Råberg, 2014; Zeller and Koella, 2017). We measured these three types of host defence in helminth infections of sticklebacks to determine (i) host effects, (ii) parasite effects, and (iii) host-parasite interaction effects on infection phenotypes.

Our first key finding was that resistance and tolerance differed among host populations, implying host genetic effects on infection outcome. Parasite infectivity (host qualitative resistance) depended on host genotype - parasite genotype interaction, whereas parasite size (a measure of parasite virulence and transmission potential as well as host quantitative resistance) was affected by the host but did not differ between parasite strains within host populations, nor according to an interaction effect. We also detected population-level differences in tolerance. Following up on our results that hosts varied in qualitative and quantitative resistance as well as in tolerance, we analysed regulatory and immune gene expression profiles for a better understanding of the molecular phenotypes. Our second key finding was that constitutive differences of gene expression profiles and other immunological and condition parameters mostly converged upon infection. In line with our hypothesis of parasite-strain specific responses to infection, this finding implies dominant effects of parasite induced phenotypic plasticity on the host side and a stronger parasite genotype main effect compared to the interaction effects.

Variation in host defence mechanisms

We present two distinct types of resistance in combinations of geographically disparate populations of hosts and parasites of the same species. First, ALX stickleback prevented infection by SKO *S. solidus*. Second, stickleback from both Alaskan populations (ALO and ALX) had higher quantitative resistance against *S. solidus* than GPS stickleback. ALX hosts also appeared to be more tolerant, as their body condition did not change with increasing parasite burden (parasite index). In contrast, body condition of ALO and GPS hosts decreased with increasing parasite index. This implies that stickleback populations (here: ALX) can have both higher qualitative resistance and tolerance. We suggest that the high tolerance is a universal property of these fish, whereas the prevention of infection is SKO *S. solidus*-specific. Tolerance is expected to correlate with high parasite prevalence (Roy and Kirchner, 2000; Best et al., 2014), which we did not observe in nature (*S. solidus* prevalence is lower in ALX than in ALO).

This could be explained by the lack of the ecological context in laboratory experiments. However, population-specific qualitative resistance against SKO *S. solidus* in laboratory trials has been reported before: two out of three Canadian stickleback populations were not infected by SKO *S. solidus* (Weber et al., 2016). If this result is *S. solidus* and/or stickleback population- or clade-specific warrants further investigation. Alaskan and Canadian stickleback belong to the Pacific clade. The fact that SKO *S. solidus* are capable of infecting marine and freshwater fish from the same clade (Weber et al., 2016; this study) suggests that the marine-freshwater divergence may contribute to resistance evolution, as suggested by Weber and colleagues (2016), but that population-specific interactions might be more important.

The terms resistance and infectivity describe capacities of the host and/or the parasite. Conclusively, infection relies on the parasite's ability to recognize and infect the host; resistance relies on the host's ability to recognize and eradicate or control the parasite. Moreover, the establishment and the maintenance of infection or resistance are multistep processes involving different host and parasite molecules (Schmid-Hempel, 2009; Duneau et al., 2011; Dybdahl et al., 2014). Concerning the qualitative resistance of ALX hosts against SKO parasites, one possible explanation could be the presence or the absence of receptors that are essential for the infection. Candidate parasite molecules are surface carbohydrates that have been shown to vary between larval stages and sibships and to correlate to infectivity and parasite growth in sticklebacks (Hammerschmidt and Kurtz, 2005). Thus, comparative studies of *S. solidus* surface molecules would be promising.

Importantly, immune defence is costly and might be selected against (Boots and Haraguchi, 1999; Duncan et al., 2011). Our statistical support for this assumption is confined to the condition factor in GPS and hepatosomatic index in ALO, but our study seems to confirm this assumption by demonstrating significantly lower body condition in exposed than in control fish.

The immediate stage of infection and the developmental status of the parasite fundamentally affect the infection phenotype. In *S. solidus* infections of sticklebacks, immune evasion is expected at early stages (until the parasite reaches the body cavity of the fish), clearance possibly only occurs within the first two weeks and immune modulation is expected above the weight threshold for sexual reproduction (50 mg) (Tierney and Crompton, 1992; Scharsack et al., 2007; Hammerschmidt and Kurtz, 2009; Barber and Scharsack, 2010; Piecyk, Ritter & Kalbe, *in review*;). An important caveat of our study is that we cannot conclude whether exposed but ultimately uninfected sticklebacks had prevented or cleared the infection. The respective parasites possibly failed to target and/or overcome the intestinal wall or were eliminated through the host's immune system. Histological specimens of exposed fish from the first two weeks post exposure (and maybe beyond) could shed light on the molecular interplay. We suggest to include specimens from qualitatively resistant hosts with surgically introduced SKO parasites. Additionally, genomics, transcriptomics and proteomics may help to identify essential loci and molecules.

Parasite-induced phenotypic plasticity transcends host genetic differences

Control fish were kept under the same laboratory conditions in order to assess whether genetic divergence of the host populations would affect their phenotype. Genetic divergence between European and Northern American stickleback as well as *Schistocephalus solidus* is well documented (Colosimo et al., 2005; Nishimura et al., 2011; Feulner et al., 2015; Fang et al., 2018). Our data indicate constitutive differences between the host populations: compared to the two Alaskan populations, European (GPS) stickleback were in better condition and had a higher baseline immunological activation as inferred from the size of the two major immune organs (SSI and HKI). Gene expression profiles differed between all host populations, but especially profiles of ALX stickleback were distinct from GPS and ALO profiles (Figure 4).

Upon *S. solidus* infection, ALX stickleback had a particular qualitative resistance: these hosts could prevent SKO infection. However, quantitative resistance did not differ between the Alaskan populations but in comparison to GPS. Parasite indices were highest in GPS hosts, confirming their low quantitative resistance against various *S. solidus* strains (Piecyk et al., *in revision*; Piecyk, Ritter & Kalbe, *in review*). Neither parasite origin, nor sympatry or allopatry had an effect on parasite size as a measure of quantitative resistance. Confirming previous data (Piecyk et al., *in revision*), proxies of immunological activation converged upon infection. Although the precise molecular mechanisms will have to be further studied, our results suggest that *Schistocephalus solidus* has a strong effect on stickleback gene expression. We conclude that the parasite-induced phenotypic plasticity transcends the genetic effects.

Environment-specific adaptations that could not be captured in the laboratory experiments

Based on wild caught sticklebacks from more than 200 Alaskan lakes, LoBue and Bell proposed a causal relationship between the conspicuous demelanized phenotype of Wolf stickleback and *Schistocephalus solidus* infection (LoBue and Bell, 1993). Even though this has been published almost three decades ago, controlled experimental infections to test this hypothesis have not been conducted. We did not detect signs of demelanization as a result of *S. solidus* infection although the parasites had reached the proposed minimal weight for sexual reproduction, which has been linked to the white phenotype (LoBue and Bell, 1993). One reason for not being able to reproduce the white phenotype in the laboratory could be that natural ecological factors that are excluded during experimental infections are important components. Cross-infection experiments in North American (Alaskan) laboratories could yield different results and controlled laboratory infections in combination with enclosures in the natural system could provide more information. It is possible that temperature shifts are essential components: the south-central Alaskan lakes are usually covered with ice from October into May and infected hosts may spend the winter under the ice (Heins et al., 2015).

Thorough field and laboratory studies should test this hypothesis. Further, transcriptomics might identify specific candidate genes and genome studies would increase our understanding of the underlying molecular mechanism.

CONCLUSION

Using three-spined stickleback (*Gasterosteus aculeatus*) hosts and *Schistocephalus solidus* parasites from distinct geographic locations we could show that main effects of the host and the parasite determine the infection phenotypes over different geographic scales (across and within continents). We identified different defence mechanisms: qualitative resistance (the inverse of parasite infection success), quantitative resistance (parasite growth suppression) and tolerance (the relationship between infection intensity and measures of host health). While qualitative resistance depended, over the scale of continents, on host-parasite interaction effects, quantitative resistance and tolerance did not. We conclude that host, parasite and interaction effects differentially affect distinct defence mechanisms.

ABBREVIATIONS

ALO: Walby Lake, Alaska, US (stickleback and *S. solidus* sampling site); ALX: Wolf Lake, Alaska, US (stickleback and *S. solidus* sampling site); CF: condition factor; GPS: Großer Plöner See, Germany (stickleback sampling site); HKI: head kidney index; HSI: hepatosomatic index; PI: parasite index; SKO: Lake Skogseidvatnet, Norway (*S. solidus* sampling site); SSI: splenosomatic index

AUTHOR CONTRIBUTIONS

DH, MB, and MK conceived the cross-infections. AP, DH, MB, and MK collected and maintained the hosts and parasites. AP, ND, MH, MK, and TH designed the experiment. AP and MH performed the experiment. AP analysed the data and wrote the manuscript. AP and OR discussed the data and the manuscript.

ACKNOWLEDGEMENTS

We are grateful to R. Derner, D. Martens, R. Schmuck, G. Schmiedeskamp, I. Schulz, M. Schwarz and N. Wildenhayn for practical support and animal husbandry. Primer sequences for *abtb1, ascl1b, kat2a, mapk13* were kindly provided by J. Gismann and M. Heckwolf.

REFERENCES

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecol. *26*, 32–46.

Arme, C., and Owen, R.W. (1967). Infections of the three-spined stickleback, Gasterosteus aculeatus L., with the plerocercoid larvae of Schistocephalus solidus (Müller, 1776), with special reference to pathological effects. Parasitology *57*, 301–314.

Ayres, J.S., and Schneider, D.S. (2008). A Signaling Protease Required for Melanization in Drosophila Affects Resistance and Tolerance of Infections. PLoS Biol. *6*, e305.

Bagamian, K.H., Heins, D.C., and Baker, J.A. (2004). Body condition and reproductive capacity of three-spined stickleback infected with the cestode Schistocephalus solidus. J. Fish Biol. *64*, 1568–1576.

Barber, I. (2005). Parasites grow larger in faster growing fish hosts. Int. J. Parasitol. *35*, 137–143.

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

Barber, I., and Nettleship, S. (2010). From 'trash fish'to supermodel: the rise and rise of the three-spined stickleback in evolution and ecology. Biologist *57*, 15–21.

Barber, I., and Scharsack, J.P. (2010). The three-spined stickleback-Schistocephalus solidus system: an experimental model for investigating host-parasite interactions in fish. Parasitology *137*, 411.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4. ArXiv14065823 Stat.

Bell, M.A., and Foster, S.A. (1994). The evolutionary biology of the threespine stickleback (Oxford University Press).

Benesh, D.P., and Kalbe, M. (2016). Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly. J. Anim. Ecol. *85*, 1004–1013.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. *57*, 289–300.

Best, A., White, A., and Boots, M. (2014). The Coevolutionary Implications of Host Tolerance. Evolution *68*, 1426–1435.

Boots, M., and Haraguchi, Y. (1999). The Evolution of Costly Resistance in Host-Parasite Systems. Am. Nat. *153*, 359–370.

Brunner, F.S., Anaya-Rojas, J.M., Matthews, B., and Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. Proc. Natl. Acad. Sci. 201619147.

Carius, H.J., Little, T.J., and Ebert, D. (2001). Genetic Variation in a Host-Parasite Association: Potential for Coevolution and Frequency-Dependent Selection. Evolution *55*, 1136–1145.

Chellappa, S., Huntingford, F.A., Strang, R.H.C., and Thomson, R.Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish Biol. *47*, 775–787.

Clarke, A.S. (1954). Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. Proc. Zool. Soc. Lond. *124*, 257–302.

Colosimo, P.F., Hosemann, K.E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., Schmutz, J., Myers, R.M., Schluter, D., and Kingsley, D.M. (2005). Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. Science *307*, 1928–1933.

Cumnock, K., Gupta, A.S., Lissner, M., Chevee, V., Davis, N.M., and Schneider, D.S. (2018). Host Energy Source Is Important for Disease Tolerance to Malaria. Curr. Biol. *28*, 1635-1642.e3.

Duncan, A.B., Fellous, S., and Kaltz, O. (2011). Reverse Evolution: Selection Against Costly Resistance in Disease-Free Microcosm Populations of Paramecium Caudatum. Evolution *65*, 3462–3474.

Duneau, D., Luijckx, P., Ben-Ami, F., Laforsch, C., and Ebert, D. (2011). Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. BMC Biol. *9*, 11.

Dybdahl, M.F., Jenkins, C.E., and Nuismer, S.L. (2014). Identifying the Molecular Basis of Host-Parasite Coevolution: Merging Models and Mechanisms. Am. Nat. *184*, 1–13.

Eizaguirre, C., Lenz, T.L., Kalbe, M., and Milinski, M. (2012). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. Nat. Commun. *3*, 621.

Fang, B., Merilä, J., Ribeiro, F., Alexandre, C.M., and Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. Mol. Phylogenet. Evol.

Feulner, P.G.D., Chain, F.J.J., Panchal, M., Huang, Y., Eizaguirre, C., Kalbe, M., Lenz, T.L., Samonte, I.E., Stoll, M., Bornberg-Bauer, E., et al. (2015). Genomics of Divergence along a Continuum of Parapatric Population Differentiation. PLOS Genet. *11*, e1004966.

Fox, J., and Weisberg, S. (2016). car: Companion to applied regression. R Package Version 21.2.

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

Gaujoux, R., & Seoighe, C. (2010). A flexible R package for nonnegative matrix factorization. *BMC Bioinformatics*, *11*(1), 367.

Gibson, G. (2005). The synthesis and evolution of a supermodel. Science *307*, e1891.

Hammerschmidt, K., and Kurtz, J. (2005). Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: Individual variation and fitness consequences. Int. J. Parasitol. *35*, 1499–1507.

Hammerschmidt, K., and Kurtz, J. (2009). Chapter 5 Ecological Immunology of a Tapeworms' Interaction with its Two Consecutive Hosts. In Advances in Parasitology, Joanne P. Webster, ed. (Academic Press), pp. 111–137.

Heins, D.C. (2012). Fecundity compensation in the three-spined stickleback Gasterosteus aculeatus infected by the diphyllobothriidean cestode Schistocephalus solidus. Biol. J. Linn. Soc. *106*, 807–819.

Heins, D.C., and Baker, J.A. (2003). Reduction of egg size in natural populations of threespine stickleback infected with a cestode macroparasite. J. Parasitol. *89*, 1–6.

Heins, D.C., Singer, S.S., and Baker, J.A. (1999). Virulence of the cestode Schistocephalus solidus and reproduction in infected threespine stickleback, Gasterosteus aculeatus. Can. J. Zool. *77*, 1967–1974.

Heins, D.C., Baker, J.A., Toups, M.A., and Birden, E.L. (2010). Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode Schistocephalus solidus. Biol. J. Linn. Soc. *100*, 835–846.

Heins, D.C., Barry, K.A., and Petrauskas, L.A. (2014). Consistency of host responses to parasitic infection in the three-spined stickleback fish infected by the diphyllobothriidean cestode Schistocephalus solidus. Biol. J. Linn. Soc. *113*, 958–968.

Heins, D.C., Eidam, D.M., and Baker, J.A. (2015). Timing of Infections in the Threespine Stickleback (Gasterosteus aculeatus) by Schistocephalus solidus in Alaska. J. Parasitol. *102*, 286–289.

Heins, D.C., Moody, K.N., and Miller, S. (2018). Are solo infections of the diphyllobothriidean cestode Schistocephalus solidus more virulent than multiple infections? Parasitology 1–8.

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., and Vandesompele, J. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. *8*, R19.

Hibbeler, S., Scharsack, J.P., and Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback Gasterosteus aculeatus. BMC Mol. Biol. *9*, 18.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. Biom. J. *50*, 346–363.

Huang, Y., Chain, F.J.J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T.L., Samonte, I.E., Stoll, M., Bornberg-Bauer, E., Reusch, T.B.H., et al. (2016). Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. Mol. Ecol. n/a-n/a.

Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J., Swofford, R., Pirun, M., Zody, M.C., White, S., et al. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. Nature *484*, 55–61.

Kalbe, M., Eizaguirre, C., Scharsack, J.P., and Jakobsen, P.J. (2016). Reciprocal cross infection of sticklebacks with the diphyllobothriidean cestode Schistocephalus solidus reveals consistent population differences in parasite growth and host resistance. Parasit. Vectors *9*, 130.

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

Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. *82*.

Lambrechts, L., Fellous, S., and Koella, J.C. (2006). Coevolutionary interactions between host and parasite genotypes. Trends Parasitol. *22*, 12–16.

Lazzaro, B.P., and Little, T.J. (2009). Immunity in a variable world. Philos. Trans. R. Soc. B Biol. Sci. *364*, 15–26.

Lenz, T.L. (2015). Transcription in space – environmental vs. genetic effects on differential immune gene expression. Mol. Ecol. *24*, 4583–4585.

LoBue, C.P., and Bell, M.A. (1993). Phenotypic Manipulation by the Cestode Parasite Schistocephalus solidus of Its Intermediate Host, Gasterosteus aculeatus, the Threespine Stickleback. Am. Nat. *142*, 725–735.

Lohman, B.K., Steinel, N.C., Weber, J.N., and Bolnick, D.I. (2017). Gene Expression Contributes to the Recent Evolution of Host Resistance in a Model Host Parasite System. Front. Immunol. *8*.

Lüscher, A., and Wedekind, C. (2002). Size-dependent discrimination of mating partners in the simultaneous hermaphroditic cestode Schistocephalus solidus. Behav. Ecol. *13*, 254–259.

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

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

Nagar, A.E., and MacColl, A.D.C. (2016). Parasites contribute to ecologically dependent postmating isolation in the adaptive radiation of three-spined stickleback. Proc R Soc B *283*, 20160691.

Neuwirth, E. (2014). RColorBrewer: ColorBrewer palettes. R Package Version 11-2 https://cran.R-project.org/package=RColorBrewer.

Nishimura, N., Heins, D.C., Andersen, R.O., Barber, I., and Cresko, W.A. (2011). Distinct Lineages of Schistocephalus Parasites in Threespine and Ninespine Stickleback Hosts Revealed by DNA Sequence Analysis. PLoS ONE *6*, e22505.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. *29*, e45.

Poulin, R., and Morand, S. (2000). The Diversity of Parasites. Q. Rev. Biol. *75*, 277–293.

Råberg, L. (2014). How to Live with the Enemy: Understanding Tolerance to Parasites. PLOS Biol. *12*, e1001989.

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

Råberg, L., Graham, A.L., and Read, A.F. (2009). Decomposing health: tolerance and resistance to parasites in animals. Philos. Trans. R. Soc. Lond. B Biol. Sci. *364*, 37–49.

Read, A.F., Graham, A.L., and Råberg, L. (2008). Animal Defenses against Infectious Agents: Is Damage Control More Important Than Pathogen Control. PLoS Biol. *6*, e1000004.

Robertson, S., Bradley, J.E., and MacColl, A.D.C. (2015). Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. Mol. Ecol. Resour. n/a-n/a.

Roy, B.A., and Kirchner, J.W. (2000). Evolutionary Dynamics of Pathogen Resistance and Tolerance. Evolution *54*, 51–63.

Scharsack, J.P., Koch, K., and Hammerschmidt, K. (2007). Who is in control of the stickleback immune system: interactions between Schistocephalus solidus and its specific vertebrate host. Proc. R. Soc. B Biol. Sci. *274*, 3151–3158.

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. Philos. Trans. R. Soc. B Biol. Sci. *364*, 85–98.

Schmid-Hempel, P. (2011). Evolutionary Parasitology The Integrated Study of Infections, Immunology, Ecology, and Genetics (Oxford University Press, Inc.: New York, NY, USA).

Schneider, D.S., and Ayres, J.S. (2008). Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat. Rev. Immunol. *8*, 889–895.

Schulenburg, H., Kurtz, J., Moret, Y., and Siva-Jothy, M.T. (2009). Introduction. Ecological immunology. Philos. Trans. R. Soc. B Biol. Sci. *364*, 3–14.

Smyth, J.D. (1946). Studies on Tapeworm Physiology I. The Cultivation of Schistocephalus Solidus In Vitro. J. Exp. Biol. *23*, 47–70.

Smyth, J.D. (1954). Studies on tapeworm physiology. VII. Fertilization of< i> Schistocephalus solidus in vitro</i>. Exp. Parasitol. *3*, 64–71.

Stearns, S.C., and Medzhitov, R. (2016). Evolutionary medicine (Sinauer Associates, Incorporated, Publishers).

Sternberg, E.D., Li, H., Wang, R., Gowler, Camden, and Roode, J.C. de (2013). Patterns of Host-Parasite Adaptation in Three Populations of Monarch Butterflies Infected with a Naturally Occurring Protozoan Disease: Virulence, Resistance, and Tolerance. Am. Nat. *182*, E235–E248.

Stutz, W.E., Schmerer, M., Coates, J.L., and Bolnick, D.I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. Mol. Ecol. *24*, 4629–4646.

Tierney, J.F., and Crompton, D.W.T. (1992). Infectivity of Plerocercoids of Schistocephalus solidus (Cestoda: Ligulidae) and Fecundity of the Adults in an Experimental Definitive Host, Gallus gallus. J. Parasitol. *78*, 1049–1054.

Vandesompele, J., Preter, K. De, Poppe, B., Roy, N. Van, and Paepe, A. De (2002). Accurate normalization of real-time quantitative RT -PCR data by geometric averaging of multiple internal control genes. 1–12.

Weber, J.N., Kalbe, M., Shim, K.C., Erin, N.I., Steinel, N.C., Ma, L., Bolnick, D.I., Nuismer, S.L., and Michalakis, Y. (2016). Resist Globally, Infect Locally: A Transcontinental Test of Adaptation by Stickleback and Their Tapeworm Parasite. Am. Nat. E000–E000.

Weber, J.N., Steinel, N.C., Shim, K.C., and Bolnick, D.I. (2017). Recent evolution of extreme cestode growth suppression by a vertebrate host. Proc. Natl. Acad. Sci. U. S. A. *114*, 6575–6580.

Wedekind, C. (1997). The infectivity, growth, and virulence of the cestode Schistocephalus solidus in its first intermediate host, the copepod Macrocyclops albidus. Parasitology *115*, 317–324.

Wedekind, C., Strahm, D., and SCHÄRER, L. (1998). Evidence for strategic egg production in a hermaphroditic cestode. Parasitology *117*, 373–382.

Wegner, K.M., Reusch, T.B.H., and Kalbe, M. (2003). Multiple parasites are driving major histocompatibility complex polymorphism in the wild. J. Evol. Biol. *16*, 224–232.

Wickham, H. (2009). Elegant graphics for data analysis (ggplot2) (New York, NY: Springer-Verlag).

Zeller, M., and Koella, J.C. (2017). The Role of the Environment in the Evolution of Tolerance and Resistance to a Pathogen. Am. Nat. *190*, 389–397.

SYNTHESIS

My doctoral research aimed to find answers to the questions how and why epidemiological traits of host-parasite interactions vary among populations. Within this framework, I focussed on defence mechanisms of hosts against helminth parasites using the three-spined stickleback (*Gasterosteus aculeatus*) as a vertebrate model organism for experimental infections.

The sticklebacks and their cestode parasites (*Schistocephalus solidus*) came from different populations across the Northern Hemisphere (Figure 1). I characterized two host populations with supposedly divergent co-evolutionary trajectories (DE and NO) in *Chapter 1* and *Chapter 2*. Given that helminth parasites can have substantial and complex immune modulatory effects on their hosts, understanding the evolutionary and ecological factors underlying host-helminth interactions is particularly important for both basic science and applied research. Only recently, medical research started to incorporate evolutionary thinking. Evolutionary medicine addresses causes of disease located in hosts (patients), in parasites, and their local environment (Stearns and Medzhitov, 2016). I hypothesized that the co-evolution of the vertebrate immune system with helminth parasites probably follows different environment- and/or phylogeny-specific trajectories. Indeed, my colleagues and I demonstrated that distinct *S. solidus* strains caused different molecular interplays and infection outcomes in divergent stickleback types. For instance, NO sticklebacks, that co-evolved with a highly virulent *S. solidus* strain, were generally more resistant and mounted a well-coordinated immune response (i.e. *The right response at the right time*; *Chapter 2*). In contrast, DE sticklebacks, that supposedly evolved under de-escalated arms race dynamics with *S. solidus*, were less resistant and their response towards a highly virulent *S. solidus* strain was un-coordinated. On a large geographic scale (*Chapter 1*), *S. solidus* size was mostly determined by the geographic origin of the parasite. Stickleback immune gene expression profiles that differed significantly between uninfected fish (implying a host genotype effect) converged upon infection (*Chapter 1 and 3*).

Figure 1. Overview of host and parasite sampling sites of all Chapters. I characterized NO (Lake Skogseidvatnet, Norway) and DE (Lake Großer Plöner See, Germany) as two divergent stickleback populations in *Chapter 1*. NO hosts showed a higher quantitative resistance against *S. solidus* than DE hosts and a well-orchestrated immune response (*Chapter 1 and 2*). NO *S. solidus* – but also *S. solidus* strains from other Atlantic populations (orange) – grew significantly faster, thus have a higher virulence, than *S. solidus* from the Baltic populations (violet). *S. solidus* growth from European Inland populations (green) was intermediate. Pacific *S. solidus* from Vancouver Island, Canada, (pink) infected both NO and DE hosts and caused significantly different immune gene expression than European parasites (*Chapter 1*). Pacific sticklebacks from Walby Lake, Alaska, (ALO) were susceptible to NO *S. solidus*, while the same parasites could not infect sticklebacks from Wolf Lake (ALX) (*Chapter 3*).

Thus, I assume that *S. solidus*-induced phenotypic plasticity, relative to the genetic underpinnings, largely determines immune gene expression profiles of infected fish (emphasising a strong parasite genotype effect). Comparing geographically isolated populations of sticklebacks and *S. solidus*, I tested whether the geographic distance could have an effect on infection outcomes (*Chapter 1 and 3*). Host genotype - parasite genotype interaction effects were weak and differed according to geographic scale. Pacific *S. solidus* could infect European hosts, whereas European *S. solidus* could not infect sticklebacks from one of the two Alaskan populations (ALX) (Figure 1). I hypothesize that this effect is population- rather than clade-specific. My results emphasize that genetic and/or phenotypic heterogeneity affect the outcome of helminth infections. I suggest further experiments to test the molecular basis and the relative importance of phenotypic plasticity in the interaction between *Gasterosteus aculeatus* and *Schistocephalus solidus* from various populations.

Host and parasite effects on epidemiological traits: immunological heterogeneity and geographic patterns

Immune systems evolve in ecological contexts and in reciprocity with parasites. Since defence is costly, it might be selected against in the absence of parasites (Sheldon and Verhulst, 1996; Boots and Haraguchi, 1999; Duncan et al., 2011). It has also been shown that trade-offs between resistance and other fitness-related traits can increase genetic divergence between populations with different exposure to parasite-mediated selection (Hasu et al., 2009; Duncan et al., 2011; Auld et al., 2013). In line with those assumptions, stickleback resistance can, at least partly, be explained by population differences of immunocompetence correlating with abundance of the respective parasite species (de Roij et al., 2011; Kalbe and Kurtz, 2006; Eizaguirre et al., 2012a; Kalbe et al., 2016).

I found in *Chapter 1* that DE sticklebacks had higher baseline immunological activation than sticklebacks from NO, which indicates constitutive differences between the populations (host genotype effect). One possible explanation is that DE sticklebacks evolved under high parasite diversity, which might favour the evolution of a constantly activated broad-spectrum, generalist type of defence (Kassen, 2002; Duncan et al., 2011; Betts et al., 2018). We expected that the low *S. solidus* prevalence in DE results from low exposure risk instead of high resistance against *S. solidus* and hypothesized that DE sticklebacks would actually be less resistant against *S. solidus* than the highly infected NO population. All tested *S. solidus* strains grew larger in DE hosts. Thus, DE sticklebacks may be inherently more susceptible to *S. solidus* infection, which is in line with the *inherent-susceptibility hypothesis* that is used to characterize hosts that are more susceptible to sympatric and allopatric parasites (Lively, 1999; King et al., 2009). In contrast, NO hosts, coming from a population with low parasite diversity and high *S. solidus* prevalence, showed a higher quantitative resistance and a well-orchestrated immune response, which was absent in DE hosts (*Chapter 1 and 2*). On another note, I cannot exclude that NO sticklebacks and/or Atlantic parasites are better adapted to our laboratory conditions, which might be why they generally performed better than other strains.

S. solidus from NO grew generally faster and, once they reached a weight that corresponds to the ability to infect the definite host and reproduce successfully (Tierney and Crompton, 1992; Hammerschmidt and Kurtz, 2009), caused an increase of co-infection probability of another helminth species, the eye fluke *Diplostomum pseudospathaceum* in both DE and NO hosts (*Chapter 2*). These results emphasize a strong parasite genotype effect. The fast growth of NO *S. solidus* is consistent with the theoretical prediction of increased parasite growth if hosts reduce but do not prevent parasite infection and growth (Gandon and Michalakis, 2000). Increased virulence would also be expected under competition among parasites in multiple infections (Schulenburg et al., 2009). Accordingly, naturally high prevalence could cause increased competition and thus faster growth, while naturally low prevalence could be linked to low co-infection probability, reduced intra-specific competition, and slow growth. Effects of intra- and inter-specific competition should be tested with controlled multiple infections and experiments involving different co-infecting parasites. Due to the costs of immune defence, selection for *S. solidus* resistance is expected to correlate with *S. solidus* prevalence. The higher prevalence of *S. solidus* in the Norwegian (NO) population compared to the German (DE) population could thus select for higher quantitative host resistance and parasite growth, i.e. virulence, in NO compared to DE. NO host resistance and parasite growth indicate directional selection (co-evolutionary arms race dynamics selecting for increased resistance and virulence), while sticklebacks and *S. solidus* from the German populations potentially evolved under de-escalated arms race dynamics (Brockhurst and Koskella, 2013; Kalbe et al., 2016).

I studied infection phenotypes of diverse *S. solidus* strains in combination with divergent host types on different geographic scales (*Chapter 1 and 3*). This approach informs about host and parasite life-history traits and potential effects of (co-evolutionary) histories and phylogeny, including evolutionary constraints.

The almost identical geographic pattern of *S. solidus* growth in NO and DE hosts and strain-specific gene expression responses (*Chapter 1 and 2*) clearly show that *S. solidus* growth and host gene expression are also parasite population (or even clade)-specific traits, again indicating a dominant parasite main effect. The four geographic areas (clustered according to *S. solidus* growth: Atlantic, Baltic, European Inland, Pacific) corresponded to *G. aculeatus* phylogeny (Fang et al., 2018). It was hypothesized that *S. solidus* phylogeny resembles that of its highly specific stickleback host. Nevertheless, an extensive investigation is still pending (Samonte et al., *unpublished data*). The phenotypic and/or genetic structuring of the parasite indicates limited gene flow, even though one could expect the opposite since *S. solidus'* definitive avian hosts have high movement potential. However, significant *S. solidus* population differentiation (and isolation-by-distance) has been reported on a small geographic scale among Alaskan lakes (Sprehn et al., 2015; Strobel et al., 2016). Moreover, different strengths of selection in each population, random genetic drift, extinction and recolonization events, as well as mutations influence the spatial distributions of alleles and traits (Gomulkiewicz et al., 2007). Thus, the geographic pattern of *S. solidus* growth and the potentially underlying evolutionary constraints warrant further investigations that focus on *S. solidus* genetics.

In order to test whether *S. solidus* (clade-specific) growth capacity is indeed a genetic and heritable trait, I suggest to run infection experiments over multiple generations. As it has already been shown that virulence of one Atlantic (NO) and one Baltic (NST) *S. solidus* population is inherited (Ritter et al. (2017), genomics and quantitative trait locus (QTL) mapping to identify the genomic locations are promising approaches. Since Baltic *S. solidus* grew significantly slower than worms from other populations and clustered localities, I assume that *S. solidus* strains intrinsically grow differently (i.e. follow different life history strategies). I also speculate that *S. solidus* from the Baltic region might reach sexual competence at lower weights than commonly assumed (*S. solidus* < 50 mg can principally reproduce; Clarke, 1954), but this remains to be investigated. It also needs to be determined why Atlantic *S. solidus* grow characteristically fast; especially since growth was not linked to latitude (*Chapter 1*).

Studying the underlying genetic specificity is key to understanding the interaction between *S. solidus* and *G. aculeatus* and their antagonistic adaptations. Future studies should incorporate various stickleback populations and *S. solidus* strains from the different geographic clusters. If we are to test for environment-, population- and/or cluster-specific local adaptation in the system, we need to compare more than two demes (Kawecki and Ebert, 2004). I would also like to emphasize that correlated traits between interacting species are not necessarily the product of co-evolution and reciprocal selection does not necessarily lead to well-matched or significantly correlated traits (Janzen, 1980; Thompson, 1994, 2005; Nuismer et al., 2010).

Accordingly, the *geographic pattern of virulence* I detected in *Chapter 1* and the specificities of immune responses in *Chapter 2* could reflect geographic variation in natural selection and host-parasite adaptations, but my results are not necessarily indicative for local adaptation or the *Geographic Mosaic Theory of Coevolution* (GMTC) (Thompson, 1994, 1999a). The GMTC predicts spatially structured mosaics of traits as a consequence from different selection and geographic remixing between subpopulations (Lively, 1999; Thompson and Cunningham, 2002; Gomulkiewicz et al., 2007). It has been argued that strong empirical tests of the predicted co-evolutionary hot spots (regions in which reciprocal selection occurs) and cold spots (regions where fitness of at least one species is unaffected by the other), selection mosaics (spatial variation in interspecific fitness functions) and trait remixing are needed to distinguish a geographic mosaic from alternative underlying processes, such as one-sided evolution or drift effects (Gomulkiewicz et al., 2007). I suggest that future studies are warranted to test (i) whether fitness of both species from different localities (populations and clades) is affected by traits mediating an interaction, (ii) if fitness functions differ across these localities, and (iii) to characterize the molecular mechanisms. Importantly, evolution can favour different trait combinations with the same functional output (known as many-to-one mapping) (Thompson et al., 2017; Bolnick et al., 2018). Thus, detailed mechanistic studies of multiple stickleback - *S. solidus* pairs are essential.

My results suggest that a combination of host and parasite genotype effects underlie the infection phenotypes of *S. solidus* and *G. aculeatus*. Interaction effects were weak at regional scales (within continents; *Chapter 1 and 2*) but apparent between continents (*Chapter 1 and 3*). Host and parasite effects further depended on the trait of interest (qualitative resistance versus quantitative resistance). Genotype- and trait-specific strength of interaction effects have also been reported for other host-parasite systems (Grech et al., 2006; Wolinska and King, 2009). However, most host-parasite associations cannot be tested on various geographic scales and studies on vertebrate hosts and their parasites are often confined to few individuals. By revealing specificities according to genotype and epidemiological trait in helminth infections of vertebrate hosts, I conclude that the results of this thesis are of importance for the broader fields of medical and host-parasite research.

Phenotypic plasticity and genetic adaptation

It is impossible to fully disentangle the host and the parasite effects on *S. solidus* growth because the parasite's size is a measure of virulence (as well as parasite fitness and transmission potential) and host quantitative resistance (Arme & Owen, 1967; Tierney and Crompton, 1992; Wedekind et al., 1998; Lüscher and Wedekind, 2002; Heins and Baker, 2003; Bagamian et al., 2004; Heins, 2012; Weber et al., 2017). Moreover, in addition to the genetic determination, my results also demonstrate that each species' phenotypic plasticity in response to different host and parasite types needs to be considered.

Phenotypic plasticity is, in a strict sense, defined as intra-individual variation, which includes the influence of the genome and the environment (West-Eberhard, 2003). In the sense of *environmental responsiveness*, phenotypic plasticity is commonly defined as the ability of a single genotype to express different phenotypes in response to environmental cues (reaction norm). In the case of species interactions, each species constitutes the environment to which the other species may respond (interaction norms) (Thompson, 1988; Agrawal, 2001). Parasites are by definition tightly linked to their hosts that form the parasite's environment of at least one developmental stage.

In this regard, developmental plasticity (i.e. irreversible phenotypes are induced during development; Schneider and Meyer, 2017) is crucial for *S. solidus* because over-exploitation might ultimately decrease transmission probability (Parker et al., 2003; Michaud et al., 2006). Life history theory further predicts that growth retardation could pay off upon higher investment in maintenance and repair mechanisms, which is especially important when the host possesses high quantitative resistance. *S. solidus* growth covaries with the growth of its host, which might partly be attributed to the feeding regime or nutrition status (i.e. available resources), and to the number of conspecifics (at least in the copepod) (Barber, 2005; Michaud et al., 2006; Barber et al., 2008). Disentangling the effects of *G. aculeatus* and *S. solidus* on phenotypic plasticity could be possible if distinct factors, such as space, resource availability, or host immune responses, were manipulated separately. One option would be the establishment of an *in vitro* system to replace the stickleback host to manipulate space or resource availability. If we identified candidate genes conferring plastic responses in *S. solidus* infections of sticklebacks, another option would be to use CRISPR-Cas for specific genetic modifications of sticklebacks and/or *S. solidus*. Analyses of the molecular mechanisms could be used to identify host and parasite genetics and transcriptional networks that are involved in *S. solidus* growth adjustment and/or limitation. Plastic responses can be adaptive or non-adaptive, which is determined in relation to the *optimal value* and the relative fitness in the respective environment (Ghalambor et al., 2007). Thus, testing for adaptive versus non-adaptive plasticity relies on fitness measurements (or at least measurements of suites of fitness related traits; Ghalambor et al., 2007) of both the host and the parasite.

Sticklebacks are known for their high level of phenotypic plasticity. For instance, the stickleback's plasticity has been shown to contribute to parallelism in morphological response to different salinities, freshwater colonization and lake-stream divergence (Morris et al., 2014; Mazzarella et al., 2015; Oke et al., 2016). Transplant experiments have revealed that candidate immune genes of sticklebacks are more strongly regulated by environmentally-mediated phenotypic plasticity than by genetic adaptations (Stutz et al., 2015).

This effect could indicate adaptive plasticity; however, the authors neither determined parasite loads, nor fitness. My thesis confirms the relative importance of induced plasticity by demonstrating that gene expression profiles that differed between uninfected fish from different populations mostly converged upon infection. In other words, the parasite-induced phenotypic plasticity transcends host genetic effects in *S. solidus – G. aculeatus* associations. Importantly, data from *Chapter 2* demonstrate that the gene expression in response to *S. solidus* infection depends on parasite strain and time and/or development. In summary, these results indicate that the parasite genotype and phenotype mostly determine the infection outcome in this helminth-host association. However, since I could not measure host and parasite fitness, I cannot conclude whether this effect is adaptive for the host and/or the parasite or not.

One caveat of our experiments is that I used F1 offspring of laboratory-bred wild-caught hosts and parasites, so non-genetic parental effects in response to the environment in the wild and/or the laboratory could have influenced our results. Experiments over multiple generations could be used to determine short-term acclimatization effects and long-term adaptations. While common garden experiments are needed to exclude the effect of environmental variation between treatments, we also need to consider the developmental status of the stickleback. I found that condition and immunological parameters differed significantly between nine-month-old DE and NO controls (*Chapter 1*), whereas I did not detect these differences in adult fish (one and a half years old) from the same populations (*Chapter 2*). I suggest that age-specific immunological activation and body condition as well as differences between fish families explain this effect. Different costs of immune function in relation to age have been described for various systems and parasite infections have been linked to stickleback age in a natural system (Pennycuick, 1971; Wolinska and King, 2009). However, one study on wild-caught sticklebacks reported that genome-wide and immune gene expression are better explained by season than by age (Brown et al., 2016). I am not aware of any experiment explicitly testing the assumption of a link between stickleback age and immunity. Further, in order to draw valid conclusions, various fish families from replicate populations would have to be tested.

We used several fish families as representatives for each population because natural selection acts on the population level and we aimed to draw ecologically relevant conclusions. I accounted for the variance that would be explained by family effects by using fish family as a random effect in the (generalized) linear mixed effect models. The variance terms differed, which indicates variation among fish families. Possible explanations for family effects involve sequence diversity of genes from the major histocompatibility complex (MHC). MHC gene diversity has been shown to influence the stickleback - *S. solidus* interaction (Kurtz et al., 2004) and MHC genotyping could be a starting point in order to study stickleback family effects in more detail.

To conclude, phenotypic plasticity has been shown to be of central importance for host-parasite infection dynamics, but the relative importance in comparison to genotype effects is still unclear. This thesis adds on to our understanding by revealing that the parasite-induced phenotypic plasticity transcends host genetic effects in *S. solidus – G. aculeatus* associations. Future studies should determine whether and, if so, which genetic and/or epigenetic mechanisms of the host and the parasite control the parasite's growth and other epidemiological traits.

Community context

Laboratory experiments may fail to capture important environmental effects of the natural system. Indeed, laboratory artefacts have been reported for *S. solidus* infections of sticklebacks and attributed to the benign conditions in the laboratory, such as absence of predation, social interactions, fluctuating environments and resource limitation (Candolin and Voigt, 2001). It has increasingly been recognized that community frameworks are important in order to understand interspecific interactions (Graham, 2008; Telfer et al., 2010; Brockhurst et al., 2014; Betts et al., 2016). Especially helminth parasites can directly or indirectly alter their host's susceptibility to macroparasites and microbes and *vice versa* (Lello et al., 2004; Graham, 2008; Broadhurst et al., 2012; Pedersen and Antonovics, 2013; Reynolds et al., 2015; Giacomin et al., 2015; Gause and Maizels, 2016; Benesh and Kalbe, 2016).

We tested the influenceof *S. solidus* immune modulation on co-infection probability of another helminth parasite, the eye fluke *Diplostomum pseudospathaceum* (*Chapter 2*), and showed that chronic *S. solidus* infection can have systemic effects. Testing one co-infecting species is, of course, just a first step towards a community ecology perspective. Importantly, it has been shown that the outcome on co-infection probabilities can be species-specific and probably depends on the number, type and species of all co-infecting parasites (Benesh and Kalbe, 2016). Co-infecting parasites can cooperate or compete, alter the degree of co-adaptation and virulence, and may have context-dependent beneficial or detrimental effects on each other and on their hosts (Telfer et al., 2010; Betts et al., 2016). Enclosures or mesocosm experiments with diverse parasite communities would be appropriate for a thorough investigation of the ecological context. The same experimental approach could be used to test the idea of a generalist defence strategy of DE sticklebacks versus a specialist defence strategy of NO sticklebacks.

We need to acknowledge that the community context includes antagonists and symbionts. The gut community is a prominent example of an interplay between pathogens, commensals, and beneficial microbes. Future studies should incorporate hologenome concepts (encompassing the host genome, its organelles' genomes, and its microbiome) and host holobiont – parasite holobiont interactions (Dheilly, 2014; Bordenstein and Theis, 2015; Theis et al., 2016). Studies on diversity, ecology and evolution of stickleback- and *Schistocephalus*-associated microbes are promising to refine our picture of this host-parasite model system. Samples from Chapter 3 shall thus be used to identify and characterize stickleback- and *S. solidus*-associated microbes and to determine host and parasite genotype effects on microbial compositions (conducted by M. Hahn and N. Dheilly).

Sticklebacks have recently been established as model organisms for host-microbe interactions (Bolnick et al., 2014a, 2014b; Smith et al., 2015; Milligan-Myhre et al., 2016). Interestingly, wild-type stickleback MHC class II polymorphism has been linked to the composition and diversity of gut microbiota (Bolnick et al., 2014).

The authors found that microbial diversity decreased with MHC diversity and speculated that macroparasite-driven selection on MHC genes could alter the host's microbiota, and *vice versa*. I suggest to test this assumption with samples from our controlled infection experiments.

Cestode microbiomes have rarely be investigated (but see Izvekova, 2005; Poddubnaya and Izvekova, 2005; Korneva, 2008; Korneva and Plotnikov, 2012). However, signs of bacterial infestation within the eggs of lab-bred *S. solidus* have been reported (P. Jakobsen, M. Kalbe, T. Henrich, P. Rausch; pers. comm.). The bacterial abundance increased over time, suggesting that these bacteria might play a role in the cestode's development. 454 sequencing of lab-bred *S. solidus* plerocercoids from wild-caught NO sticklebacks indicated highest sequence similarities with the genus *Ohtaekwangia* (class *Bacteroidetes*). We screened adult worms and eggs as well as fish tissues for presence of bacteria of the *Bacteroidetes* phylum. I supported the hypothesized presence of *Ohtaekwangia*-like bacteria in *S. solidus* eggs with *Bacteriodetes*-specific PCR primers and sequence analysis of the ~600 bp fragment. Bacteria from adult cestodes and stickleback organs clustered into other clades. Parasites from different geographic locations, populations, families, individuals, and another *Schistocephalus* species (*Schistocephalus pungitii* that specifically infects nine-spined sticklebacks, *Pungitius pungitius*) were sampled and could add novel aspects to the specificities of helminth-host interactions (*unpublished data*).

Specificities of the host-helminth interplay

The data of all three Chapters indicate that qualitative resistance (*S. solidus* infection success) is not necessarily linked to quantitative resistance (*S. solidus* growth) in sticklebacks. This has been proposed previously as, for example, MHC diversity negatively correlates with *S. solidus* parasite index but seems to have no influence on infection success (Kurtz et al., 2004). I found in *Chapter 3* that sticklebacks from an Alaskan population (ALX) were both more resistant (by means of qualitative resistance) and more tolerant, indicating that the two defence mechanisms are not mutually exclusive. This could be counter-intuitive because resistance and tolerance have different effects on host-parasite ecology and co-evolution. However, one particular cost of resistance is that overproduction of defence molecules can have immunopathogenic effects (Graham et al., 2005). In this regard, virulence has been defined to result from both infection-induced immunopathology and direct effects of the parasite (Long and Boots, 2001).

Helminth infections are special as these parasites are exceptional immune modulators. Helminth infections can protect from immune dysregulatory diseases such as autoimmune disorders and allergy (McSorley et al., 2013). Treating human patients with helminths, their eggs, or products (known as helminth therapy) is already applied in clinical settings (Summers et al., 2005, 2005b; Croese et al., 2006; Liu et al., 2009a, 2009b; Bager et al., 2010; Bourke et al., 2012; Maizels and McSorley, 2016; Smallwood et al., 2017). In line with (co-)evolutionary thinking, it has been suggested that only certain helminth species could have beneficial effects in helminth therapies (Leonardi-Bee et al., 2006; Cooper, 2009; Helmby, 2015). Following up on the question of how specific helminth immune modulatory effects could be, we used different types (or 'strains') of the same host and parasite species in controlled experimental infections. Indeed, my colleagues and I could demonstrate that those hosts that co-evolved with a high growth parasite (NO) up-regulated T regulatory genes in concordance with pro-inflammatory activities if infected with their sympatric parasite (and DE sticklebacks did not). This effect became apparent when the high growth (NO) *S. solidus* strain became infective to the definitive host (*Chapter 2*).

I assume that the T regulatory response at this stage of infection is beneficial for the host and the parasite by facilitating the persistence of the parasite and preventing immunopathological responses (tolerance). Our results thus indicate that different strains of the same helminth species can have profoundly different effects on their hosts and that these effects are further host type- and time-dependent.

Schistocephalus solidus specifically infects three-spined sticklebacks and experimental transfer to fish species other than *G. aculeatus* causes death of the parasite within 2-10 days (Bråten, 1966; Orr et al., 1969). This extreme host specificity provides an outstanding model to study helminth immune modulation. Sticklebacks are principally able to eliminate *S. solidus* up to 17 days post infection. Several mechanisms by which sticklebacks potentially limit *S. solidus* burden have been proposed: digestive enzymes and immune cells attack the parasite within the first 14 to 24 hours; proliferation of head kidney monocytes increases among exposed sticklebacks after 7 days; adaptive immunity, such as MHC molecules, are suggested to be involved after several weeks; transcriptome data revealed up-regulation of effectors and receptors of innate immunity (e.g. toll-like receptors and macrophage-associated genes) and complement components after 50 days; the respiratory burst peaks from 47-67 days post exposure (Kurtz et al., 2004; Hammerschmidt & Kurtz, 2007; Barber and Scharsack, 2010; Haase et al., 2016; Stewart et al., 2017). Overall, our data confirms this sequence. The trajectories of immunological activation were linked to ontogeny and size of a highly virulent *S. solidus* strain. In the same experiment, infection with a low growth strain (up to ~ 50 mg) neither affected host gene expression profiles nor co-infection probabilities (*Chapter 2*). My results from *Chapter 1 and 3* demonstrate that immunological activation and quantitative resistance (parasite growth) were *S. solidus* strain-specific but not correlated after eight weeks. Mechanisms of tolerance and concomitant immunity may thus be important at late stages of *S. solidus* infection.

Notably, the link between immunological activation and parasite growth is complex: fast growing parasites might cause an elevated immune response; but growing evidence suggests that immunological activation could also favour parasite growth directly through increased uptake of nutrients due to higher influx of cells and immune modulators or indirectly through adjustment of the parasite's life history strategy (Babayan et al., 2010; Kalbe et al., 2016). For instance, the filarial nematode *Litomosoides sigmodontis* adjusts its development and reproduction to the presence of immune cells involved in anti-helminth attack in infected mice and schistosome development depends on signals from the host immune system (Davies et al., 2001; Cheng et al., 2008; Babayan et al., 2010; Lamb et al., 2010; Tang et al., 2013). Accordingly, helminth infections and the host's immune response are double-edged swords that need to be studied in further detail. A mechanistic understanding will be key to assess the costs and benefits of helminth therapies.

In this thesis, I analysed host regulatory and immune gene expression in response to *S. solidus* exposure and infection. It is well known *"that, immunologically, more is not necessarily better"* (Viney et al. 2005), especially in the context of immunopathology. The expression levels of candidate genes are, of course, just a first step to characterize the molecular interplay between sticklebacks and *S. solidus*. In addition to analyses of transcriptomic responses, we should try to understand the underlying gene regulatory mechanisms as it has been shown that gene regulatory networks are essential in rapid responses and evolutionary adaptation (Stern et al., 2007; López-Maury et al., 2008; van Gestel and Weissing, 2018). The idea of modular gene expression patterns also justified the multivariate approach to analyse the gene expression data of this thesis. Future experiments within the scope of genomics, transcriptomics, and proteomics should study sequence variants, localize the respective RNAs and proteins in the organisms (e.g. using *in situ* hybridization or immunohistochemistry), and characterize the function of the respective molecules at the interface of host and parasite.

The International Helminth Genomes Consortium (2018) recently published a comprehensive study of 81 helminth genomes; new potential drug targets and compounds were identified. Notably, a *Schistocephalus solidus* specimen from Neustädter Binnenwasser, Germany, was included. Transcriptome data from experimental infections using *S. solidus* from the same origin have already been analysed (Fahmy et al., *in prep*.) and whole transcriptome data of infected fish from the same study are available (T. Henrich & N. Erin and others). Following up on those investigations, studies on protein interactions could help to identify and characterize the proteins involved in the molecular crosstalk between hosts and helminths. In summary, in addition to distinct defence mechanisms on geographical scales, specificities in the helminth-host interplay occur at different levels of the infection process. The future challenge is to link effector molecules to epidemiological traits, such as resistance, tolerance, infectivity and virulence.

Concluding remarks

It has been claimed that *"we need to expand [basic] research on the practical consequences"* (Bolnick et al., 2018). However, the original idea of basic research is that "*Insight must precede application*" (Max Planck). Accordingly, applied science should incorporate basic research. This has been recognized in evolutionary medicine (Nesse et al., 2010). Focussing on helminth therapy, this thesis is an example of how important basic research, here in the field of host-parasite interaction, is for treatment and prevention strategies in human health. A thorough consideration of how host and parasite genotype and/or phenotype affect immune modulation in vertebrates is essential for both treatment of helminth infections and helminth therapy. Further examples of the necessity of (co-)evolutionary perspectives encompass the evolution of antibiotic resistance, veterinary medicine, agriculture, and conservation implications.

Future studies should focus on host-helminth genetic adaptations, the significance and the mechanisms of plasticity, and the molecular crosstalk. Additional host and parasite populations may be incorporated to further test for local adaptation and the *Geographic Mosaic Theory of Coevolution*, as well as to test the generalisability and the ecological relevance of my results. Future work needs to be backed up with extensive field data to ascertain the relevance in the natural system. Within the context of natural differences between populations, it is actually under debate whether trajectories of host-parasite interactions could be generalised at all (Auld and Brand, 2017; Bolnick et al., 2018). The results of this thesis, such as *S. solidus* strain-specific stickleback gene expression, indicate that individual characteristics of each stickleback – *S. solidus* interaction in their respective habitat influence their interaction. However, the fact that gene expression profiles that differed among uninfected fish mostly converged upon *S. solidus* infection (irrespective of the continent of origin!) suggests a strong relative importance of parasite-induced phenotypic plasticity in the interaction between three-spined sticklebacks *Gasterosteus aculeatus* and their cestode *Schistocephalus solidus*.

The investigation of replicate populations from different habitats (e.g. high and low *S. solidus* prevalence/parasite diversity) and multiple locations would allow to partition the variance of epidemiological traits among habitats, locations, and habitat x location interactions (Bolnick et al., 2018).

Last but not least, experimental co-evolution with appropriate controls and treatments allowing for one-sided adaptation are required (and under way) to test for the co-evolutionary potential in the stickleback-*S. solidus* system. The (co-)evolutionary history is important to understand present-day patterns. However, adaptations could also occur fast (maybe due to phenotypic plasticity), altering our understanding of vertebrate co-evolution with parasites. An experimental approach over multiple generations allows to study short-term and long-term effects in the light of trans-generational plasticity and to distinguish between acclimatization and adaptation. This approach further allows to measure host and parasite fitness (lifetime reproductive success and the reproductive success of subsequent generations). It also needs to be tested whether fitness of both species (from different populations and clades) is indeed affected by traits mediating an interaction.

REFERENCES

Agrawal, A. A. (2001). Phenotypic Plasticity in the Interactions and Evolution of Species. *Science*, *294*(5541), 321–326. https://doi.org/10.1126/science.1060701

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In *Second international symposium on information theory* (pp. 267–281). Retrieved from http://ci.nii.ac.jp/naid/10011153980/

Aken, B. L., Ayling, S., Barrell, D., Clarke, L., Curwen, V., Fairley, S., Hourlier, T. (2016). The Ensembl gene annotation system. *Database*, *2016*.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*(17), 3389–3402.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Anderson, R. M., & May, R. M. (1985). Vaccination and herd immunity to infectious diseases. *Nature*, *318*. Retrieved from https://www.nature.com/articles/318323a0

Anthony, R. M., Rutitzky, L. I., Urban, J. F., Stadecker, M. J., & Gause, W. C. (2007). Protective immune mechanisms in helminth infection. *Nature Reviews Immunology*, *7*(12), 975–987. https://doi.org/10.1038/nri2199

Arme, C., & Owen, R. W. (1967). Infections of the three-spined stickleback, Gasterosteus aculeatus L., with the plerocercoid larvae of Schistocephalus solidus (Müller, 1776), with special reference to pathological effects. *Parasitology*, *57*(02), 301–314. https://doi.org/10.1017/S0031182000072103

Auld, S. K. J. R., Penczykowski, R. M., Housley Ochs, J., Grippi, D. C., Hall, S. R., & Duffy, M. A. (2013). Variation in costs of parasite resistance among natural host populations. *Journal of Evolutionary Biology*, *26*(11), 2479–2486. https://doi.org/10.1111/jeb.12243

Auld, S.K.J.R., and Brand, J. (2017). Environmental variation causes different (co) evolutionary routes to the same adaptive destination across parasite populations. Evol. Lett. *1*, 245–254.

Ayres, J. S., & Schneider, D. S. (2008). A Signaling Protease Required for Melanization in Drosophila Affects Resistance and Tolerance of Infections. *PLoS Biology*, *6*(12), e305. https://doi.org/10.1371/journal.pbio.0060305

Babayan, S.A., Read, A.F., Lawrence, R.A., Bain, O., and Allen, J.E. (2010). Filarial Parasites Develop Faster and Reproduce Earlier in Response to Host Immune Effectors That Determine Filarial Life Expectancy. PLoS Biol *8*, e1000525.

Backiel, T., & Le Cren, E. D. (1978). Some density relationships for fish population parameters. *Ecology of Freshwater Fish Production*, 279–302.

Bagamian, K. H., Heins, D. C., & Baker, J. A. (2004). Body condition and reproductive capacity of three-spined stickleback infected with the cestode Schistocephalus solidus. *Journal of Fish Biology*, *64*(6), 1568–1576. https://doi.org/10.1111/j.0022-1112.2004.00411.x

Bager, P., Arnved, J., Rønborg, S., Wohlfahrt, J., Poulsen, L. K., Westergaard, T., Melbye, M. (2010). Trichuris suis ova therapy for allergic rhinitis: A randomized, double-blind, placebo-controlled clinical trial. *Journal of Allergy and Clinical Immunology*, *125*(1), 123-130.e3. https://doi.org/10.1016/j.jaci.2009.08.006

Barber, I. (2005). Parasites grow larger in faster growing fish hosts. *International Journal for Parasitology*, *35*(2), 137–143. https://doi.org/10.1016/j.ijpara.2004.11.010

Barber, I. (2013). Sticklebacks as model hosts in ecological and evolutionary parasitology. *Trends in Parasitology*, *29*(11), 556–566. https://doi.org/10.1016/j.pt.2013.09.004

Barber, I., & Nettleship, S. (2010). From 'trash fish'to supermodel: the rise and rise of the three-spined stickleback in evolution and ecology. *Biologist*, *57*(1), 15–21.

Barber, I., & Scharsack, J. P. (2010). The three-spined stickleback-Schistocephalus solidus system: an experimental model for investigating host-parasite interactions in fish. *Parasitology*, *137*(3), 411.

Barber, I., Wright, H. A., Arnott, S. A., & Wootton, R. J. (2008). Growth and energetics in the stickleback–*Schistocephalus* host–parasite system: a review of experimental infection studies. *Behaviour*, *145*(4), 647–668. https://doi.org/10.1163/156853908792451403

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models using lme4. *ArXiv:1406.5823 [Stat]*. Retrieved from http://arxiv.org/abs/1406.5823

Bell, M. A., & Foster, S. A. (1994). *The evolutionary biology of the threespine stickleback*. Oxford University Press.

Ben-Ami, F., Regoes, R. R., & Ebert, D. (2008). A quantitative test of the relationship between parasite dose and infection probability across different host–parasite combinations. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1636), 853–859. https://doi.org/10.1098/rspb.2007.1544

Benesh, D. P., & Kalbe, M. (2016). Experimental parasite community ecology: intraspecific variation in a large tapeworm affects community assembly. *Journal of Animal Ecology*, *85*(4), 1004–1013. https://doi.org/10.1111/1365-2656.12527

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289–300.

Best, A., White, A., & Boots, M. (2014). The Coevolutionary Implications of Host Tolerance. *Evolution*, *68*(5), 1426–1435. https://doi.org/10.1111/evo.12368

Betts, A., Gray, C., Zelek, M., MacLean, R. C., & King, K. C. (2018). High parasite diversity accelerates host adaptation and diversification. *Science*, *360*(6391), 907–911. https://doi.org/10.1126/science.aam9974

Betts, A, Rafaluk, C., & King, K. C. (2016). Host and Parasite Evolution in a Tangled Bank. *Trends in Parasitology*, *32*(11), 863–873. https://doi.org/10.1016/j.pt.2016.08.003

Birrer, S. C., Reusch, T. B. H., & Roth, O. (2012). Salinity change impairs pipefish immune defence. *Fish & Shellfish Immunology*, *33*(6), 1238–1248. https://doi.org/10.1016/j.fsi.2012.08.028

Blanford, S., Thomas, M. B., Pugh, C., & Pell, J. K. (2003). Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. *Ecology Letters*, *6*(1), 2–5. https://doi.org/10.1046/j.1461-0248.2003.00387.x

Bolger, T., & Connolly, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology*, *34*(2), 171–182. https://doi.org/10.1111/j.1095-8649.1989.tb03300.x

Bolnick, D.I., Barrett, R.D.H., Oke, K.B., Rennison, D.J., and Stuart, Y.E. (2018). (Non)Parallel Evolution. Annu Rev Ecol Evol Syst *49*, 303–330.

Bolnick, D. I., Snowberg, L. K., Caporaso, J. G., Lauber, C., Knight, R., & Stutz, W. E. (2014). Major Histocompatibility Complex class IIb polymorphism influences gut microbiota composition and diversity. *Molecular Ecology*, *23*(19), 4831–4845. https://doi.org/10.1111/mec.12846

Bolnick, D. I., Snowberg, L. K., Hirsch, P. E., Lauber, C. L., Org, E., Parks, B., … Svanbäck, R. (2014). Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nature Communications*, *5*, 4500. https://doi.org/10.1038/ncomms5500

Boots, M., Best, A., Miller, M. R., & White, A. (2009). The role of ecological feedbacks in the evolution of host defence: what does theory tell us? *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *364*(1513), 27–36. https://doi.org/10.1098/rstb.2008.0160

Boots, M., & Haraguchi, Y. (1999). The Evolution of Costly Resistance in Host-Parasite Systems. *The American Naturalist*, *153*(4), 359–370. https://doi.org/10.1086/303181

Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes. *PLOS Biol*, *13*(8), e1002226. https://doi.org/10.1371/journal.pbio.1002226

Bourke, C. D., Mutapi, F., Nausch, N., Photiou, D. M. F., Poulsen, L. K., Kristensen, B., Bager, P. (2012). Trichuris suis ova therapy for allergic rhinitis does not affect allergen-specific cytokine responses despite a parasite-specific cytokine response. *Clinical & Experimental Allergy*, *42*(11), 1582–1595. https://doi.org/10.1111/j.1365-2222.2012.04063.x

Bråten, T. (1966). Host specificity in Schistocephalus solidus. *Parasitology*, *56*(04), 657–664. https://doi.org/10.1017/S0031182000071687

Broadhurst, M. J., Ardeshir, A., Kanwar, B., Mirpuri, J., Gundra, U. M., Leung, J. M., Loke, P. (2012). Therapeutic Helminth Infection of Macaques with Idiopathic Chronic Diarrhea Alters the Inflammatory Signature and Mucosal Microbiota of the Colon. *PLOS Pathogens*, *8*(11), e1003000. https://doi.org/10.1371/journal.ppat.1003000

Brockhurst, M. A., Chapman, T., King, K. C., Mank, J. E., Paterson, S., & Hurst, G. D. D. (2014). Running with the Red Queen: the role of biotic conflicts in evolution. *Proceedings of the Royal Society of London B: Biological Sciences*, *281*(1797), 20141382. https://doi.org/10.1098/rspb.2014.1382

Brockhurst, M. A., Fenton, A., Roulston, B., & Rainey, P. B. (2006). The impact of phages on interspecific competition in experimental populations of bacteria. *BMC Ecology*, *6*(1), 19. https://doi.org/10.1186/1472-6785-6-19

Brockhurst, M. A., & Koskella, B. (2013). Experimental coevolution of species interactions. *Trends in Ecology & Evolution*, *28*(6), 367–375. https://doi.org/10.1016/j.tree.2013.02.009

Brown, M., Hablützel, P., Friberg, I. M., Thomason, A. G., Stewart, A., Pachebat, J. A., & Jackson, J. A. (2016). Seasonal immunoregulation in a naturally-occurring vertebrate. *BMC Genomics*, *17*, 369. https://doi.org/10.1186/s12864-016-2701-7

Brunner, F. S. (2016). *Parasitism along the speciation continuum - Variation of parasite effects across speciation stages of Gasterosteus aculeatus and their modification by environmental changes*. Queen Mary University of London.

Brunner, F. S., Anaya-Rojas, J. M., Matthews, B., & Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. *Proceedings of the National Academy of Sciences*, 201619147. https://doi.org/10.1073/pnas.1619147114

Brunner, F. S., Schmid-Hempel, P., & Barribeau, S. M. (2014). Protein-poor diet reduces host-specific immune gene expression in Bombus terrestris. *Proc. R. Soc. B*, *281*(1786), 20140128. https://doi.org/10.1098/rspb.2014.0128

Buckling, A., & Rainey, P. B. (2002). Antagonistic coevolution between a bacterium and a bacteriophage. *Proceedings. Biological Sciences / The Royal Society*, *269*(1494), 931–936. https://doi.org/10.1098/rspb.2001.1945

Bull, J. J. (1994). Perspective: Virulence. *Evolution*, *48*(5), 1423. https://doi.org/10.2307/ 2410237

Burdon, J. J., & Jarosz, A. M. (1991). Host-Pathogen Interactions in Natural Populations of Linum marginale and Melampsora lini: I. Patterns of Resistance and Racial Variation in a Large Host Population. *Evolution*, *45*(1), 205–217. https://doi.org/10.2307/2409494

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. *The Journal of Parasitology*, *83*(4), 575–583. https://doi.org/10.2307/3284227

Calandra, T., & Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature Reviews Immunology*, *3*(10), 791–800. https://doi.org/1 0.1038/nri1200

Candolin, U., and Voigt, H.-R. (2001). No effect of a parasite on reproduction in stickleback males: a laboratory artefact? Parasitology *122*, 457–464.

Carius, H. J., Little, T. J., & Ebert, D. (2001). Genetic Variation in a Host-Parasite Association: Potential for Coevolution and Frequency-Dependent Selection. *Evolution*, *55*(6), 1136–1145. https://doi.org/10.1111/j.0014-3820.2001.tb00633.x

Carroll, M. C. (2004). The complement system in regulation of adaptive immunity. *Nature Immunology*, *5*(10), 981–986. https://doi.org/10.1038/ni1113

Chappell, L. H., Hardie, L. J., & Secombes, C. J. (1994). Diplostomiasis: the disease and host-parasite interactions. *Parasitic Diseases of Fish*, 59–86.

Chellappa, S., Huntingford, F. A., Strang, R. H. C., & Thomson, R. Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. *Journal of Fish Biology*, *47*(5), 775–787. https://doi.org/10.1111/j.1095-8649.1995.tb06002.x

Cheng, Y., Song, W., Liu, W., Lei, J., Mo, H., Ruppel, A., and Li, Y. (2008). The effects of T cell deficiency on the development of worms and granuloma formation in mice infected with Schistosoma japonicum. Parasitol. Res. *102*, 1129–1134.

Clarke, A. S. (1954). Studies on the life cycle of the pseudophyllidean cestode Schistocephalus solidus. *Proceedings of the Zoological Society of London*, *124*(2), 257–302. https://doi.org/10.1111/j.1469-7998.1954.tb07782.x

Colosimo, P. F., Hosemann, K. E., Balabhadra, S., Villarreal, G., Dickson, M., Grimwood, J., … Kingsley, D. M. (2005). Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. *Science*, *307*(5717), 1928–1933. https://doi.org/10.1126/science.1107239

Cooper, P.J. (2009). Interactions between helminth parasites and allergy. Curr. Opin. Allergy Clin. Immunol. *9*, 29–37.

Croese, J., O'Neil, J., Masson, J., Cooke, S., Melrose, W., Pritchard, D., & Speare, R. (2006). A proof of concept study establishing Necator americanus in Crohn's patients and reservoir donors. *Gut*, *55*(1), 136–137. https://doi.org/10.1136/gut.2005.079129

Cumnock, K., Gupta, A. S., Lissner, M., Chevee, V., Davis, N. M., & Schneider, D. S. (2018). Host Energy Source Is Important for Disease Tolerance to Malaria. *Current Biology*, *28*(10), 1635-1642.e3. https://doi.org/10.1016/j.cub.2018.04.009

Dargent, F., Scott, M. E., Hendry, A. P., & Fussmann, G. F. (2013). Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1773), 20132371. https://doi.org/10.1098/rspb.2013.2371

Davies, S.J., Grogan, J.L., Blank, R.B., Lim, K.C., Locksley, R.M., and McKerrow, J.H. (2001). Modulation of Blood Fluke Development in the Liver by Hepatic CD4+ Lymphocytes. Science *294*, 1358–1361.

de Roij, J., Harris, P. D., & MacColl, A. D. C. (2011). Divergent resistance to a monogenean flatworm among three-spined stickleback populations. *Functional Ecology*, *25*(1), 217–226. https://doi.org/10.1111/j.1365-2435.2010.01775.x

D'haeseleer, P. (2005). How does gene expression clustering work? *Nature Biotechnology*, *23*(12), 1499–1502.

Dheilly, N. M. (2014). Holobiont–Holobiont Interactions: Redefining Host–Parasite Interactions. *PLoS Pathog*, *10*(7), e1004093. https://doi.org/10.1371/journal.ppat. 1004093

Dubinina, M. N. (1980). Tapeworms (Cestoda, Ligulidae) of the fauna of the USSR., vii + 320 pp.

Duffy, M. A., & Forde, S. E. (2009). Ecological feedbacks and the evolution of resistance. *Journal of Animal Ecology*, *78*(6), 1106–1112. https://doi.org/10.1111/j.1365-2656.2009.01568.x

Duncan, A. B., Fellous, S., & Kaltz, O. (2011). Reverse Evolution: Selection Against Costly Resistance in Disease-Free Microcosm Populations of Paramecium Caudatum. *Evolution*, *65*(12), 3462–3474. https://doi.org/10.1111/j.1558-5646.2011.01388.x

Duneau, D., Luijckx, P., Ben-Ami, F., Laforsch, C., & Ebert, D. (2011). Resolving the infection process reveals striking differences in the contribution of environment, genetics and phylogeny to host-parasite interactions. *BMC Biology*, *9*(1), 11. https://doi.org/10.1186/1741-7007-9-11

Dybdahl, M. F., Jenkins, C. E., & Nuismer, S. L. (2014). Identifying the Molecular Basis of Host-Parasite Coevolution: Merging Models and Mechanisms. *The American Naturalist*, *184*(1), 1–13. https://doi.org/10.1086/676591

Ebert, D., & Hamilton, W. D. (1996). Sex against virulence: the coevolution of parasitic diseases. *Trends in Ecology & Evolution*, *11*(2), 79–82. https://doi.org/10.1016/0169-5347(96)81047-0

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012a). Divergent selection on locally adapted major histocompatibility complex immune genes experimentally proven in the field. *Ecology Letters*, *15*(7), 723–731. https://doi.org/10.1111/j.1461-0248.2012.01791.x

Eizaguirre, C., Lenz, T. L., Kalbe, M., & Milinski, M. (2012b). Rapid and adaptive evolution of MHC genes under parasite selection in experimental vertebrate populations. *Nature Communications*, *3*, 621. https://doi.org/10.1038/ncomms1632

Eizaguirre, C., Lenz, T. L., Sommerfeld, R. D., Harrod, C., Kalbe, M., & Milinski, M. (2011). Parasite diversity, patterns of MHC II variation and olfactory based mate choice in diverging three-spined stickleback ecotypes. *Evolutionary Ecology*, *25*(3), 605–622. https://doi.org/10.1007/s10682-010-9424-z

Faltýnková, A., Nasincová, V., & Kablásková, L. (2007). Larval trematodes (Digenea) of the great pond snail, Lymnaea stagnalis (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. *Parasite (Paris, France)*, *14*(1), 39–51.

Fang, B., Merilä, J., Ribeiro, F., Alexandre, C. M., & Momigliano, P. (2018). Worldwide phylogeny of three-spined sticklebacks. *Molecular Phylogenetics and Evolution*. https://doi.org/10.1016/j.ympev.2018.06.008

Fellowes, M. D. E., Kraaijeveld, A. R., & Godfray, H. C. J. (1998). Trade–off associated with selection for increased ability to resist parasitoid attack in Drosophila melanogaster. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1405), 1553–1558. https://doi.org/10.1098/rspb.1998.0471

Feulner, P. G. D., Chain, F. J. J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T. L., … Bornberg-Bauer, E. (2013). Genome-wide patterns of standing genetic variation in a marine population of three-spined sticklebacks. *Molecular Ecology*, *22*(3), 635–649. https://doi.org/10.1111/j.1365-294X.2012.05680.x

Feulner, P. G. D., Chain, F. J. J., Panchal, M., Huang, Y., Eizaguirre, C., Kalbe, M., … Milinski, M. (2015). Genomics of Divergence along a Continuum of Parapatric Population Differentiation. *PLOS Genetics*, *11*(2), e1004966. https://doi.org/10.1371/journal.pgen.1004966

Fischer, U., Koppang, E. O., & Nakanishi, T. (2013). Teleost T and NK cell immunity. *Fish & Shellfish Immunology*, *35*(2), 197–206. https://doi.org/10.1016/j.fsi.2013.04.018

Forde, S. E., Beardmore, R. E., Gudelj, I., Arkin, S. S., Thompson, J. N., & Hurst, L. D. (2008). Understanding the limits to generalizability of experimental evolutionary models. *Nature*, *455*(7210), 220–223. https://doi.org/10.1038/nature07152

Fox, J, & Weisberg, S. (2011). An {R} Companion to Applied Regression. *Second Edition. Thousand Oaks CA: Sage*. Retrieved from http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Franke, F., Rahn, A. K., Dittmar, J., Erin, N., Rieger, J. K., Haase, D., Scharsack, J. P. (2014). In vitro leukocyte response of three-spined sticklebacks (Gasterosteus aculeatus) to helminth parasite antigens. *Fish & Shellfish Immunology*, *36*(1), 130–140. https://doi.org/10.1016/j.fsi.2013.10.019

Frischknecht, M. (1993). The breeding colouration of male three-spined sticklebacks (Gasterosteus aculeatus) as an indicator of energy investment in vigour. *Evolutionary Ecology*, *7*(5), 439–450. https://doi.org/10.1007/BF01237640

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature*, *399*(6736), 541–548.

Gandon, S., & Michalakis, Y. (2000). Evolution of parasite virulence against qualitative or quantitative host resistance. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1447), 985–990. https://doi.org/10.1098/rspb.2000.1100

Gaujoux, R., & Seoighe, C. (2010). A flexible R package for nonnegative matrix factorization. *BMC Bioinformatics*, *11*(1), 367.

Gause, W. C., & Maizels, R. M. (2016). Macrobiota — helminths as active participants and partners of the microbiota in host intestinal homeostasis. *Current Opinion in Microbiology*, *32*, 14–18. https://doi.org/10.1016/j.mib.2016.04.004

Giacomin, P., Croese, J., Krause, L., Loukas, A., & Cantacessi, C. (2015). Suppression of inflammation by helminths: a role for the gut microbiota? *Phil. Trans. R. Soc. B*, *370*(1675), 20140296. https://doi.org/10.1098/rstb.2014.0296

Gibson, G. (2005). The synthesis and evolution of a supermodel. *Science*, *307*(1890), e1891.

Gomulkiewicz, R., Drown, D. M., Dybdahl, M. F., Godsoe, W., Nuismer, S. L., Pepin, K. M., Ridenhour B. J., Smith, C. I., Yoder, J. B. (2007). Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity*, *98*(5), 249–258. https://doi.org/10.1038/sj.hdy.6800949

Gomulkiewicz, R., Thompson, J. N., Holt, R. D., Nuismer, S. L., Hochberg, M. E., & McCauley, A. E. D. E. (2000). Hot Spots, Cold Spots, and the Geographic Mosaic Theory of Coevolution. *The American Naturalist*, *156*(2), 156–174. https://doi.org/ 10.1086/303382

Graham, A. L. (2013). Optimal immunity meets natural variation: the evolutionary biology of host defence. *Parasite Immunology*, *35*(11), 315–317. https://doi.org/10.1111/ pim.12073

Graham, A. L. (2008). Ecological rules governing helminth–microparasite coinfection. *Proceedings of the National Academy of Sciences*, *105*(2), 566–570. https://doi.org/10.1073/pnas.0707221105

Graham, A. L., Allen, J. E., & Read, A. F. (2005). Evolutionary Causes and Consequences of Immunopathology. *Annual Review of Ecology, Evolution, and Systematics*, *36*(1), 373–397. https://doi.org/10.1146/annurev.ecolsys.36.102003.152622

Grech, K., Watt, K., & Read, A. F. (2006). Host–parasite interactions for virulence and resistance in a malaria model system. *Journal of Evolutionary Biology*, *19*(5), 1620–1630. https://doi.org/10.1111/j.1420-9101.2006.01116.x

Haase, D., Rieger, J. K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., & Reusch, T. B. H. (2014). Specific Gene Expression Responses to Parasite Genotypes Reveal Redundancy of Innate Immunity in Vertebrates. *PLoS ONE*, *9*(9), e108001. https://doi.org/10.1371/journal.pone.0108001

Haase, D., Rieger, J. K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., … Reusch, T. B. H. (2016). Comparative transcriptomics of stickleback immune gene responses upon infection by two helminth parasites, Diplostomum pseudospathaceum and Schistocephalus solidus. *Zoology*, *119*(4), 307–313. https://doi.org/10.1016/ j.zool.2016.05.005

Hamilton, W D, Axelrod, R., & Tanese, R. (1990). Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences of the United States of America*, *87*(9), 3566–3573.

Hamilton, W. D. (1980). Sex versus Non-Sex versus Parasite. *Oikos*, *35*(2), 282–290. https://doi.org/10.2307/3544435

Hammerschmidt, K., & Kurtz, J. (2005). Surface carbohydrate composition of a tapeworm in its consecutive intermediate hosts: Individual variation and fitness consequences. *International Journal for Parasitology*, *35*(14), 1499–1507. https://doi.org/10.1016/ j.ijpara.2005.08.011

Hammerschmidt, K., & Kurtz, J. (2007). Schistocephalus solidus: Establishment of tapeworms in sticklebacks – fast food or fast lane? *Experimental Parasitology*, *116*(2), 142–149. https://doi.org/10.1016/j.exppara.2006.12.013

Hammerschmidt, K., & Kurtz, J. (2009). Chapter 5 Ecological Immunology of a Tapeworms' Interaction with its Two Consecutive Hosts. In Joanne P. Webster (Ed.), *Advances in Parasitology* (Vol. Volume 68, pp. 111–137). Academic Press. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065308X08006052

Hansen, M. H. H., & Koella, J. C. (2003). Evolution of tolerance: the genetic basis of a host's resistance against parasite manipulation. *Oikos*, *102*(2), 309–317. https://doi.org/10.1034/j.1600-0706.2003.12537.x

Hasu, T., Benesh, D. P., & Valtonen, E. T. (2009). Differences in parasite susceptibility and costs of resistance between naturally exposed and unexposed host populations. *Journal of Evolutionary Biology*, *22*(4), 699–707. https://doi.org/10.1111/j.1420-9101.2009.01704.x

Heath, D. D., Holcman, B., & Shaw, R. J. (1994). Echinococcus granulosus: the mechanism of oncosphere lysis by sheep complement and antibody. *International Journal for Parasitology*, *24*(7), 929–935. https://doi.org/10.1016/0020-7519(94)90156-2

Heins, D. C., Eidam, D. M., & Baker, J. A. (2015). Timing of Infections in the Threespine Stickleback (Gasterosteus aculeatus) by Schistocephalus solidus in Alaska. *Journal of Parasitology*, *102*(2), 286–289. https://doi.org/10.1645/14-675

Heins, D. C. (2012). Fecundity compensation in the three-spined stickleback Gasterosteus aculeatus infected by the diphyllobothriidean cestode Schistocephalus solidus. *Biological Journal of the Linnean Society*, *106*(4), 807–819. https://doi.org/10.1111/j.1095-8312.2012.01907.x

Heins, D. C., & Baker, J. A. (2003). Reduction of egg size in natural populations of threespine stickleback infected with a cestode macroparasite. *Journal of Parasitology*, *89*(1), 1–6. https://doi.org/10.1645/0022-3395(2003)089[0001: ROESIN]2.0.CO;2

Heins, D. C., Baker, J. A., Toups, M. A., & Birden, E. L. (2010). Evolutionary significance of fecundity reduction in threespine stickleback infected by the diphyllobothriidean cestode Schistocephalus solidus. *Biological Journal of the Linnean Society*, *100*(4), 835–846. https://doi.org/10.1111/j.1095-8312.2010.01486.x

Heins, D. C., Barry, K. A., & Petrauskas, L. A. (2014). Consistency of host responses to parasitic infection in the three-spined stickleback fish infected by the diphyllobothriidean cestode Schistocephalus solidus. *Biological Journal of the Linnean Society*, *113*(4), 958–968. https://doi.org/10.1111/bij.12392

Heins, D. C., Moody, K. N., & Miller, S. (2018). Are solo infections of the diphyllobothriidean cestode Schistocephalus solidus more virulent than multiple infections? *Parasitology*, 1–8. https://doi.org/10.1017/S003118201800094X

Heins, D. C, Singer, S. S., & Baker, J. A. (1999). Virulence of the cestode Schistocephalus solidus and reproduction in infected threespine stickleback, Gasterosteus aculeatus. *Canadian Journal of Zoology*, *77*(12), 1967–1974. https://doi.org/10.1139/z99-180

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007a). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, *8*, R19. https://doi.org/10.1186/gb-2007-8-2-r19

Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., & Vandesompele, J. (2007b). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*, *8*(2), R19. https://doi.org/10.1186/gb-2007-8-2-r19

Helmby, H. (2015). Human helminth therapy to treat inflammatory disorders- where do we stand? *BMC Immunology*, *16*, 12. https://doi.org/10.1186/s12865-015-0074-3

Hibbeler, S., Scharsack, J. P., & Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback Gasterosteus aculeatus. *BMC Molecular Biology*, *9*(1), 18. https://doi.org/10.1186/1471-2199-9-18

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363.

Huang, Y., Chain, F. J. J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T. L., … Feulner, P. G. D. (2016). Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. *Molecular Ecology*, n/a-n/a. https://doi.org/10.1111/mec.13520

International Helminth Genomes Consortium. (2018). Comparative genomics of the major parasitic worms. *Nature Genetics*. https://doi.org/10.1038/s41588-018-0262-1

Izvekova, G. I. (2005). Activity of Carbohydrases of Symbiotic Microflora and Their Role in Processes of Digestion of Fish and Their Parasitizing Cestodes (on the Example of Pike and Triaenophorus nodulosus). *Journal of Evolutionary Biochemistry and Physiology*, *41*(4), 406–414. https://doi.org/10.1007/s10893-005-0076-5

Jager, I., & Schorring, S. (2006). Multiple Infections: Relatedness and Time Between Infections Affect the Establishment and Growth of the Cestode Schistocephalus Solidus in Its Stickleback Host. *Evolution*, *60*(3), 616–622. https://doi.org/10.1111/j.0014-3820.2006.tb01141.x

Janzen, D. H. (1980). When is it coevolution. *Evolution*, *34*(3), 611–612.

Johnson, P. C. D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. *Methods in Ecology and Evolution*, *5*(9), 944–946. https://doi.org/10.1111/2041-210X.12225

Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., … Kingsley, D. M. (2012). The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, *484*(7392), 55–61. https://doi.org/10.1038/nature10944

Kalbe, M., & Kurtz, J. (2006). Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke Diplostomum pseudospathaceum. *Parasitology*, *132*(01), 105–116. https://doi.org/10.1017/S0031182005008681

Kalbe, M., Wegner, K. M., & Reusch, T. B. H. (2002). Dispersion patterns of parasites in 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*, *60*(6), 1529–1542. https://doi.org/10.1111/j.1095-8649.2002.tb02445.x

Kalbe, M., Eizaguirre, C., Scharsack, J. P., & Jakobsen, P. J. (2016). Reciprocal cross infection of sticklebacks with the diphyllobothriidean cestode Schistocephalus solidus reveals consistent population differences in parasite growth and host resistance. *Parasites & Vectors*, *9*, 130. https://doi.org/10.1186/s13071-016-1419-3

Kaltz, O., & Shykoff, J. A. (2002). Within- and among-population variation in infectivity, latency and spore production in a host–pathogen system. *Journal of Evolutionary Biology*, *15*(5), 850–860. https://doi.org/10.1046/j.1420-9101.2002.00433.x

Karvonen, A., Seppälä, O., & Valtonen, E. T. (2004). Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology*, *129*(4), 473–478. https://doi.org/10.1017/S0031182004006006

Kassen, R. (2002). The experimental evolution of specialists, generalists, and the maintenance of diversity. *Journal of Evolutionary Biology*, *15*(2), 173–190. https://doi.org/10.1046/j.1420-9101.2002.00377.x

Kaufmann, J., Lenz, T. L., Milinski, M., & Eizaguirre, C. (2014). Experimental parasite infection reveals costs and benefits of paternal effects. *Ecology Letters*, *17*(11), 1409–1417. https://doi.org/10.1111/ele.12344

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241.

Khan, A. R., & Fallon, P. G. (2013). Helminth therapies: Translating the unknown unknowns to known knowns. *International Journal for Parasitology*, *43*(3–4), 293–299. https://doi.org/10.1016/j.ijpara.2012.12.002

King, K. C., Delph, L. F., Jokela, J., & Lively, C. M. (2009). The Geographic Mosaic of Sex and the Red Queen. *Current Biology*, *19*(17), 1438–1441. https://doi.org/10.1016/j.cub.2009.06.062

Kissick, H. T., Dunn, L. K., Ghosh, S., Nechama, M., Kobzik, L., & Arredouani, M. S. (2014). The Scavenger Receptor MARCO Modulates TLR-Induced Responses in Dendritic Cells. *PLoS ONE*, *9*(8), e104148. https://doi.org/10.1371/journal.pone.0104148

Korneva, J. V. (2008). Nanobacteria associated with mucous intestines of freshwater fishes and tegument of their parasites (Cestoda). *Acta Parasitologica*, *53*(3), 312–314. https://doi.org/10.2478/s11686-008-0041-8

Korneva, Z. V., & Plotnikov, A. O. (2012). Ultrastructure adaptations of symbiotic bacteria associated with freshwater fish and their cestode parasites. *Inland Water Biology*, *5*(2), 178–183. https://doi.org/10.1134/S1995082912020071

Kraal, G., van der Laan, L. J. W., Elomaa, O., & Tryggvason, K. (2000). The macrophage receptor MARCO. *Microbes and Infection*, *2*(3), 313–316. https://doi.org/10.1016/S1286-4579(00)00296-3

Kurtz, J., Kalbe, M., Aeschlimann, P. B., Häberli, M. A., Wegner, K. M., Reusch, T. B. H., & Milinski, M. (2004). Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1535), 197–204. https://doi.org/10.1098/rspb.2003.2567

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13). https://doi.org/10.18637/jss.v082.i13

Lamb, E.W., Walls, C.D., Pesce, J.T., Riner, D.K., Maynard, S.K., Crow, E.T., Wynn, T.A., Schaefer, B.C., and Davies, S.J. (2010). Blood Fluke Exploitation of Non-Cognate CD4+ T Cell Help to Facilitate Parasite Development. PLOS Pathog *6*, e1000892.

Lambrechts, L., Fellous, S., & Koella, J. C. (2006). Coevolutionary interactions between host and parasite genotypes. *Trends in Parasitology*, *22*(1), 12–16. https://doi.org/10.1016/ j.pt.2005.11.008

Lambrechts, L., Halbert, J., Durand, P., Gouagna, L. C., & Koella, J. C. (2005). Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitoes to Plasmodium falciparum. *Malaria Journal*, *4*(1), 3. https://doi.org/ 10.1186/ 1475-2875-4-3

Lazzaro, B. P., & Little, T. J. (2009). Immunity in a variable world. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 15–26. https://doi.org/10.1098/rstb.2008.0141

Leemans, J. C., te Velde, A. A., Florquin, S., Bennink, R. J., de Bruin, K., van Lier, R. A. W., … Hamann, J. (2004). The Epidermal Growth Factor-Seven Transmembrane (EGF-TM7) Receptor CD97 Is Required for Neutrophil Migration and Host Defense. *The Journal of Immunology*, *172*(2), 1125–1131. https://doi.org/10.4049/jimmunol.172.2.1125

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. https://doi.org/10.1111/2041-210X.12512

Lello, J., Boag, B., Fenton, A., Stevenson, I. R., & Hudson, P. J. (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature*, *428*(6985), 840–844. https://doi.org/10.1038/nature02490

Lenz, T. L. (2015). Transcription in space – environmental vs. genetic effects on differential immune gene expression. *Molecular Ecology*, *24*(18), 4583–4585. https://doi.org/10.1111/mec.13356

Lenz, T. L., Eizaguirre, C., Rotter, B., Kalbe, M., & Milinski, M. (2013). Exploring local immunological adaptation of two stickleback ecotypes by experimental infection and transcriptome-wide digital gene expression analysis. *Molecular Ecology*, *22*(3), 774–786. https://doi.org/10.1111/j.1365-294X.2012.05756.x

Leonardi-Bee, J., Pritchard, D., and Britton, J. (2006). Asthma and Current Intestinal Parasite Infection. Am. J. Respir. Crit. Care Med. *174*, 514–523.

Lescak, E. A., Bassham, S. L., Catchen, J., Gelmond, O., Sherbick, M. L., Hippel, F. A. von, & Cresko, W. A. (2015). Evolution of stickleback in 50 years on earthquake-uplifted islands. *Proceedings of the National Academy of Sciences*, *112*(52), E7204–E7212. https://doi.org/10.1073/pnas.1512020112

Liu, Q., Sundar, K., Mishra, P. K., Mousavi, G., Liu, Z., Gaydo, A., Gause, W. C. (2009a). Helminth Infection Can Reduce Insulitis and Type 1 Diabetes through CD25- and IL-10-Independent Mechanisms. *Infection and Immunity*, *77*(12), 5347–5358. https://doi.org/10.1128/IAI.01170-08

Liu, Z., Liu, Q., Bleich, D., Salgame, P., & Gause, W. C. (2009b). Regulation of type 1 diabetes, tuberculosis, and asthma by parasites. *Journal of Molecular Medicine*, *88*(1), 27–38. https://doi.org/10.1007/s00109-009-0546-0

Lively, C. M. (1999). Migration, Virulence, and the Geographic Mosaic of Adaptation by Parasites. *The American Naturalist*, *153*(S5), S34–S47. https://doi.org/10.1086/303210

Lively, C. M. (1996). Host-Parasite Coevolution and Sex. *BioScience*, *46*(2), 107–114. https://doi.org/10.2307/1312813

LoBue, C. P., & Bell, M. A. (1993). Phenotypic Manipulation by the Cestode Parasite Schistocephalus solidus of Its Intermediate Host, Gasterosteus aculeatus, the Threespine Stickleback. *The American Naturalist*, *142*(4), 725–735.

Lohman, B. K., Steinel, N. C., Weber, J. N., & Bolnick, D. I. (2017). Gene Expression Contributes to the Recent Evolution of Host Resistance in a Model Host Parasite System. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01071

Long, G. H., & Boots, M. (2011). How can immunopathology shape the evolution of parasite virulence? *Trends in Parasitology*, *27*(7), 300–305. https://doi.org/10.1016/ j.pt.2011.03.012

López-Maury, L., Marguerat, S., and Bähler, J. (2008). Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat. Rev. Genet. *9*, 583–593.

Lüscher, A., & Milinski, M. (2003). Simultaneous hermaphrodites reproducing in pairs self-fertilize some of their eggs: an experimental test of predictions of mixed-mating and Hermaphrodite's Dilemma theory. *Journal of Evolutionary Biology*, *16*(5), 1030–1037. https://doi.org/10.1046/j.1420-9101.2003.00552.x

Lüscher, A., & Wedekind, C. (2002). Size-dependent discrimination of mating partners in the simultaneous hermaphroditic cestode Schistocephalus solidus. *Behavioral Ecology*, *13*(2), 254–259. https://doi.org/10.1093/beheco/13.2.254

MacColl, A. D. C. (2009). Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, *32*(1), 153–160. https://doi.org/10.1111/j.1600-0587.2008.05486.x

Maerten, P., Shen, C., Bullens, D. M. A., Van Assche, G., Van Gool, S., Geboes, K., Ceuppens, J. L. (2005). Effects of interleukin 4 on CD25+CD4+ regulatory T cell function. *Journal of Autoimmunity*, *25*(2), 112–120. https://doi.org/10.1016/ j.jaut.2005.04.001

Maizels, R. M. (2005). Infections and allergy — helminths, hygiene and host immune regulation. *Current Opinion in Immunology*, *17*(6), 656–661. https://doi.org/10.1016/ j.coi.2005.09.001

Maizels, R. M., Bundy, D. A., Selkirk, M. E., Smith, D. F., & Anderson, R. M. (1993). Immunological modulation and evasion by helminth parasites in human populations. *Nature*, *365*(6449), 797–805.

Maizels, R. M., & McSorley, H. J. (2016). Regulation of the host immune system by helminth parasites. *Journal of Allergy and Clinical Immunology*, *138*(3), 666–675. https://doi.org/10.1016/j.jaci.2016.07.007

Maizels, R. M., & Nussey, D. H. (2013). Into the wild: digging at immunology's evolutionary roots. *Nature Immunology*, *14*(9), ni.2643. https://doi.org/10.1038/ni.2643

Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nature Reviews Immunology*, *3*(9), 733–744. https://doi.org/10.1038/nri1183

Mäkinen, H. S., Cano, J. M., & Merilä, J. (2006). Genetic relationships among marine and freshwater populations of the European three-spined stickleback (Gasterosteus aculeatus) revealed by microsatellites. *Molecular Ecology*, *15*(6), 1519–1534. https://doi.org/10.1111/j.1365-294X.2006.02871.x

Maxson, J. E., Gotlib, J., Pollyea, D. A., Fleischman, A. G., Agarwal, A., Eide, C. A., Tyner, J. W. (2013). Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML. *New England Journal of Medicine*, *368*(19), 1781–1790. https://doi.org/10.1056/NEJMoa1214514

Mazzarella, A.B., Voje, K.L., Hansson, T.H., Taugbøl, A., and Fischer, B. (2015). Strong and parallel salinity-induced phenotypic plasticity in one generation of threespine stickleback. J. Evol. Biol. *28*, 667–677.

McFadden, C., Morgan, R., Rahangdale, S., Green, D., Yamasaki, H., Center, D., & Cruikshank, W. (2007). Preferential Migration of T Regulatory Cells Induced by IL-16. *The Journal of Immunology*, *179*(10), 6439–6445. https://doi.org/10.4049/jimmunol.179.10.6439

McKinnon, J. S., & Rundle, H. D. (2002). Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution*, *17*(10), 480–488. https://doi.org/10.1016/S0169-5347(02)02579-X

McSorley, H. J., Hewitson, J. P., & Maizels, R. M. (2013). Immunomodulation by helminth parasites: Defining mechanisms and mediators. *International Journal for Parasitology*, *43*(3), 301–310. https://doi.org/10.1016/j.ijpara.2012.11.011

Meakins, R.H., and Walkey, M. (1975). The effects of parasitism by the plerocercoid of Schistocephalus solidus Muller 1776 (Pseudophyllidea) on the respiration of the three-spined stickleback Gasterosteus aculeatus L. J. Fish Biol. *7*, 817–824.

Michaud, M., Milinski, M., Parker, G. A., & Chubb, J. C. (2006). Competitive Growth Strategies in Intermediate Hosts: Experimental Tests of a Parasite life-History Model Using the Cestode, Schistocephalus solidus. *Evolutionary Ecology*, *20*(1), 39–57. https://doi.org/10.1007/s10682-005-3274-0

Milinski, M., & Bakker, T. C. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature*, *344*(6264), 330.

Milligan-Myhre, K., Small, C. M., Mittge, E. K., Agarwal, M., Currey, M., Cresko, W. A., & Guillemin, K. (2016). Innate immune responses to gut microbiota differ between oceanic and freshwater threespine stickleback populations. *Disease Models & Mechanisms*, *9*(2), 187–198. https://doi.org/10.1242/dmm.021881

Morran, L.T., Schmidt, O.G., Gelarden, I.A., Parrish, R.C., and Lively, C.M. (2011). Running with the Red Queen: Host-Parasite Coevolution Selects for Biparental Sex. Science *333*, 216–218.

Morris, M. R. J., Richard, R., Leder, E. H., Barrett, R. D. H., Aubin-Horth, N., & Rogers, S. M. (2014). Gene expression plasticity evolves in response to colonization of freshwater lakes in threespine stickleback. *Molecular Ecology*, *23*(13), 3226–3240. https://doi.org/10.1111/mec.12820

Mosmann, T. R., & Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today*, *17*(3), 138–146. https://doi.org/10.1016/0167-5699(96) 80606-2

Mostowy, R., & Engelstadter, J. (2011). The impact of environmental change on host-parasite coevolutionary dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1716), 2283–2292. https://doi.org/10.1098/rspb.2010.2359

Mulcahy, G., O'Neill, S., Donnelly, S., & Dalton, J. P. (2004). Helminths at mucosal barriers—interaction with the immune system. *Advanced Drug Delivery Reviews*, *56*(6), 853–868. https://doi.org/10.1016/j.addr.2003.10.033

Murphy, K., & Weaver, C. (2017). *Janeway's immunobiology* (9th Edition). Garland Science.

Nagar, A. E., & MacColl, A. D. C. (2016). Parasites contribute to ecologically dependent postmating isolation in the adaptive radiation of three-spined stickleback. *Proc. R. Soc. B*, *283*(1836), 20160691. https://doi.org/10.1098/rspb.2016.0691

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Nesse, R. M., Bergstrom, C. T., Ellison, P. T., Flier, J. S., Gluckman, P., Govindaraju, D. R., Valle, D. (2010). Making evolutionary biology a basic science for medicine. *Proceedings of the National Academy of Sciences*, *107*(suppl 1), 1800–1807. https://doi.org/10.1073/pnas.0906224106

Nesse, R. M. (2013). Tinbergen's four questions, organized: a response to Bateson and Laland. *Trends in Ecology & Evolution*, *28*(12), 681–682. https://doi.org/10.1016/j.tree.2013.10.008

Neuwirth, E. (2014). *RColorBrewer Palettes. R package version 1.1-2.* Retrieved from http://CRAN.R-project.org/package=RColorBrewer

Neuwirth, Erich. (2014). RColorBrewer: ColorBrewer palettes. *R Package Version 1.1-2*, https://cran.R-project.org/package=RColorBrewer. https://doi.org/citeulike-article-id:5433478

Nishimura, N., Heins, D. C., Andersen, R. O., Barber, I., & Cresko, W. A. (2011). Distinct Lineages of Schistocephalus Parasites in Threespine and Ninespine Stickleback Hosts Revealed by DNA Sequence Analysis. *PLoS ONE*, *6*(7), e22505. https://doi.org/10.1371/journal.pone.0022505

Nuismer, S. L., Gomulkiewicz, R., & Ridenhour, B. J. (2010). When Is Correlation Coevolution? *The American Naturalist*, *175*(5), 525–537. https://doi.org/10.1086/651591

Nutman, T. B. (2015). Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. *Parasite Immunology*, *37*(6), 304–313. https://doi.org/10.1111/pim.12194

Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., … Wagner, H. (2015). *vegan: community ecology package. 2.0-0. R package version 2.3-0.*

Orr, T. S. C., Hopkins, C. A., & Charles, G. H. (1969). Host specificity and rejection of Schistocephalus solidus. *Parasitology*, *59*(3), 683–690. https://doi.org/10.1017/S0031182000031206

O'Shea, J. J., & Paul, W. E. (2010). Mechanisms Underlying Lineage Commitment and Plasticity of Helper CD4+ T Cells. *Science*, *327*(5969), 1098–1102. https://doi.org/10.1126/science.1178334

Papkou, A., Guzella, T., Yang, W., Koepper, S., Pees, B., Schalkowski, R., Barg, M.-C., Rosenstiel, P.C., Teotónio, H., and Schulenburg, H. (2019). The genomic basis of Red Queen dynamics during rapid reciprocal host–pathogen coevolution. Proc. Natl. Acad. Sci. *116*, 923–928.

Parker, G. A., Chubb, J. C., Roberts, G. N., Michaud, M., & Milinski, M. (2003). Optimal growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionary Biology*, *16*(1), 47–54. https://doi.org/10.1046/j.1420-9101.2003.00504.x

Paterson, S., Vogwill, T., Buckling, A., Benmayor, R., Spiers, A. J., Thomson, N. R., Brockhurst, M. A. (2010). Antagonistic coevolution accelerates molecular evolution. *Nature*, *464*(7286), 275–278. https://doi.org/10.1038/nature08798

Pedersen, A. B., & Antonovics, J. (2013). Anthelmintic treatment alters the parasite community in a wild mouse host. *Biology Letters*, *9*(4), 20130205. https://doi.org/10.1098/rsbl.2013.0205

Pennycuick, L. (1971). Differences in the parasite infections in three-spined sticklebacks (Gasterosteus aculeatus L.) of different sex, age and size. *Parasitology*, *63*(03), 407–418. https://doi.org/10.1017/S0031182000079932

Peón, A. N., Ledesma-Soto, Y., & Terrazas, L. I. (2016). Regulation of immunity by Taeniids: lessons from animal models and in vitro studies. *Parasite Immunology*, *38*(3), 124–135. https://doi.org/10.1111/pim.12289

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), e45. https://doi.org/10.1093/nar/29.9.e45

Pinheiro, J., Bates, D., DebRoy, S., & Sarkar, D. (2015). R Core Team (2014) nlme: linear and nonlinear mixed effects models. R package version 3.1-121. *Available at h Ttp://CRAN. R-Project. Org/Package= Nlme*.

Poddubnaya, L. G., & Izvekova, G. I. (2005). Detection of bacteria associated with the tegument of caryophyllidean cestodes. *Helminthologia*, *42*(1), 9–14.

Poulin, R., & Morand, S. (2000). The Diversity of Parasites. *The Quarterly Review of Biology*, *75*(3), 277–293.

Råberg, L. (2014). How to Live with the Enemy: Understanding Tolerance to Parasites. *PLOS Biology*, *12*(11), e1001989. https://doi.org/10.1371/journal.pbio.1001989

Råberg, L., Graham, A. L., & Read, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *364*(1513), 37–49. https://doi.org/10.1098/rstb.2008.0184

Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling Genetic Variation for Resistance and Tolerance to Infectious Diseases in Animals. *Science*, *318*(5851), 812–814. https://doi.org/10.1126/science.1148526

Rauch, G., Kalbe, M., & B.H. Reusch, T. (2008). Partitioning average competition and extreme-genotype effects in genetically diverse infections. *Oikos*, *117*(3), 399–405. https://doi.org/10.1111/j.2007.0030-1299.16301.x

Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology*, *2*(3), 73–76. https://doi.org/10.1016/0966-842X(94)90537-1

Read, A. F., Graham, A. L., & Råberg, L. (2008). Animal Defenses against Infectious Agents: Is Damage Control More Important Than Pathogen Control. *PLoS Biology*, *6*(12), e1000004. https://doi.org/10.1371/journal.pbio.1000004

Restif, O., & Koella, J. C. (2003). Shared Control of Epidemiological Traits in a Coevolutionary Model of Host-Parasite Interactions. *The American Naturalist*, *161*(6), 827–836. https://doi.org/10.1086/375171

Reynolds, L. A., Finlay, B. B., & Maizels, R. M. (2015). Cohabitation in the Intestine: Interactions among Helminth Parasites, Bacterial Microbiota, and Host Immunity. *The Journal of Immunology*, *195*(9), 4059–4066. https://doi.org/10.4049/jimmunol.1501432

Rhodes, L. D., Wallis, S., & Demlow, S. E. (2009). Genes associated with an effective host response by Chinook salmon to Renibacterium salmoninarum. *Developmental & Comparative Immunology*, *33*(2), 176–186. https://doi.org/10.1016/j.dci.2008.08.006

Ritter, M., Kalbe, M., & Henrich, T. (2017). Virulence in the three-spined stickleback specific parasite Schistocephalus solidus is inherited additively. *Experimental Parasitology*, *180*(Supplement C), 133–140. https://doi.org/10.1016/j.exppara.2017.02.016

Robertson, S., Bradley, J. E., & MacColl, A. D. C. (2015). Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. *Molecular Ecology Resources*, n/a-n/a. https://doi.org/10.1111/1755-0998.12497

Rodríguez-Sosa, M., Satoskar, A. R., Calderón, R., Gomez-Garcia, L., Saavedra, R., Bojalil, R., & Terrazas, L. I. (2002). Chronic Helminth Infection Induces Alternatively Activated Macrophages Expressing High Levels of CCR5 with Low Interleukin-12 Production and Th2-Biasing Ability. *Infection and Immunity*, *70*(7), 3656–3664. https://doi.org/10.1128/IAI.70.7.3656-3664.2002

Roy, B. A., & Kirchner, J. W. (2000). Evolutionary Dynamics of Pathogen Resistance and Tolerance. *Evolution*, *54*(1), 51–63. https://doi.org/10.1111/j.0014-3820.2000.tb00007.x

Salvaudon, L., Héraudet, V., & Shykoff, J. A. (2005). Parasite-host fitness trade-offs change with parasite identity: genotype-specific interactions in a plant-pathogen system. *Evolution; International Journal of Organic Evolution*, *59*(12), 2518–2524.

Salvaudon, L., Héraudet, V., & Shykoff, J. A. (2007). Genotype-specific interactions and the trade-off between host and parasite fitness. *BMC Evolutionary Biology*, *7*(1), 189. https://doi.org/10.1186/1471-2148-7-189

Schärer, L., & Wedekind, C. (1999). Lifetime reproductive output in a hermaphrodite cestode when reproducing alone or in pairs: a time cost of pairing. *Evolutionary Ecology*, *13*(4), 381–394. https://doi.org/10.1023/A:1006789110502

Scharsack, J. P., Franke, F., Erin, N. I., Kuske, A., Büscher, J., Stolz, H., … Kalbe, M. (2016). Effects of environmental variation on host–parasite interaction in three-spined sticklebacks (Gasterosteus aculeatus). *Zoology*, *119*(4), 375–383. https://doi.org/10.1016/j.zool.2016.05.008

Scharsack, J. P., Kalbe, M., Harrod, C., & Rauch, G. (2007). Habitat-specific adaptation of immune responses of stickleback (Gasterosteus aculeatus) lake and river ecotypes. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1617), 1523–1532. https://doi.org/10.1098/rspb.2007.0210

Scharsack, J. P., & Kalbe, M. (2014). Differences in susceptibility and immune responses of three-spined sticklebacks (Gasterosteus aculeatus) from lake and river ecotypes to sequential infections with the eye fluke Diplostomum pseudospathaceum. *Parasites & Vectors*, *7*(1), 109.

Scharsack, J. P., Koch, K., & Hammerschmidt, K. (2007). Who is in control of the stickleback immune system: interactions between Schistocephalus solidus and its specific vertebrate host. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1629), 3151–3158. https://doi.org/10.1098/rspb.2007.1148

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*(1513), 357–366. https://doi.org/10.1098/rspb.2002.2265

Schmid-Hempel, P. (2009). Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 85–98. https://doi.org/10.1098/ rstb.2008.0157

Schmid-Hempel, P. (2011). *Evolutionary Parasitology The Integrated Study of Infections, Immunology, Ecology, and Genetics*. Oxford University Press, Inc.: New York, NY, USA.

Schneider, D. S., & Ayres, J. S. (2008). Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. *Nature Reviews Immunology*, *8*(11), 889–895. https://doi.org/10.1038/nri2432

Schneider, R. F., & Meyer, A. (2017). How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology*, *26*(1), 330–350. https://doi.org/10.1111/mec.13880

Schulenburg, H., Kurtz, J., Moret, Y., & Siva-Jothy, M. T. (2009). Introduction. Ecological immunology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1513), 3–14. https://doi.org/10.1098/rstb.2008.0249

Schulte, R.D., Makus, C., Hasert, B., Michiels, N.K., and Schulenburg, H. (2010). Multiple reciprocal adaptations and rapid genetic change upon experimental coevolution of an animal host and its microbial parasite. Proc. Natl. Acad. Sci. *107*, 7359–7364.

Seppälä, O., Karvonen, A., & Tellervo Valtonen, E. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behaviour*, *68*(2), 257–263. https://doi.org/10.1016/j.anbehav.2003.10.021

Sheldon, B. C., & Verhulst, S. (1996). Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, *11*(8), 317–321. https://doi.org/10.1016/0169-5347(96)10039-2

Sitjà-Bobadilla, A. (2008). Living off a fish: a trade-off between parasites and the immune system. *Fish & Shellfish Immunology*, *25*(4), 358–372. https://doi.org/10.1016/ j.fsi.2008.03.018

Smallwood, T. B., Giacomin, P. R., Loukas, A., Mulvenna, J. P., Clark, R. J., & Miles, J. J. (2017). Helminth Immunomodulation in Autoimmune Disease. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.00453

Smith, C. C., Snowberg, L. K., Gregory Caporaso, J., Knight, R., & Bolnick, D. I. (2015). Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. *The ISME Journal*. https://doi.org/10.1038/ismej.2015.64

Smyth, J. D. (1946). Studies on Tapeworm Physiology I. The Cultivation of Schistocephalus Solidus In Vitro. *Journal of Experimental Biology*, *23*(1), 47–70.

Smyth, J. D. (1954). Studies on tapeworm physiology. VII. Fertilization of *Schistocephalus solidus in vitro*. *Experimental Parasitology*, *3*(1), 64–71.

Sprehn, C. G., Blum, M. J., Quinn, T. P., & Heins, D. C. (2015). Landscape Genetics of Schistocephalus solidus Parasites in Threespine Stickleback (Gasterosteus aculeatus) from Alaska. *PLoS ONE*, *10*(4), e0122307. https://doi.org/10.1371/ journal.pone.0122307

Stearns, S. C., & Medzhitov, R. (2016). *Evolutionary medicine*. Sinauer Associates, Incorporated, Publishers.

Stern, S., Dror, T., Stolovicki, E., Brenner, N., and Braun, E. (2007). Genome-wide transcriptional plasticity underlies cellular adaptation to novel challenge. Mol. Syst. Biol. *3*, 106.

Sternberg, E. D., Li, H., Wang, R., Gowler, Camden, & Roode, J. C. de. (2013). Patterns of Host-Parasite Adaptation in Three Populations of Monarch Butterflies Infected with a Naturally Occurring Protozoan Disease: Virulence, Resistance, and Tolerance. *The American Naturalist*, *182*(6), E235–E248. https://doi.org/10.1086/673442

Stewart, A., Jackson, J., Barber, I., Eizaguirre, C., Paterson, R., van West, P., … Cable, J. (2017). Chapter Two - Hook, Line and Infection: A Guide to Culturing Parasites, Establishing Infections and Assessing Immune Responses in the Three-Spined Stickleback. In D. Rollinson & J. R. Stothard (Eds.), *Advances in Parasitology* (Vol. 98, pp. 39–109). Academic Press. https://doi.org/10.1016/bs.apar.2017.07.001

Strobel, H. M., Alda, F., Sprehn, C. G., Blum, M. J., & Heins, D. C. (2016). Geographic and host-mediated population genetic structure in a cestode parasite of the three-spined stickleback. *Biological Journal of the Linnean Society*, *119*(2), 381–396. https://doi.org/10.1111/bij.12826

Studer, A., Thieltges, D. W., & Poulin, R. (2010). Parasites and global warming: net effects of temperature on an intertidal host-parasite system. *Marine Ecology Progress Series*, *415*, 11–22.

Stutz, W. E., Schmerer, M., Coates, J. L., & Bolnick, D. I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. *Molecular Ecology*, *24*(18), 4629–4646. https://doi.org/10.1111/mec.13295

Summers, R. W., Elliott, D. E., Urban, J. F., Thompson, R., & Weinstock, J. V. (2005a). Trichuris suis therapy in Crohn's disease. *Gut*, *54*(1), 87–90. https://doi.org/10.1136/gut.2004.041749

Summers, R. W., Elliott, D. E., Urban, J. F., Thompson, R. A., & Weinstock, J. V. (2005b). Trichuris suis therapy for active ulcerative colitis: A randomized controlled trial. *Gastroenterology*, *128*(4), 825–832. https://doi.org/10.1053/j.gastro.2005.01.005

Tabbara, I. A. (1993). Granulocyte colony-stimulating factor. *Southern Medical Journal*, *86*(3), 350–355.

Tang, H., Ming, Z., Liu, R., Xiong, T., Grevelding, C.G., Dong, H., and Jiang, M. (2013). Development of Adult Worms and Granulomatous Pathology Are Collectively Regulated by T- and B-Cells in Mice Infected with Schistosoma japonicum. PLOS ONE *8*, e54432.

Telfer, S., Lambin, X., Birtles, R., Beldomenico, P., Burthe, S., Paterson, S., & Begon, M. (2010). Species Interactions in a Parasite Community Drive Infection Risk in a Wildlife Population. *Science*, *330*(6001), 243–246. https://doi.org/10.1126/science.1190333

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C. G., … Bordenstein, S. R. (2016). Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes. *MSystems*, *1*(2), e00028-16. https://doi.org/10.1128/mSystems.00028-16

Thompson, J. N. (1988). Variation in Interspecific Interactions. *Annual Review of Ecology and Systematics*, *19*, 25.

Thompson, J. N. (1994). The coevolutionary process University of Chicago Press. *Chicago, Illinois, USA*.

Thompson, J. N. (1998). Rapid evolution as an ecological process. *Trends in Ecology & Evolution*, *13*(8), 329–332.

Thompson, J. N. (1999a). Specific Hypotheses on the Geographic Mosaic of Coevolution. *The American Naturalist*, *153*(S5), S1–S14. https://doi.org/10.1086/303208

Thompson, J. N. (1999b). The Evolution of Species Interactions. *Science*, *284*(5423), 2116–2118. https://doi.org/10.1126/science.284.5423.2116

Thompson, J. N., & Cunningham, B. M. (2002). Geographic structure and dynamics of coevolutionary selection. *Nature*, *417*(6890), 735–738. https://doi.org/10.1038/nature00810

Thompson, J. N. (2005). *The geographic mosaic of coevolution*. University of Chicago Press.

Thompson, C.J., Ahmed, N.I., Veen, T., Peichel, C.L., Hendry, A.P., Bolnick, D.I., and Stuart, Y.E. (2017). Many-to-one form-to-function mapping weakens parallel morphological evolution. Evolution *71*, 2738–2749.

Tierney, J. F., & Crompton, D. W. T. (1992). Infectivity of Plerocercoids of Schistocephalus solidus (Cestoda: Ligulidae) and Fecundity of the Adults in an Experimental Definitive Host, Gallus gallus. *The Journal of Parasitology*, *78*(6), 1049–1054. https://doi.org/10.2307/3283228

Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift Für Tierpsychologie*, *20*(4), 410–433.

van Gestel, J., and Weissing, F.J. (2018). Is plasticity caused by single genes? Nature *555*, E19–E20.

van der Veen, I. T., & Kurtz, J. (2002). To avoid or eliminate: cestode infections in copepods. *Parasitology*, *124*(4), 465–474. https://doi.org/10.1017/S0031182001001275

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

Van Valen, L. (1974). Molecular evolution as predicted by natural selection. *Journal of Molecular Evolution*, *3*(2), 89–101. https://doi.org/10.1007/BF01796554

Vandesompele, J., Preter, K. De, Poppe, B., Roy, N. Van, & Paepe, A. De. (2002). Accurate normalization of real-time quantitative RT -PCR data by geometric averaging of multiple internal control genes, 1–12.

Vandesompele, J., Preter, K. D., Pattyn, F., Poppe, B., Roy, N. V., Paepe, A. D., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), 1–12. https://doi.org/10.1186/gb-2002-3-7-research0034

Vos, T., Allen, C., Arora, M., Barber, R. M., Bhutta, Z. A., Brown, A., … Murray, C. J. L. (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet*, *388*(10053), 1545–1602. https://doi.org/10.1016/S0140-6736(16)31678-6

Weaver, C. T., Harrington, L. E., Mangan, P. R., Gavrieli, M., & Murphy, K. M. (2006). Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties. *Immunity*, *24*(6), 677–688. https://doi.org/10.1016/j.immuni.2006.06.002

Weber, J. N., Kalbe, M., Shim, K. C., Erin, N. I., Steinel, N. C., Ma, L., … Michalakis, Y. (2016). Resist Globally, Infect Locally: A Transcontinental Test of Adaptation by Stickleback and

Their Tapeworm Parasite. *The American Naturalist*, E000–E000. https://doi.org/10.1086/689597

Weber, J. N., Steinel, N. C., Shim, K. C., & Bolnick, D. I. (2017). Recent evolution of extreme cestode growth suppression by a vertebrate host. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(25), 6575–6580. https://doi.org/10.1073/pnas.1620095114

Wedekind, C. (1997). The infectivity, growth, and virulence of the cestode Schistocephalus solidus in its first intermediate host, the copepod Macrocyclops albidus. *Parasitology*, *115*(03), 317–324. https://doi.org/10.1017/S0031182097001406

Wedekind, C., Strahm, D., & Schärer, L. (1998). Evidence for strategic egg production in a hermaphroditic cestode. *Parasitology*, *117*(04), 373–382. https://doi.org/null

Wegner, K. M., Reusch, T. B. H., & Kalbe, M. (2003). Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of Evolutionary Biology*, *16*(2), 224–232. https://doi.org/10.1046/j.1420-9101.2003.00519.x

Weinstock, J. V., & Elliott, D. E. (2013). Translatability of helminth therapy in inflammatory bowel diseases. *International Journal for Parasitology*, *43*(3), 245–251. https://doi.org/10.1016/j.ijpara.2012.10.016

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. Oxford University Press.

Wickham, H. (2009). *Elegant graphics for data analysis (ggplot2)*. New York, NY: Springer-Verlag.

Wickham, Hadley. (2011). The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, *40*(1), 1–29.

Williams, G. C. (1966). Adaptation and Natural Selection. Princeton University Press. *Princeton, NJ*.

Williams, G. C., & Nesse, R. M. (1991). The Dawn of Darwinian Medicine. *The Quarterly Review of Biology*, *66*(1), 1–22. https://doi.org/10.1086/417048

World Health Organization. (2018). *World health statistics 2018: monitoring health for the SDGs*. Retrieved from http://apps.who.int/iris/bitstream/handle/10665/272596/9789241565585-eng.pdf?ua=1

Zeller, M., & Koella, J. C. (2017). The Role of the Environment in the Evolution of Tolerance and Resistance to a Pathogen. *The American Naturalist*, *190*(3), 389–397. https://doi.org/10.1086/692759

Zhu, L., Nie, L., Zhu, G., Xiang, L., & Shao, J. (2012). Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. *Developmental & Comparative Immunology*, *39*(1–2), 39–62. https://doi.org/10.1016/j.dci.2012.04.001

ACKNOWLEDGEMENTS

First and foremost, I thank **Dr. Martin Kalbe** and **Dr. Olivia Roth** for being my PhD supervisors and encouraging me continuously. Martin died way too young on May 5, 2018 shortly before his 54th birthday. He has been an outstanding person, supervisor and scientist. His passion for parasitology and his generosity were inspirational. I am grateful for Martin's and Olivia's guidance through the intense years of my PhD.

I'd like to thank my further thesis committee members, **Prof. Dr. Hinrich Schulenburg** and **Prof. Dr. Arne Nolte**, for helpful and encouraging conversations. I am grateful to **Prof. Dr. Manfred Milinski** and the Max Planck Institute for Evolutionary Biology, **Prof. Dr. Thorsten Reusch** and the GEOMAR, the International Max Planck Research School for Evolutionary Biology, and our lovely administration for making this thesis possible by providing such excellent working conditions and funding as well as support through ups and downs. Further, I'd like to thank **Dr. Mario Hasler**, **Dr. Kenyon Mobley**, **Dr. Lutz Becks**, **Dr. Irene Samonte** and **Dr. Nina Hafer** for useful discussion.

Martin, deine Tür stand mir immer offen und Du hast mich stets unterstützt. Ich möchte Dir danken, dass ich ein paar Jahre mit Dir arbeiten und so viel von Dir lernen durfte. Dein Verlust ist schmerzlich.

Livi, auch Du hast mich während der letzen Jahre stets unterstützt und die volle Betreuung meiner Doktorarbeit übernommen, als Martin es nicht mehr konnte. Ich danke Dir von Herzen. Ohne Dich, deinen Rat und deine tatkräftige Unterstützung hätte diese Arbeit nicht entstehen können. Du verlierst Dich nicht in Kleinigkeiten und kämpfst für deine Doktoranden wie ein Löwin. Das hat mir wahnsinnig geholfen. Ich bin gern zurück gekommen in die Pipefish group.

Dieses Projekt wäre nicht möglich gewesen ohne tatkräftige und professionelle Unterstützung von **Withe Derner**, **Anja Baade** aka Hasselmeyer, **Regina Leipnitz**, **Helga Luttmann**, **Daniel Martens**, **Isabel Moreau**, **Gisela Schmiedeskamp**, **Ines Schultz**, **Michael Schwarz**, **Nina Wildenhayn**. Ihr Lieben, ihr seid unersetzlich! Mit Euch zu arbeiten war eine große Freude. Auch wenn die Aufgaben noch so unüberwindbar schienen, gemeinsam haben wir es geschafft. Ich bedanke mich vor allem dafür, dass ihr mich nie nur als Doktorandin gesehen habt, sondern immer als Mensch. Ihr habt eine Arbeitsatmosphäre geschaffen, in der die Motivation und die Freude nie ganz verloren gingen. **Ralf** und **Gerhard**, auch Euch gebührt ein großer Dank für die Hilfe und die professionelle Pflege der Tiere, seien es "goldene Kühe" oder nicht.

Marc, gemeinsam halten wir die Flagge der Evolutionsökologie hoch. Allen Widrigkeiten zum Trotz sind wir nicht im Chaos versunken, sondern haben großartige Projekte auf die Beine gestellt. Ich würd sagen, Ritter's Razor und Piecyk's Perfectionism haben sich gut ergänzt. Danke für deine Meinung und deine Offenheit, deinen ausgesprochen guten Musikgeschmack und dein qPCR-Talent.

Jamie, **Stella**, **Ben**, thank you so much for having such a great time in the office. **Felix** und **Lara**, danke für Eure artübergreifende Hilfe in allen Lebenslagen. **Henry, Kosmas**, **Melanie** und **Véro**, ihr seid die besten Büronachbarn, die man sich vorstellen kann. Danke für den Kaffesupply und die guten Worte. **Henry**, es war mir eine Freude mit Dir auf der Zielgeraden einzulaufen. Hab vielen Dank für deine Zeit und den umfassenden und kreativen Zusammenhalt. **Melanie**, Du Stichlingsprofi, gemeinsam haben wir in New York unsere Projekte vorgestellt und in Kyoto die letzten Ergebnisse präsentiert. Ich danke Dir für die zahlreichen gewinnbringenden Diskussionen. Auch an **Ralf**, **Kim**, **Zora** und den Rest der **pipefish group** geht ein dickes Dankeschön. **Diana**, **Conny**, **Svend** und **Fabian**, danke für Eure freundliche Unterstützung. **Emma** und **Annika**, aka The Swedish Ladies, ich danke Euch für die Jahre der body-mind-and-spirit-Unterstützung. **Sophie**, we went through ups and downs of our theses. You are more than a friend, you are family. Hope to see you soon.

Lieber **Emil**, wir sind in den letzten Jahren durch Höhen und Tiefen gegangen und haben alle Herausforderungen gemeistert. Du begleitest mich schon mein gesamtes Studium und hast mich mit deiner Wissbegierde, deinen Ideen und deiner Liebenswürdigkeit stets motiviert weiter zu machen. Danke für alles. Du bist der beste Sohn der Welt!

Tillmann, Du resilienter Superheld, wir haben es geschafft, in anstrengenden und in beflügelnden Zeiten den Überblick zu behalten und uns gemeinsame Momente zu schaffen. Unvergesslich sind mir unsere Momente am und auf dem Wasser, unser nächtliches Philosophieren über phänotypische Plastizität und genetische Anpassungen, Utopien und Dystopien, Aristoteles & Sokrates, Adorno & Horkheimer – Danke!

Danke auch an alle weiteren Freunde und Familienmitglieder, die an mich geglaubt haben.

SUPPLEMENTARY INFORMATION

Chapter 1

Specificity of resistance and geographic patterns of virulence in a vertebrate host-parasite system

Agnes Piecyk, Olivia Roth, Martin Kalbe

Table S1. Parasite sampling sites

ID	Origin		Geo-coordinates
ECH	Echo Lake, Vancouver Island	Canada	49°98'N, 125°41'W
ISC	Lake Myvatn	Iceland	65°39'N, 16°57'W
NU	North Uist (Loch Eubhal/ Grogary)	Scotland	57°34'N, 07°17'W
SKO	Skogseidvatnet	Norway	60°13'N, 05°53'E
SP	Xinzo de Limia	Spain	42°08'N, 07°39'W
IBB	Ibbenbürener Aa	Germany	52°17'N, 07°36'E
NST	Neustädter Binnenwasser	Germany	54°06'N, 10°48'E
GOT	Gotland	Sweden	57°54'N, 18°56'E
OBB	Obbola	Sweden	63°39'N, 20°17'E

SI.1 Supplementary information on infection rates

If not stated otherwise, infection rates were calculated by using the number of infected individuals as proportional data in generalized mixed effects models (GLMMs) with binomial error structure and logit link function using the glmer() function of the *lme4* R package (Bates et al., 2014). Significantly different groups were identified with glht() post hoc tests from the *multcomp* package (Hothorn et al., 2008). Infection rates differed considerably between parasite sibships and fish families; some parasite sibships failed to infect any fish. According to our experimental design, however, we did not test for fish family or parasite sibship effects. Parasite sibship was included as a random factor in analyses of infection rates in copepods; the random term 'round' (i.e. parasite sibship x fish family combination) was included in all analyses of the interaction between *S. solidus* and its fish hosts.

As expected for the unspecific first intermediate host, *S. solidus* from every origin managed to infect *M. albidus* copepods. We tested for potential differences in infection rates in copepods between the two years of the experiment by using data from parasite sibships that were used in both years (Table S2). Indeed, infection rates of parasites from NU and SKO were significantly higher in 2014 (NU: $z = 4.472$, $p < 0.0001$; SKO: $z = 6.214$, $p < 0.0001$). Testing each year separately, infection rates did not differ significantly between parasite populations in 2014; in 2015, ISC *S. solidus* infected significantly more copepods than parasites from GOT ($z = -5.289$, $p < 0.001$), NU ($z = 4.416$, $p < 0.001$), OBB ($z = 3.615$, $p < 0.01$), SKO ($z = 3.948$, $p < 0.01$), SP ($z = 4.115$, $p < 0.01$); IBB *S. solidus* infected significantly more copepods than *S. solidus* from GOT ($z = -4.638$, $p < 0.001$), NU ($z = 3.76$, $p < 0.01$), SKO ($z = 3.275$, $p = 0.029$), and SP ($z = 3.453$, $p = 0.016$); ECH *S. solidus* infected significantly more copepods than *S. solidus* from GOT ($z = 4.148$, $p < 0.01$) and NU ($z = 3.299$, $p = 0.027$). Using the sibship of the parasite as explanatory instead

of the origin improved the model fit, pointing towards sibship- rather than origin-effects. Interestingly, Pacific (ECH) parasites had the highest infection rates in copepods and the lowest infection rates in sticklebacks. However, overall, and consistent with previous publications (Hammerschmidt and Kurtz, 2005), infection rates in copepods did not influence infection rates in fish.

Infection rates in fish did not differ significantly between the two years of the experiment (DE data; $X^2_5 = 9.42$, $p = 0.094$). *S. solidus* origin influenced the infection rates in NO hosts ($X^2_8 = 21.619$, $p = 0.006$). This was driven by significant differences between infections with NU versus ECH parasites ($z = -3.446$, $p = 0.016$). NU *S. solidus* had the overall highest infection rate (average: 40 %) and ECH *S. solidus* had the lowest infection rate (average: 9 %). The variance terms for the random effect differed between the experiments, which indicates different parasite sibship x fish family effects; namely, lower variance in DE in *contrast 1*. Fish from the naturally highly parasitized Norwegian (NO) population ate considerably less infected copepods than DE fish, so we tested for a possible link between the number of ingested copepods and infection success. There was no consistent pattern; the number of infected copepods correlated with an increase or decrease of the infection rates, dependent on the origin of the parasite and the fish population (not shown). Accordingly and in line with the literature (Wedekind and Milinski, 1996), our data does not indicate avoidance behaviour.

Table S2. Infection rates of *S. solidus* in its first intermediate host (*M. albidus*)

Year of the experiment	Parasite sibship	Parasite origin	Infected copepods	Uninfected copepods	Infection rate	Average per origin and experiment
2014	ECH_3x10	ECH	51	38	0.57	
2014	ECH_6x23	ECH	69	17	0.80	
2014	ECH_9x14	ECH	61	17	0.78	0.72
2014	GOT_10x12	GOT	44	42	0.51	
2014	GOT_13x8	GOT	24	62	0.28	
2014	GOT_1x5	GOT	48	38	0.56	0.45
2014	NST_13x14	NST	51	29	0.64	
2014	NST_2x7	NST	46	37	0.55	
2014	NST_8x9	NST	43	80	0.35	0.51
2014	NU_10x14	NU	60	28	0.68	
2014	NU_4x12	NU	21	65	0.24	
2014	NU_8x17	NU	56	21	0.73	0.55
2014	OBB_11x48	OBB	39	73	0.35	
2014	OBB_18x20	OBB	54	30	0.64	
2014	OBB_5x16	OBB	48	25	0.66	0.55
2014	SKO_18x49	SKO	45	36	0.56	
2014	SKO_18x57	SKO	52	33	0.61	
2014	SKO_57x58	SKO	49	36	0.58	0.58
2014	SP_10x12	SP	55	33	0.63	
2014	SP_14x19	SP	19	23	0.45	
2014	SP_1x13	SP	22	66	0.25	0.44
2015	ECH_3x10	ECH	49	24	0.67	
2015	ECH_6x23	ECH	60	28	0.68	
2015	ECH_9x14	ECH	53	32	0.62	0.66
2015	GOT_13x8	GOT	28	71	0.28	
2015	GOT_1x5	GOT	39	71	0.35	
2015	GOT_9x6	GOT	34	64	0.35	0.33
2015	IBB_35	IBB	76	56	0.58	
2015	IBB_39	IBB	117	55	0.68	
2015	IBB_41	IBB	123	35	0.78	0.68
2015	ISC_59	ISC	103	31	0.77	
2015	ISC_61	ISC	121	47	0.72	
2015	ISC_70	ISC	124	55	0.69	0.73
2015	NST_13x14	NST	76	66	0.54	
2015	NST_2x7	NST	86	65	0.57	
2015	NST_8x9	NST	92	80	0.53	0.55
2015	NU_10x14	NU	36	54	0.40	
2015	NU_5x18	NU	30	85	0.26	
2015	NU_8x17	NU	57	50	0.53	0.40
2015	OBB_11x48	OBB	44	50	0.47	
2015	OBB_18x20	OBB	33	54	0.38	
2015	OBB_5x16	OBB	46	43	0.52	0.45
2015	SKO_18x57	SKO	63	111	0.36	
2015	SKO_26x44	SKO	106	41	0.72	
2015	SKO_57x58	SKO	43	131	0.25	0.44
2015	SP_10x12	SP	55	75	0.42	
2015	SP_1x13	SP	44	44	0.50	
2015	SP_8x17	SP	43	82	0.34	0.42

Table S3. Infection rates and *S. solidus* size in DE and NO *G. aculeatus*

Year of the experiment	Fish family	Fish origin	Parasite sibship	Parasite origin	Total fish	Exposed fish	Infected fish	Uninfected fish	Infection rate	Mean weight	Mean PI
2014	GPS_16x6	DE	ECH_3x10	ECH	20	19	2	17	0.11	88.3	13.53
2014	GPS_16x6	DE	GOT_10x12	GOT	20	20	3	17	0.15	48.53	6.8
2014	GPS_16x6	DE	NST_8x9	NST	20	20	9	11	0.45	31.63	5.14
2014	GPS_16x6	DE	NU_8x17	NU	20	20	6	14	0.30	75.28	11.62
2014	GPS_16x6	DE	OBB_11x48	OBB	20	19	4	15	0.21	42.88	6.43
2014	GPS_16x6	DE	SKO_57x58	SKO	20	20	5	15	0.25	102.72	15.33
2014	GPS_16x6	DE	SP_14x19	SP	18	17	7	10	0.41	61.27	10.97
2014	GPS_24x29	DE	ECH_9x14	ECH	20	20	2	18	0.10	96.4	13.15
2014	GPS_24x29	DE	GOT_13x8	GOT	20	20	0	20	0.00	na	na
2014	GPS_24x29	DE	NST_13x14	NST	20	20	4	16	0.20	25.6	4.18
2014	GPS_24x29	DE	NU_10x14	NU	20	20	4	16	0.20	80.5	15.2
2014	GPS_24x29	DE	OBB_18x20	OBB	20	20	2	18	0.10	18.7	3.74
2014	GPS_24x29	DE	SKO_18x49	SKO	20	20	0	20	0.00	na	na
2014	GPS_24x29	DE	SP_10x12	SP	20	18	0	18	0.00	na	na
2014	GPS_5x3	DE	ECH_6x23	ECH	20	19	3	16	0.16	68.57	12.34
2014	GPS_5x3	DE	GOT_1x5	GOT	20	19	5	14	0.26	41.5	6.75
2014	GPS_5x3	DE	NST_2x7	NST	20	20	4	16	0.20	42.6	6.71
2014	GPS_5x3	DE	NU_4x12	NU	18	17	1	16	0.06	94.96	13.73
2014	GPS_5x3	DE	OBB_5x16	OBB	20	19	4	15	0.21	23.83	4.48
2014	GPS_5x3	DE	SKO_18x57	SKO	20	20	7	13	0.35	98.27	14.74
2014	GPS_5x3	DE	SP_1x13	SP	20	20	1	19	0.05	75	9.96
2015	GPS_117x111	DE	IBB_39	IBB	20	19	10	9	0.53	89.95	11.04
2015	GPS_117x111	DE	ISC_70	ISC	20	19	5	14	0.26	106.74	14.19
2015	GPS_117x111	DE	NST_8x9	NST	20	20	8	12	0.40	38.5	5.1
2015	GPS_117x111	DE	SKO_57x58	SKO	13	12	3	9	0.25	109.53	14.95
2015	GPS_125x105	DE	IBB_35	IBB	20	20	5	15	0.25	75.56	15.89
2015	GPS_125x105	DE	ISC_59	ISC	20	20	2	18	0.10	70.95	16.47
2015	GPS_125x105	DE	NST_2x7	NST	20	18	2	16	0.11	47.3	8.87
2015	GPS_125x105	DE	SKO_26x44	SKO	20	20	4	16	0.20	131.3	21.55
2015	GPS_22x4	DE	IBB_41	IBB	20	20	3	17	0.15	87.63	13.32
2015	GPS_22x4	DE	ISC_61	ISC	20	16	5	11	0.31	89.34	13.8
2015	GPS_22x4	DE	NST_13x14	NST	20	20	10	10	0.50	48.07	7.56
2015	GPS_22x4	DE	SKO_18x57	SKO	20	20	7	13	0.35	136.99	20.29
2015	SKO_10x6	NO	ECH_3x10	ECH	20	18	4	14	0.22	25.15	4.12
2015	SKO_10x6	NO	GOT_13x8	GOT	19	17	4	13	0.24	12.83	2.18
2015	SKO_10x6	NO	IBB_41	IBB	20	17	2	15	0.12	46.7	7.08
2015	SKO_10x6	NO	ISC_61	ISC	20	18	6	12	0.33	50.93	7.59
2015	SKO_10x6	NO	NST_13x14	NST	20	16	4	12	0.25	3.85	0.85
2015	SKO_10x6	NO	NU_5x18	NU	14	14	7	7	0.50	59.57	9.01
2015	SKO_10x6	NO	OBB_18x20	OBB	20	17	10	7	0.59	6.89	1.18
2015	SKO_10x6	NO	SKO_18x57	SKO	20	14	8	6	0.57	69.08	10.91
2015	SKO_10x6	NO	SP_8x17	SP	20	18	5	13	0.28	45.18	7.43
2015	SKO_11x2	NO	ECH_9x14	ECH	20	17	0	17	0.00	na	na
2015	SKO_11x2	NO	GOT_1x5	GOT	20	19	2	17	0.11	10.3	1.71
2015	SKO_11x2	NO	IBB_39	IBB	20	17	2	15	0.12	55.85	8.75
2015	SKO_11x2	NO	ISC_70	ISC	20	14	5	9	0.36	45.04	6.7
2015	SKO_11x2	NO	NST_8x9	NST	20	16	2	14	0.13	10.95	1.75
2015	SKO_11x2	NO	NU_8x17	NU	20	16	6	10	0.38	88.65	12.31
2015	SKO_11x2	NO	OBB_11x48	OBB	20	15	1	14	0.07	2.8	0.5
2015	SKO_11x2	NO	SKO_57x58	SKO	20	19	2	17	0.11	123	13.65
2015	SKO_11x2	NO	SP_10x12	SP	20	16	2	14	0.13	51.8	7.16
2015	SKO_4x17	NO	ECH_6x23	ECH	20	19	1	18	0.05	42.9	7.28
2015	SKO_4x17	NO	GOT_9x6	GOT	20	17	1	16	0.06	0.6	0.11
2015	SKO_4x17	NO	IBB_35	IBB	20	17	3	14	0.18	53.97	8.62
2015	SKO_4x17	NO	ISC_59	ISC	20	10	1	9	0.10	89.3	13.89
2015	SKO_4x17	NO	NST_2x7	NST	20	15	2	13	0.13	7.2	1.22
2015	SKO_4x17	NO	NU_10x14	NU	20	16	5	11	0.31	68.42	10.8
2015	SKO_4x17	NO	OBB_5x16	OBB	20	18	1	17	0.06	3.4	0.51
2015	SKO_4x17	NO	SKO_26x44	SKO	20	14	4	10	0.29	83.78	12.78
2015	SKO_4x17	NO	SP_1x13	SP	20	16	2	14	0.13	34.15	5.46

Table S4. Host and parasite effects and their interaction on infection rates

Host effect, parasite effect and interaction				
Data subset	Explanatory	Df	Chisq	p-value
Contrast 1 (simultaneously infected DE and NO hosts)	*S. solidus* origin	3	0.8817	0.82985
	Host population	1	2.2658	0.13226
	S. solidus origin : host population	3	6.4206	0.09285
Parasite effect				
Data subset	Explanatory	Df	Chisq	p-value
Contrast 2 (DE in 2014)	*S. solidus* origin	6	7.1518	0.307
Contrast 3 (NO in 2015)	*S. solidus* origin	8	21.619	0.00567

Differences between the two years of the experiment				
Data subset	Explanatory	Df	Chisq	p-value
DE hosts infected in 2014 and 2015	*S. solidus* origin	1	1.1665	0.28013
	Round	5	9.416	0.09358
	S. solidus origin : round	3	3.4414	0.32844

Sticklebacks from two different host populations (DE and NO) were exposed to *S. solidus* parasites from nine different locations in three experiments over two consecutive years (2014 and 2015). The infection rates were analysed as proportional data (accounting for the copepods that were not ingested) with binomial error structure. We tested for differences between the years by using data of hosts that were exposed to the same sibships in the two years of the experiment. The respective generalized linear model (GLM) included 'round' (fish family x parasite sibship combination) and the interaction with *S. solidus* origin as an explanatory. Host and parasite effects were analysed with GLMMs including 'round' as random effect.

SI.2 Supplementary information on parasite indices (contrast 1)

Table S5. The effect of host and parasite population on parasite indices

Explanatory	numDF	denDF	F-value	p-value	R^2
Host population	1	95	23.48201	< 0.0001	0.8934325
S. solidus origin	3	95	78.93636	< 0.0001	
Host population : *S. solidus* origin	3	95	0.99526	0.3986	

Sticklebacks were infected with single *S. solidus* parasites from NST, IBB, ISC, or SKO. The linear mixed model (LMM) included 'round', i.e. host and parasite genotype combinations, as random intercept. The R^2 includes the effect of the random term and was calculated according to (Nakagawa and Schielzeth, 2013; Johnson, 2014; Lefcheck, 2016).

Table S6. Post hoc testing using manually defined contrast to determine differences between fish populations.

	Parasite origin	Estimate	Std. Err	t-value	Pr(>|t|)
DE vs NO fish	IBB	-5.1459	1.0619	-4.846	< 0.0001
	ISC	-6.5330	1.0289	-6.350	< 0.0001
	NST	-5.9784	0.7431	-8.045	< 0.0001
	SKO	-7.7660	1.1629	-6.678	< 0.0001

Table S7. Post hoc testing using manually defined contrast to determine differences between origin of the parasites.

	Parasite origins			Estimate	Std. Error	t-value	Pr(>|t|)
DE fish	ISC	vs	IBB	1.6325	0.9230	1.769	0.4925
	NST	vs	IBB	-5.9838	0.7002	-8.545	<0.001
	SKO	vs	IBB	6.2738	1.0076	6.227	<0.001
	NST	vs	ISC	-7.6163	0.8279	-9.200	<0.001
	SKO	vs	ISC	4.6413	1.1015	4.214	<0.001
	SKO	vs	NST	12.2576	0.9186	13.344	<0.001
NO fish	ISC	vs	IBB	0.2453	1.1679	0.210	1.0000
	NST	vs	IBB	-6.8163	1.0961	-6.219	<0.001
	SKO	vs	IBB	3.6537	1.2217	2.991	0.0353
	NST	vs	ISC	-7.0617	0.9627	-7.335	<0.001
	SKO	vs	ISC	3.4083	1.1063	3.081	0.0273
	SKO	vs	NST	10.4700	1.0327	10.139	<0.001

SI.3 Supplementary information on host condition and immunological parameters

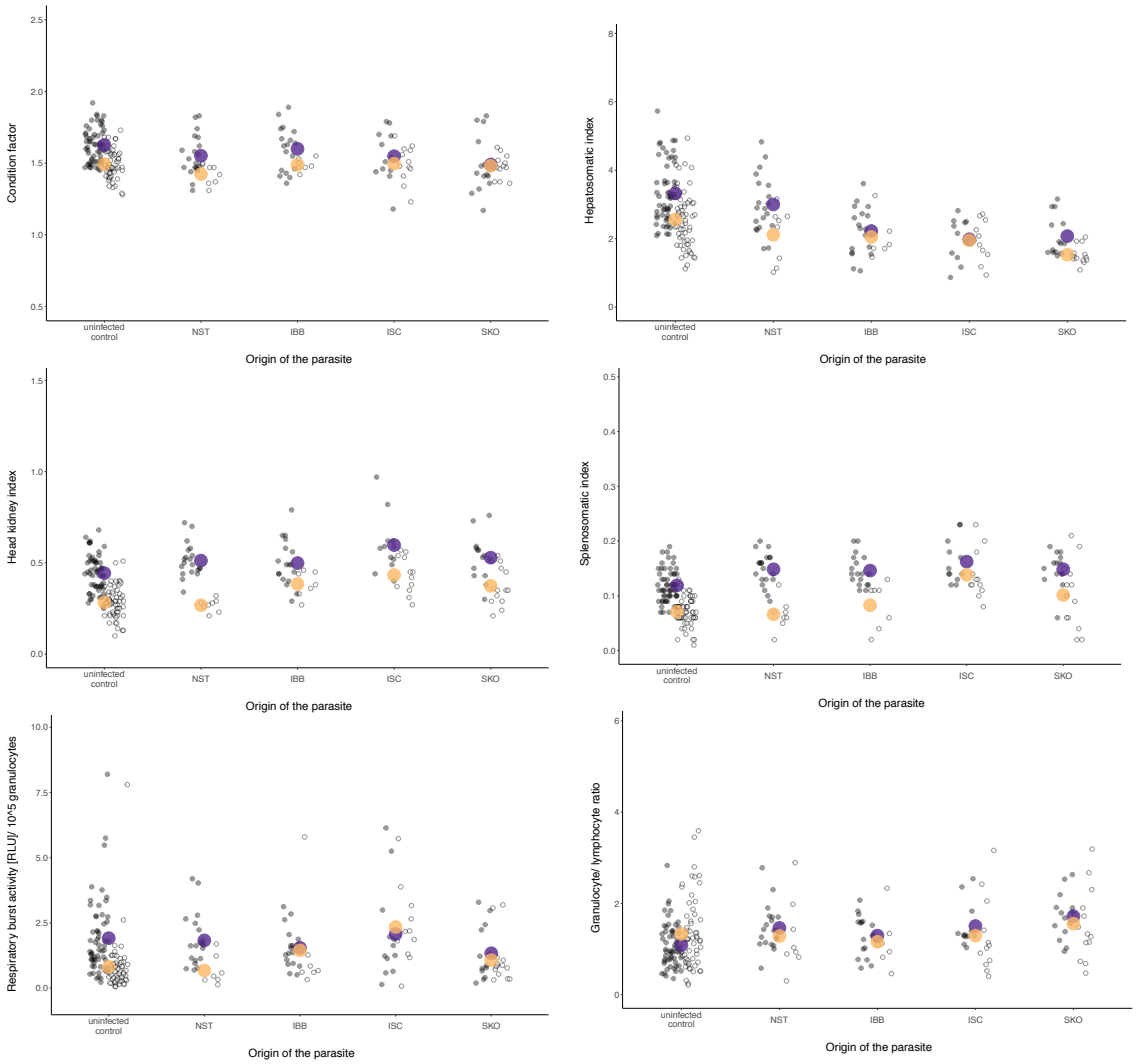


Figure S1. Phenotypic differences between NO (orange) and DE (violet) sticklebacks (*contrast 1*). The fish were either sham-exposed or infected with single *S. solidus* parasites from the Baltic (NST: Neustädter Binnenwasser, Germany), the European Inland (IBB: Ibbenbürener Aa, Germany), or the Atlantic region (ISC: Lake Myvatn, Iceland; SKO: Lake Skogseidvatnet, Norway). The parasite sibships, i.e. genotypes, were the same for both host populations. The fish were dissected 55 (+/- 2) DPE.

We determined the overall condition (condition factor, CF, the ratio between the observed weight *W* (in g) and the expected weight at a given length *L* (in cm): $CF = 100 * W/L^b$. The expected weight depends on the exponent *b*, which is characteristic for each fish population and was calculated by regression analysis of logarithm-transformed data of the length and the weight of all fish from each experiment, (Frischknecht, 1993)) and estimates of metabolic reserves (hepatosomatic index, $HSI = 100 * W_L/ W$, with W_L representing the weight of the liver, (Chellappa et al., 1995)) and immunological activity (splenosomatic index, $SSI = 100 * W_S/ W$, with W_s representing the weight of the spleen, (Seppänen et al., 2009); head kidney index, HKI, the weight of the head kidney in relation to body weight). Numbers of granulocytes and lymphocytes in 0.5 mL head kidney leukocyte (HKL) cell suspensions were used to calculate the granulocyte to lymphocyte (G/L) ratio as a proxy for the activity of the innate versus the adaptive immune system. Relative light units (RLUs) in a lucigenin-enhanced chemi-luminescence assay quantify the production of reactive oxygen species (ROS) and hence phagocytic capacity of HKL.

Cell suspensions of HKL were prepared by forcing tissue samples through a 40 µm nylon mesh (BD Falcon, USA). The cells were transferred to a 96 deep well plate and rinsed twice in R-90 (90% (v/v) RPMI 1640 in distilled water) at 600 *g* for 10 min at 4 °C. Total cell numbers were determined by a modified protocol (Scharsack et al., 2004) of the Standard cell dilution assay (Pechhold et al., 1994). Therefore, each sample was supplemented with 2 mg/L propidium iodide (Sigma Aldrich) and 3 x 10^4 green fluorescent reference particles (4 µm, Polyscience, USA). FSC/SSC characteristics were measured in linear mode for one minute or for up to 10,000 events using a Becton Dickinson FACS Calibur and BD CellQuest™ pro software (Version 6.0). Propidium iodide positive (i.e. dead) cells and cellular debris (low FSC characteristics) were excluded from further analyses. Granulocytes and leukocytes were identified according to their FSC/SSC profiles. The numbers of viable granulocytes and lymphocytes in 0.5 mL were used to calculate the granulocyte to lymphocyte ratio (G/L ratio) (Kurtz et al., 2004). A lucigenin-enhanced chemiluminescence (CL) assay (Scott and Klesius, 1981; Kurtz et al., 2004) was used for functional analysis of innate immune activity. The CL assay measures the phagocytic capacity of HKL by quantifying the respiratory burst reaction in relative luminescence units (RLUs). Briefly, 10^5 live cells per sample were supplemented with 50 µg lucigenin (Sigma M 8010) and incubated at 18 °C and 2% CO_2 for 30 min. Zymosan (Sigma Z 4250) was added at a final concentration of 0.75 µg/µL to stimulate the production of reactive oxygen species (ROS).

Chemiluminescence was measured every 3 min for 3.5 hours (Berthold Technologies luminometer) and the area under the kinetic curve (calculated with Win Glow 2000 professional software) was used for analyses. The RLU was standardized by division by the mean RLU of the negative controls (wells containing buffer without head kidney cells) for each day and by division by the number of vital granulocytes of the respective sample. Unfortunately, we could not obtain enough cells from every fish (data was missing from 13 samples) and thus analysed production of reactive oxygen species of a total of 1430 different samples. Controls (medium without cells) were missing for one round in 2015. Values for those controls were inferred from data from empty wells in relation to controls.

Testing these condition and immunity related indices in each experiment, DE sticklebacks (*contrast 2*; Figure S2) showed significantly elevated immune parameters if they were infected with Pacific *S. solidus*: the head kidneys were larger (LMM; $p < 0.001$), the G/L ratio was significantly higher in comparison to all but SKO-infected fish (LMM; $p < 0.001$) and the head kidney's potential to produce reactive oxygen species was higher in comparison to controls (LMM; $p < 0.001$) and SKO-parasite infected fish (LMM; $p = 0.005$). The fish had significantly lower body condition than their respective controls if infected with Spanish parasites (LMM, $p < 0.001$) (Figure S2). In *contrast 1*, DE fish had significantly lower body condition than respective controls if infected with SKO-parasites (LMM; $p = 0.003$). The Hepatosomatic index was significantly smaller when fish were infected with fast growing *S. solidus* from IBB, ISC or SKO (LMMs; $p < 0.001$). Compared to controls, spleens were enlarged if fish were infected with parasites from ISC (LMM; $p < 0.001$), NST (LMM; $p < 0.001$) or SKO (LMM; $p = 0.003$). Head kidneys were larger in ISC-infected fish than in control fish (LMM; $p < 0.001$) and the G/L ratio was significantly higher in SKO-infected fish than in control fish (LMM; $p < 0.001$) (Figure S3).

Relative to the control, NO sticklebacks had significantly lower Hepatosomatic indices when they were infected with sympatric (SKO-) *S. solidus* parasites (LMM; $p < 0.001$). The Splenosomatic index was higher in ISC-parasite infected fish in comparison to controls and NST-parasite infected fish (LMMs; each $p < 0.001$). Head kidney related immune parameters did not differ between infected and uninfected NO sticklebacks (Figure S4).

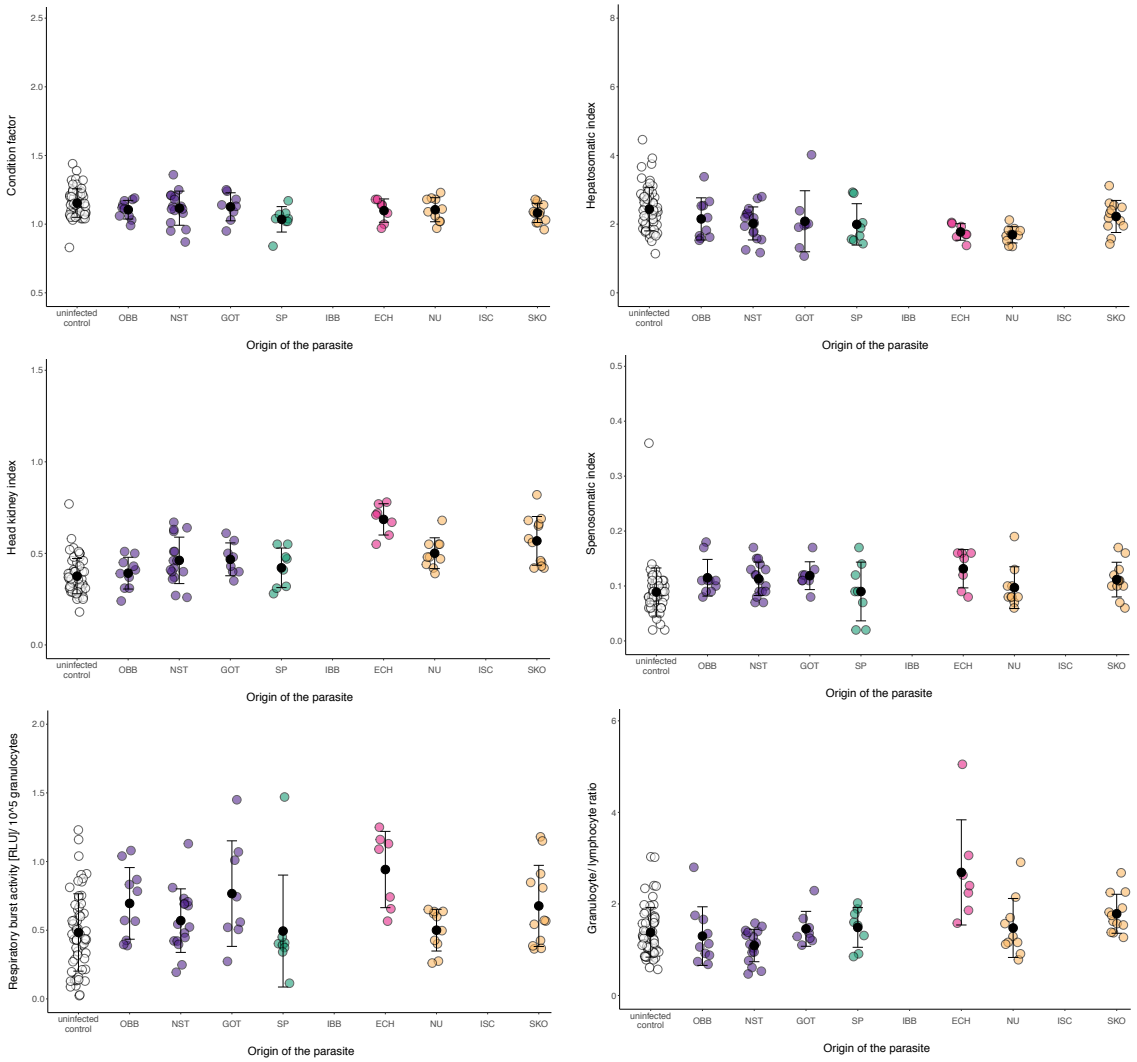


Figure S2. Phenotypic differences between sham-exposed and *S. solidus* infected DE sticklebacks (*contrast 2*).

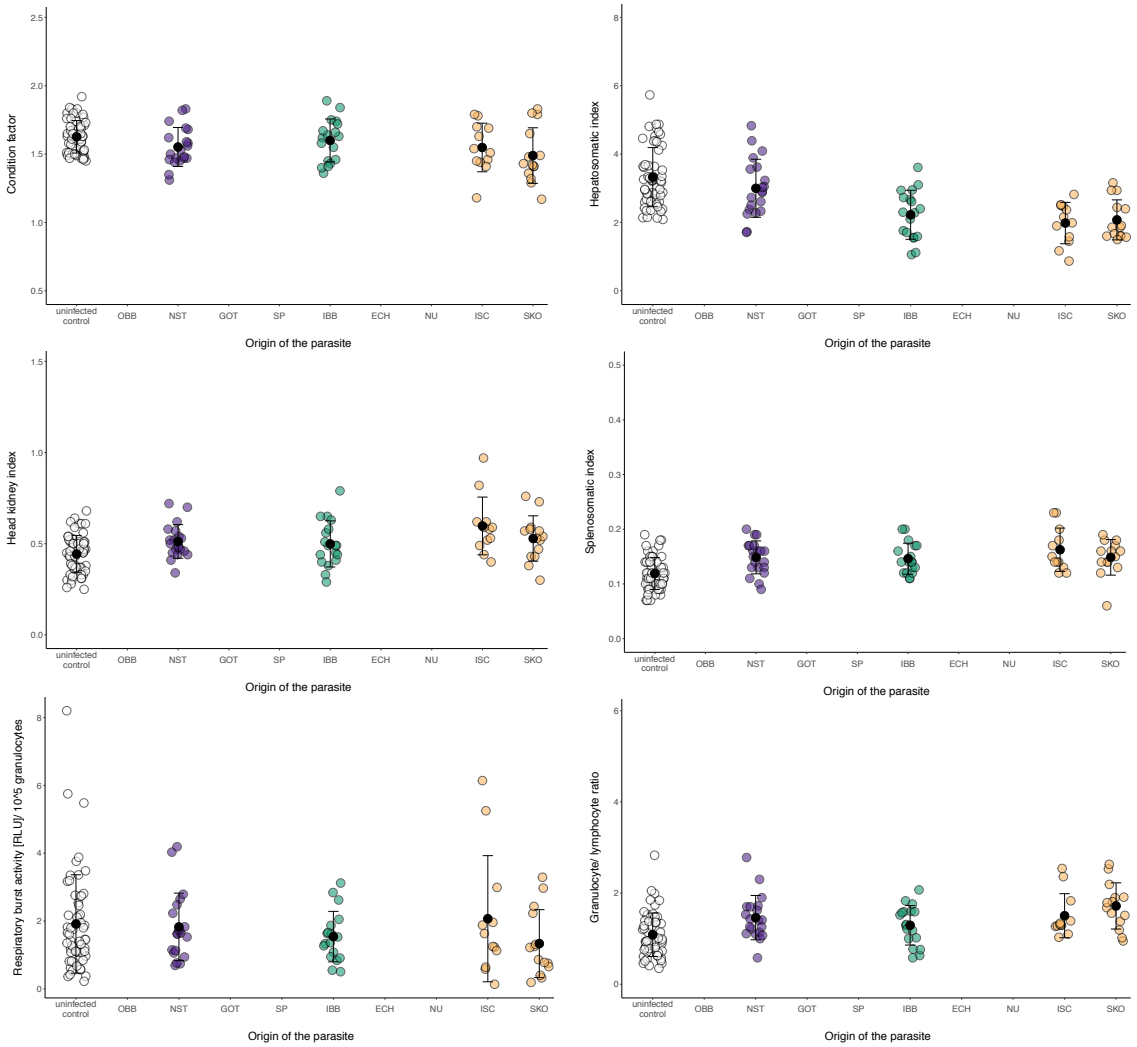


Figure S3. Phenotypic differences between sham-exposed and *S. solidus* infected DE sticklebacks (DE in *contrast 1*).

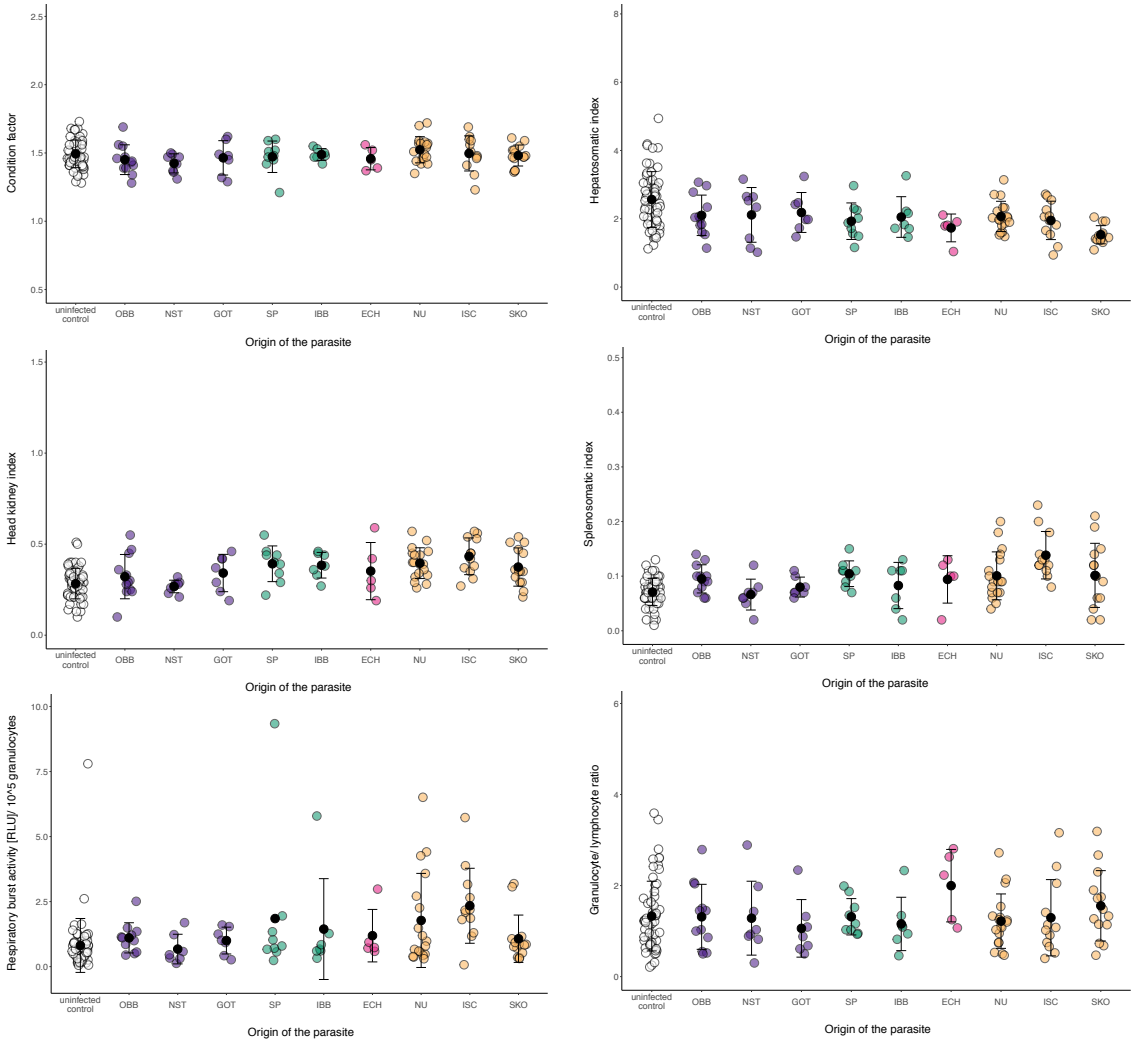


Figure S4. Phenotypic differences between sham-exposed and *S. solidus* infected NO sticklebacks (*contrast 3*).

SI.4 Supplementary information on host immune gene expression (contrast 1)

SI.4.1 Stickleback immune gene expression differences between populations

Table S8. Differentially expressed immune genes of control DE and NO sticklebacks (*contrast 1*)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2	
all genes	1	10.226	4.4637	0.0012	0.08704	
innate	1	4.249	3.3172	0.009699	0.06595	
ANOVA results		numDF	denDF	F-value	p-value	pseudo R2
	marco	1	44	1.034201	0.3147	0.202792
	mst1ra	1	44	0.988256	0.3256	0.1088608
	mif1	1	44	0.003197	0.9552	0.2315523
	il-1β	1	44	0.202999	0.6545	0.04337287
	tnfr1	1	44	0.7095827	0.4041	0.175504
	saal1	1	44	0.7411738	0.3940	0.1033716
	tlr2	1	44	0.411762	0.5244	0.1649114
	csf3r	1	44	7.288786	0.0098	0.4030412
	p22^{phox}	1	44	19.317685	**0.0001**	0.4384317
	nkef-b	1	44	1.349697	0.2516	0.05610734
	sla1	1	44	9.537701	**0.0035**	0.3700301
	cd97	1	44	3.454024	0.0698	
adaptive	1	3.9233	6.7610	0.0018	0.12663	
	stat4	1	44	3.703683	0.0608	0.07364854
	stat6	1	44	5.313142	0.0259	0.319497
	igm	1	44	11.776301	**0.0013**	0.3308283
	cd83	1	44	0.071832	0.7899	0.03668696
	foxp3	1	44	0.7612114	0.3877	0.2346003
	tgf-β	1	44	0.3527227	0.5556	0.127027
	tcr-β	1	44	54.47217	**< 0.0001**	0.5979209
	il16	1	44	2.9887492	0.0909	0.2852176
	mhcII	1	44	6.409371	0.0150	0.6314256
complement	1	2.0539	4.7784	0.0039	0.09302	
	cfb	1	44	5.792049	0.0204	0.3207292
	c7	1	44	20.639223	**< 0.0001**	0.3323971
	c9	1	44	0.8890409	0.3509	0.1934673

All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S9. Differentially expressed immune genes of ISC-infected DE and NO sticklebacks (*contrast 1*)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	6.123	2.8019	0.015798	0.10076
innate	1	4.661	3.5770	0.0044	0.12548

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	18	0.8257869	0.3755	0.2335003
mst1ra	1	18	1.891570	0.1859	0.4391818
mif1	1	18	0.1941722	0.6647	0.2549664
il-1β	1	18	19.989167	**0.0003**	0.4332452
tnfr1	1	18	0.222879	0.6425	0.3809059
saal1	1	18	0.003260	0.9551	0.5787717
tlr2	1	18	0.032950	0.8580	0.267587
csf3r	1	18	4.140233	0.0569	0.1009758
p22^{phox}	1	18	0.284175	0.6005	0.3422705
nkef-b	1	18	3.658218	0.0718	0.3553208
sla1	1	18	0.000222	0.9883	0.3292233
cd97	1	18	0.195377	0.6637	0.2935738
adaptive	not significant after FDR correction				
complement	not significant after FDR correction				

Sticklebacks were infected with single *S. solidus* plerocercoids from an Icelandic (ISC) population. All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

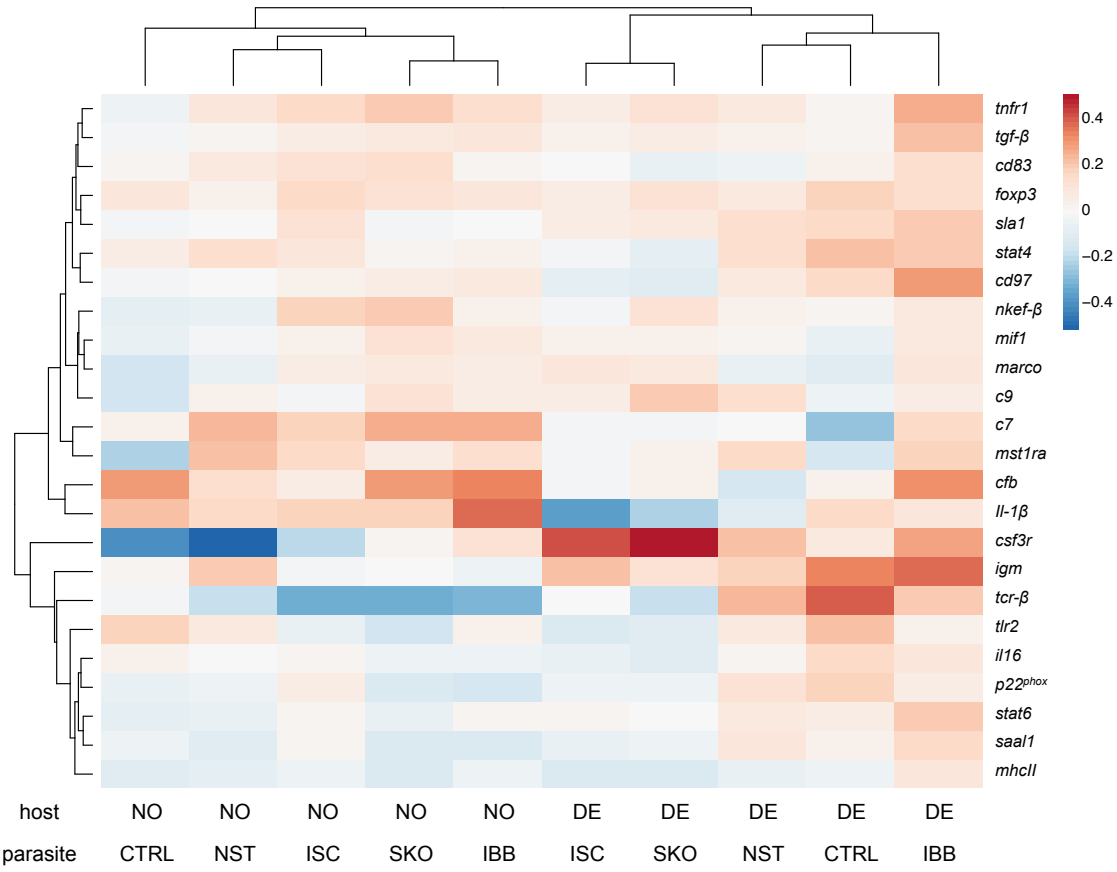


Figure S6. Heatmap showing average gene expression values in spleen samples from sham-exposed (CTRL) and *S. solidus* infected DE and NO sticklebacks. Calibrated normalized relative quantities (CNRQ) were log10-transformed. The heatmap (function aheatmap() from *NMF*) was based on Euclidian distances; rows and columns were clustered according to similarity.

SI.5 Supplementary information for contrast 2 and contrast 3

Table S10. The effect of *S. solidus* origin on parasite indices in DE fish (*contrast 2*)

Explanatory			R^2	numDF	denDF	F-value	p-value
S. solidus origin			0.7841811	6	62	42.39099	< 0.0001
				Estimate	Std. Error	z-value	Pr(>|z|)
GOT	vs	ECH		-6.1425	1.0848	-5.663	<0.001
NST	vs	ECH		-7.5550	0.9467	-7.980	<0.001
NU	vs	ECH		0.1340	1.0296	0.130	1.00000
OBB	vs	ECH		-7.8000	1.0296	-7.576	<0.001
SKO	vs	ECH		2.0792	0.9936	2.093	0.35358
SP	vs	ECH		-2.0675	1.0812	-1.912	0.46838
NST	vs	GOT		-1.4125	0.9046	-1.561	0.70422
NU	vs	GOT		6.2765	0.9910	6.334	<0.001
OBB	vs	GOT		-1.6575	0.9910	-1.673	0.63143
SKO	vs	GOT		8.2217	0.9536	8.622	<0.001
SP	vs	GOT		4.0750	1.0446	3.901	0.00185
NU	vs	NST		7.6890	0.8422	9.130	<0.001
OBB	vs	NST		-0.2450	0.8422	-0.291	0.99995
SKO	vs	NST		9.6342	0.7978	12.076	<0.001
SP	vs	NST		5.4875	0.9046	6.066	<0.001
OBB	vs	NU		-7.9340	0.9343	-8.492	<0.001
SKO	vs	NU		1.9452	0.8945	2.175	0.30647
SP	vs	NU		-2.2015	0.9910	-2.222	0.28062
SKO	vs	OBB		9.8792	0.8945	11.044	<0.001
SP	vs	OBB		5.7325	0.9910	5.785	<0.001
SP	vs	SKO		-4.1467	0.9536	-4.349	< 0.001

Sticklebacks were infected with single *S. solidus* from OBB, NST, GOT, SP, ECH, NU or SKO. The linear mixed model (LMM) included 'round', i.e. host and parasite genotype combinations, as random intercept. The R^2 includes the effect of the random term and was calculated according to (Nakagawa and Schielzeth, 2013; Johnson, 2014; Lefcheck, 2016). Post hoc tests are based on Tukey's all pair comparisons.

Table S11. The effect of *S. solidus* origin on parasite indices in DE fish (in *contrast 1*)

Explanatory			R^2	numDF	denDF	F-value	p-value
S. solidus origin			0.8037465	3	58	68.63429	< 0.0001
				Estimate	Std. Error	z-value	Pr(>|z|)
ISC	vs	IBB		1.5266	0.9236	1.653	0.348
NST	vs	IBB		-6.0916	0.8223	-7.408	<0.001
SKO	vs	IBB		5.9563	0.9007	6.613	<0.001
NST	vs	ISC		-7.6181	0.8934	-8.527	<0.001
SKO	vs	ISC		4.4297	0.9692	4.571	<0.001
SKO	vs	NST		12.0478	0.8638	13.947	<0.001

Sticklebacks were infected with single *S. solidus* from NST, IBB, ISC, or SKO. The linear mixed model (LMM) included 'round', i.e. host and parasite genotype combinations, as random intercept. The R^2 includes the effect of the random term and was calculated according to (Nakagawa and Schielzeth, 2013; Johnson, 2014; Lefcheck, 2016). Post hoc tests are based on Tukey's all pair comparisons.

Table S12. The effect of *S. solidus* origin on parasite indices in NO sticklebacks (*contrast 3*)

Explanatory			R^2	numDF	denDF	F-value	p-value
S. solidus origin			0.6242631	8	81	61.08925	< 0.0001
				Estimate	Std. Error	z-value	Pr(>|z|)
GOT	vs	ECH		-2.99800	1.31129	-2.286	0.30948
IBB	vs	ECH		3.46771	1.32980	2.608	0.15671
ISC	vs	ECH		2.99283	1.38830	2.156	0.39017
NST	vs	ECH		-3.58425	1.23234	-2.908	0.07195
NU	vs	ECH		5.85811	1.41111	4.151	<0.001
OBB	vs	ECH		-3.67717	1.22033	-3.013	0.05374
SKO	vs	ECH		7.08414	1.46836	4.825	<0.001
SP	vs	ECH		2.18311	1.67868	1.300	0.91545
IBB	vs	GOT		6.46571	0.77373	8.357	<0.001
ISC	vs	GOT		5.99083	0.87043	6.883	<0.001
NST	vs	GOT		-0.58625	0.59072	-0.992	0.98271
NU	vs	GOT		8.85611	0.90637	9.771	<0.001
OBB	vs	GOT		-0.67917	0.56523	-1.202	0.94534
SKO	vs	GOT		10.08214	0.99316	10.152	<0.001
SP	vs	GOT		5.18111	1.28384	4.036	0.00138
ISC	vs	IBB		-0.47488	0.89808	-0.529	0.99979
NST	vs	IBB		-7.05196	0.63075	-11.180	<0.001
NU	vs	IBB		2.39040	0.93296	2.562	0.17371
OBB	vs	IBB		-7.14488	0.60694	-11.772	< 0.001
SKO	vs	IBB		3.61643	1.01748	3.554	0.00908
SP	vs	IBB		-1.28460	1.30274	-0.986	0.98338
NST	vs	ISC		-6.57708	0.74621	-8.814	<0.001
NU	vs	ISC		2.86528	1.01460	2.824	0.09069
OBB	vs	ISC		-6.67000	0.72620	-9.185	<0.001
SKO	vs	ISC		4.09131	1.09282	3.744	0.00466
SP	vs	ISC		-0.80972	1.36240	-0.594	0.99951
NU	vs	NST		9.44236	0.78784	11.985	<0.001
OBB	vs	NST		-0.09292	0.34448	-0.270	1.00000
SKO	vs	NST		10.66839	0.88631	12.037	<0.001
SP	vs	NST		5.76736	1.20309	4.794	<0.001
OBB	vs	NU		-9.53528	0.76892	-12.401	<0.001
SKO	vs	NU		1.22603	1.12166	1.093	0.96852
SP	vs	NU		-3.67500	1.38564	-2.652	0.14060
SKO	vs	OBB		10.76131	0.86953	12.376	<0.001
SP	vs	OBB		5.86028	1.19078	4.921	<0.001
SP	vs	SKO		-4.90103	1.44390	-3.394	0.01638

Sticklebacks were either sham-exposed or infected with single *S. solidus* from OBB, NST, GOT, SP, IBB, ECH, NU, ISC or SKO. The linear mixed model (LMM) included 'round', i.e. host and parasite genotype combinations, as random intercept. The R^2 was calculated according to (Nakagawa and Schielzeth, 2013; Johnson, 2014; Lefcheck, 2016). Post hoc tests are based on Tukey's all pair comparisons.

SI.5.1 Stickleback gene expression according to clustered localities of parasites

Table S13. The effect of parasite origin on immune gene expression in *S. solidus* infected DE sticklebacks (*contrast 2*: Pacific versus Baltic parasites)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	11.612	4.7401	0.0047	0.13047
innate	1	4.442	3.8777	0.0183	0.10833
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	29	0.6705910	0.4195	0.1577212
mst1ra	1	29	0.7120762	0.4057	0.2422407
mif1	1	29	24.383022	**< 0.0001**	0.4261418
il-1β	1	29	2.646215	0.1146	0.2497382
tnfr1	1	29	19.531804	**0.0001**	0.1943971
saal1	1	29	0.6674974	0.4206	0.05301745
tlr2	1	29	0.9491798	0.3380	0.3058376
csf3r	1	29	8.230615	0.0076	0.5995144
p22^{phox}	1	29	0.3811838	0.5418	0.1390658
nkef-b	1	29	2.782856	0.1060	0.2846311
sla1	1	29	5.547274	0.0255	0.2100529
cd97	1	29	5.671517	0.0240	0.1500293
adaptive	1	3.5866	4.1608	0.0126	0.11695
stat4	1	29	0.1462244	0.7050	0.08385778
stat6	1	29	0.9020573	0.3501	0.04451725
igm	1	29	0.0193593	0.8903	0.4507765
cd83	1	29	1.8584893	0.1833	0.2673836
foxp3	1	29	6.688682	0.0150	0.2362648
tgf-β	1	29	2.9102652	0.0987	0.2368642
tcr-β	1	29	0.273943	0.6047	0.02215725
il16	1	29	4.212606	0.0492	0.2792412
mhcII	1	29	7.260669	0.0116	0.6589304
complement	1	3.5827	8.1042	0.0014	0.20684
cfb	1	29	6.734624	0.0147	0.1718487
c7	1	29	0.754282	0.3923	0.0116967
c9	1	29	10.791768	**0.0027**	0.347015

DE sticklebacks were infected with single *S. solidus* from the Pacific (ECH) or the Baltic region (OBB, NST, GOT). All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the R package piecewiseSEM (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S14. The effect of parasite origin on immune gene expression in *S. solidus* infected DE sticklebacks (*contrast 2*: Pacific versus Atlantic parasites)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	7.483	3.7018	0.0040	0.13122
innate	not significant				
adaptive	1	4.3787	5.8377	0.0002	0.19318

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
stat4	1	22	3.183325	0.0882	0.2131196
stat6	1	22	0.8429660	0.3685	0.3147911
igm	1	22	0.173993	0.6806	0.2110768
cd83	1	22	0.8383639	0.3698	0.03927397
foxp3	1	22	6.090012	0.0218	0.2836478
tgf-β	1	22	0.2983933	0.5904	0.2660784
tcr-β	1	22	2.8142746	0.1076	0.1019166
il16	1	22	2.9249314	0.1013	0.3136332
mhcII	1	22	15.711934	**0.0007**	0.3365486
complement	1	1.6097	3.6639	0.0161	0.13192
cfb	1	22	5.561498	0.0277	0.176698
c7	1	22	0.0013976	0.9705	0.01687028
c9	1	22	2.386635	0.1366	0.2022392

DE sticklebacks were infected with single *S. solidus* from the Pacific (ECH) or the Atlantic region (NU, SKO). All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the R package piecewiseSEM (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S15. The effect of parasite origin on immune gene expression in *S. solidus* infected NO stickle-backs (*contrast* 3: Pacific versus Baltic parasites)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	9.896	4.0409	0.0028	0.12559
innate	1	4.562	3.2630	0.006399	0.10441
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	25	8.392080	**0.0077**	0.2271724
mst1ra	1	25	3.77560	0.0633	0.03548697
mif1	1	25	5.541702	**0.0267**	0.6104602
il-1β	1	25	4.012346	0.0561	0.2204628
tnfr1	1	25	17.895225	**0.0003**	0.4698114
saal1	1	25	0.2236917	0.6403	0.02951859
tlr2	1	25	0.68259	0.4165	0.2310644
csf3r	1	25	3.0020583	0.0955	0.4896976
p22^{phox}	1	25	7.051231	**0.0136**	0.4252072
nkef-b	1	25	11.806990	**0.0021**	0.4074129
sla1	1	25	16.878200	**0.0004**	0.5575598
cd97	1	25	17.052705	**0.0004**	0.1673577
adaptive	1	3.6002	5.7965	0.0020	0.16799
stat4	1	25	20.719634	**0.0001**	0.3796316
stat6	1	25	2.5762096	0.1210	0.101408
igm	1	25	5.040543	0.0338	0.1895309
cd83	1	25	13.400433	**0.0012**	0.4245893
foxp3	1	25	13.907577	**0.0010**	0.4174076
tgf-β	1	25	38.95597	**< 0.0001**	0.2843239
tcr-β	1	25	0.9623782	0.3360	0.4709141
il16	1	25	20.30002	**0.0001**	0.2542416
mhcII	1	25	11.242303	**0.0025**	0.5879825
complement	not significant after FDR correction				

NO sticklebacks were infected with single *S. solidus* from the Pacific (ECH) or the Baltic region (OBB, NST, GOT). All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the R package piecewiseSEM (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S16. The effect of parasite origin on immune gene expression in *S. solidus* infected NO sticklebacks (*contrast 3*: Pacific versus Atlantic parasites)

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	11.136	3.9272	0.0037	0.07781
innate	1	4.772	2.9472	0.0142	0.05932
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	43	1.6216848	0.2097	0.1003356
mst1ra	1	43	10.335062	**0.0025**	0.06026265
mif1	1	43	0.24499306	0.6231	0.600834
il-1β	1	43	7.41588	**0.0093**	0.1414507
tnfr1	1	43	9.378793	**0.0038**	0.1669963
saal1	1	43	0.934775	0.3390	0.1185011
tlr2	1	43	2.2991785	0.1368	0.554773
csf3r	1	43	1.337027	0.2539	0.3369563
p22^{phox}	1	43	7.219358	**0.0102**	0.2022315
nkef-b	1	43	10.156009	**0.0027**	0.08408415
sla1	1	43	21.053776	**< 0.0001**	0.09562046
cd97	1	43	13.395692	**0.0007**	0.07031316
adaptive	1	4.269	5.2682	0.0037	0.10309
stat4	1	43	14.963542	**0.0004**	0.1379449
stat6	1	43	2.9758228	0.0917	0.07796727
igm	1	43	12.137119	**0.0011**	0.2510531
cd83	1	43	5.426821	**0.0246**	0.2126779
foxp3	1	43	7.771886	**0.0079**	0.1427779
tgf-β	1	43	15.66522	**0.0003**	0.06727398
tcr-β	1	43	0.191459	0.6639	0.2949225
il16	1	43	20.661348	**< 0.0001**	0.1248736
mhcII	1	43	8.220390	**0.0064**	0.275311
complement	1	2.0950	5.1591	0.007999	0.09852
cfb	1	43	3.007361	0.0901	0.1230495
c7	1	43	1.942404	0.1706	0.02336822
c9	1	43	17.776506	**0.0001**	0.2357195

NO sticklebacks were infected with single *S. solidus* from Pacific (ECH) or the Atlantic region (NU, ISC, SKO). All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations; permutations were constrained within 'round'. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the R package piecewiseSEM (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

SI.5.2 Stickleback immune gene expression in infected versus control fish

ECH-infected DE sticklebacks had significantly higher expression of three genes of innate immunity, one gene of adaptive immunity (*foxp3*) and complement *c9*; RNA levels of *tcr-β* and *mhcII* were significantly lower than in controls (Table S17). NU-infected DE sticklebacks had significantly higher expression of five innate immune genes and two complement components; again, *tcr-β* was significantly lower expressed than in controls (Table S18). SKO-infected DE sticklebacks had significantly lower expression of the genes *igm* and *tcr-β* (Table S19).

In NO hosts, four genes were significantly higher expressed upon infection with the ECH strain; only RNA levels of *tlr2* were higher in controls. Infection with NU *S. solidus* was linked to lower RNA levels of *foxp3* and *tcr-β* in comparison to controls, *mhcII* RNA levels were higher (Table S20).

Table S17. The effect of Pacific (ECH) *S. solidus* infection on immune gene expression in DE sticklebacks

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	10.968	6.6963	< 0.0001	0.17831
innate	1	5.1367	7.51	< 0.0001	0.19545
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	28	1.3223894	0.2599	0.04482254
mst1ra	1	28	4.963628	0.0341	0.1346899
mif1	1	28	22.335106	**0.0001**	0.4110788
il-1β	1	28	4.135334	0.0516	0.4828812
tnfr1	1	28	38.28464	**< 0.0001**	0.5536073
saal1	1	28	0.9519854	0.3376	0.04472742
tlr2	1	28	2.4176792	0.1312	0.05212668
csf3r	1	28	39.81470	**< 0.0001**	0.5984884
p22^{phox}	1	28	0.014436	0.9052	0.09826987
nkef-b	1	28	4.096781	0.0526	0.1156389
sla1	1	28	5.463977	0.0268	0.1726114
cd97	1	28	3.979375	0.0559	0.2699205
adaptive	1	3.7237	6.4701	0.0006	0.17312
stat4	1	28	0.7173548	0.4042	0.1566161
stat6	1	28	2.750128	0.1084	0.1536997
igm	1	28	0.577116	0.4538	0.05510709
cd83	1	28	0.8975855	0.3515	0.04130682
foxp3	1	28	10.969312	**0.0026**	0.3648484
tgf-β	1	28	6.312335	0.0180	0.1654147
tcr-β	1	28	20.170149	**0.0001**	0.6763045
il16	1	28	5.640592	0.0246	0.1530182
mhcII	1	28	7.095925	**0.0127**	0.6559287
complement	1	2.11	5.5693	0.007	0.15362
cfb	1	28	1.0761172	0.3084	0.05403997
c7	1	28	6.37071	0.0176	0.2160032
c9	1	28	15.702800	**0.0005**	0.3294452

DE sticklebacks were infected with single *S. solidus* from the Pacific region (ECH) or sham-exposed as controls. All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S18. The effect of Atlantic (NU) *S. solidus* infection on immune gene expression in DE sticklebacks

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	6.3423	4.5438	0.0022	0.11700
innate	1	3.2598	4.8912	0.0034	0.12816
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	31	21.277178	**0.0001**	0.2247061
mst1ra	1	31	41.52767	**< 0.0001**	0.3802244
mif1	1	31	2.8351053	0.1023	0.07494536
il-1β	1	31	1.3225529	0.2589	0.4956676
tnfr1	1	31	17.84915	**0.0002**	0.3553141
saal1	1	31	1.1572809	0.2903	0.03720579
tlr2	1	31	0.1355171	0.7153	0.171688
csf3r	1	31	9.019195	**0.0052**	0.3367392
p22^{phox}	1	31	0.0842680	0.7735	0.1051056
nkef-b	1	31	0.2753085	0.6035	0.1033829
sla1	1	31	0.5254048	0.4740	0.215329
cd97	1	31	3.792486	0.0606	0.1558379
adaptive	1	1.817	3.925	0.009	0.0983
stat4	1	31	0.1487538	0.7024	0.2565489
stat6	1	31	5.427391	0.0265	0.225471
igm	1	31	0.002153	0.9633	0.003609847
cd83	1	31	1.2962914	0.2636	0.3051214
foxp3	1	31	0.122438	0.7288	0.1561726
tgf-β	1	31	6.105449	0.0192	0.1567034
tcr-β	1	31	19.427037	**0.0001**	0.6016856
il16	1	31	0.0135641	0.9080	0.1486146
mhcII	1	31	4.943850	0.0336	0.2853142
complement	1	1.2655	4.7503	0.0140	0.12308
cfb	1	31	0.317478	0.5772	0.01028082
c7	1	31	13.294900	**0.0010**	0.3283917
c9	1	31	9.197163	**0.0049**	0.3806867

DE sticklebacks were infected with single *S. solidus* from Scotland (NU) or sham-exposed as controls. All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S19. The effect of Atlantic (SKO) *S. solidus* infection on immune gene expression in DE sticklebacks

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	8.252	4.8340	0.0016	0.12456
adaptive	1	4.7356	8.7604	< 0.0001	0.20144
ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
stat4	1	31	6.408154	0.0166	0.3467709
stat6	1	31	1.371914	0.2504	0.1504773
igm	1	31	21.017566	**0.0001**	0.3755734
cd83	1	31	4.057735	0.0527	0.1049793
foxp3	1	31	0.6079386	0.4415	0.3040527
tgf-β	1	31	1.734336	0.1975	0.05518428
tcr-β	1	31	80.30430	**< 0.0001**	0.7823056
il16	1	31	0.01339549	0.9086	0.008679018
mhcII	1	31	4.254542	0.0476	0.2047914
complement	1	1.2540	3.4196	0.0278	0.09219
cfb	1	31	5.623478	0.0241	0.1556643
c7	1	31	1.80347	0.1890	0.0803123
c9	1	31	0.1222875	0.7289	0.3124093

DE sticklebacks were infected with single *S. solidus* from Norway (SKO) or sham-exposed as controls. All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S20. The effect of *S. solidus* infection on immune gene expression in NO sticklebacks

CTRL vs ECH

PERMANOVA results	Df	SumsOfSqs	F.Model	Pr(<F)	R2
all genes	1	13.034	5.7731	0.006799	0.18870
innate	1	8.038	5.4255	0.0118	0.18141

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	22	7.480525	**0.0121**	0.6146342
mst1ra	1	22	55.38068	**< 0.0001**	0.420314
mif1	1	22	1.7377567	0.2010	0.6540345
il-1β	1	22	4.584579	0.0436	0.1603913
tnfr1	1	22	7.089261	**0.0142**	0.6098981
saal1	1	22	0.03122978	0.8613	0.3679037
tlr2	1	22	7.701943	**0.0110**	0.2455484
csf3r	1	22	0.7428609	0.3980	0.6157382
p22^{phox}	1	22	6.769916	0.0163	0.2206684
nkef-b	1	22	3.1618774	0.0892	0.402492
sla1	1	22	4.423985	0.0471	0.3688205
cd97	1	22	0.0128443	0.9108	0.7624587
complement	1	7.0196	7.6098	0.007799	0.23142
cfb	1	22	1.338124	0.2598	0.1066028
c7	1	22	6.080528	0.0219	0.26121
c9	1	22	11.663511	**0.0025**	0.3391339

CTRL vs NU

all genes	1	7.162	2.77725	0.008699	0.06827
adaptive	1	3.9791	5.7140	0.0016	0.13059

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
stat4	1	35	4.555951	0.0399	0.1020942
stat6	1	35	1.708545	0.1997	0.0958445
igm	1	35	4.462372	0.0419	0.5772781
cd83	1	35	3.0748604	0.0883	0.5380313
foxp3	1	35	12.229478	**0.0013**	0.3642791
tgf-β	1	35	0.00923769	0.9240	0.150044
tcr-β	1	35	18.261184	**0.0001**	0.3470945
il16	1	35	2.0088023	0.1652	0.1248851
mhcII	1	35	38.17083	**< 0.0001**	0.5933614

NO sticklebacks were infected with single *S. solidus* from the Pacific region (ECH) or Scotland (NU) or sham-exposed as controls (CTRL). All models are based on log10-transformed calibrated normalized relative quantities (CNRQ values) and include the weight of the fish as covariate to account for size related effects. If results from multivariate statistics remained significant after FDR correction, single genes were analysed with linear mixed models (LMMs) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with the function sem.model.fits() from *piecewiseSEM* (Lefcheck, 2016). Differentially expressed genes are marked in bold letters if significant after FDR correction.

SI.5.3 NMDS: infected versus control DE sticklebacks (contrast 2)

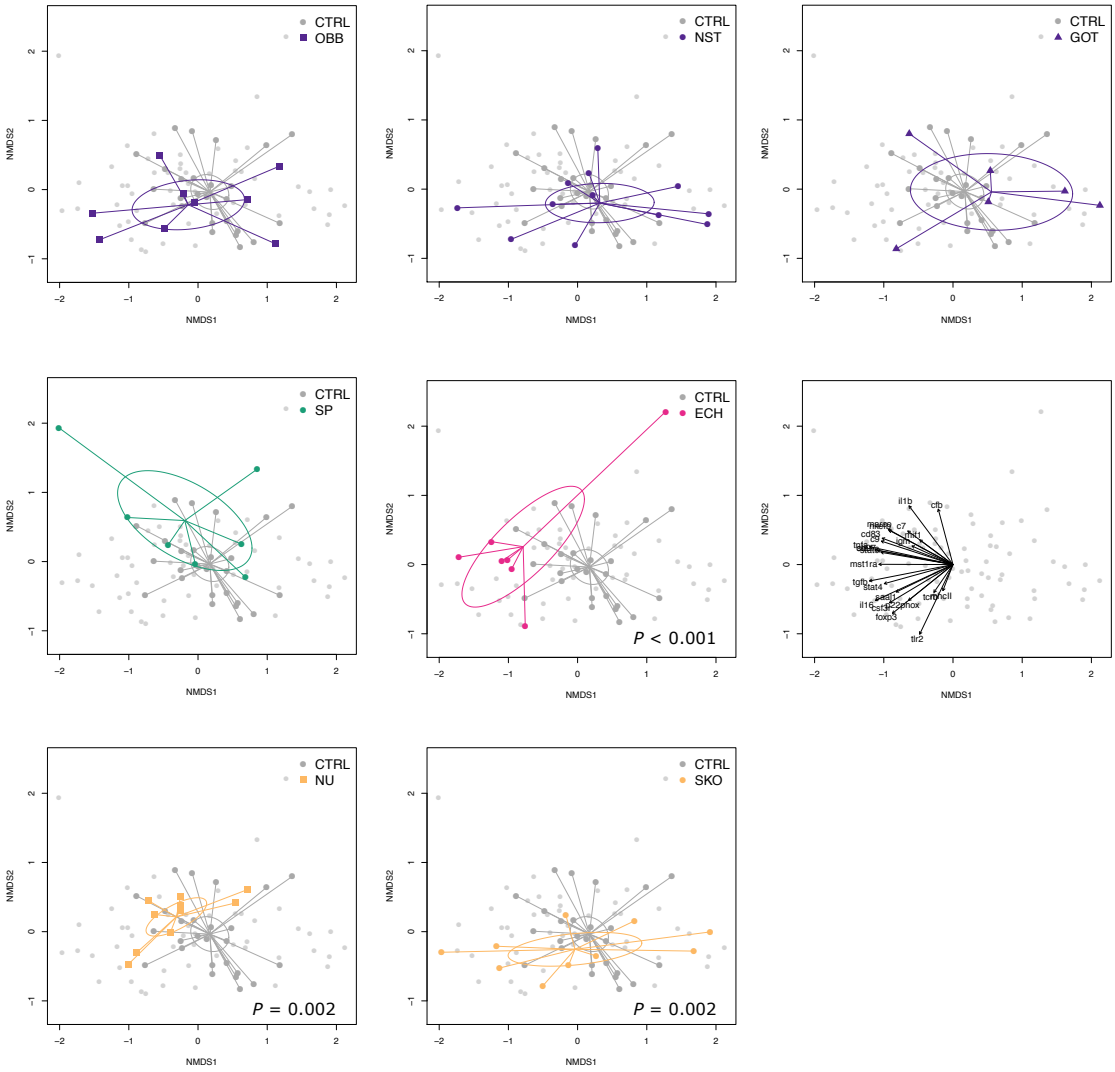


Figure S7. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions showing multivariate data from 24 immune genes of infected and sham-exposed DE sticklebacks (2014). Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

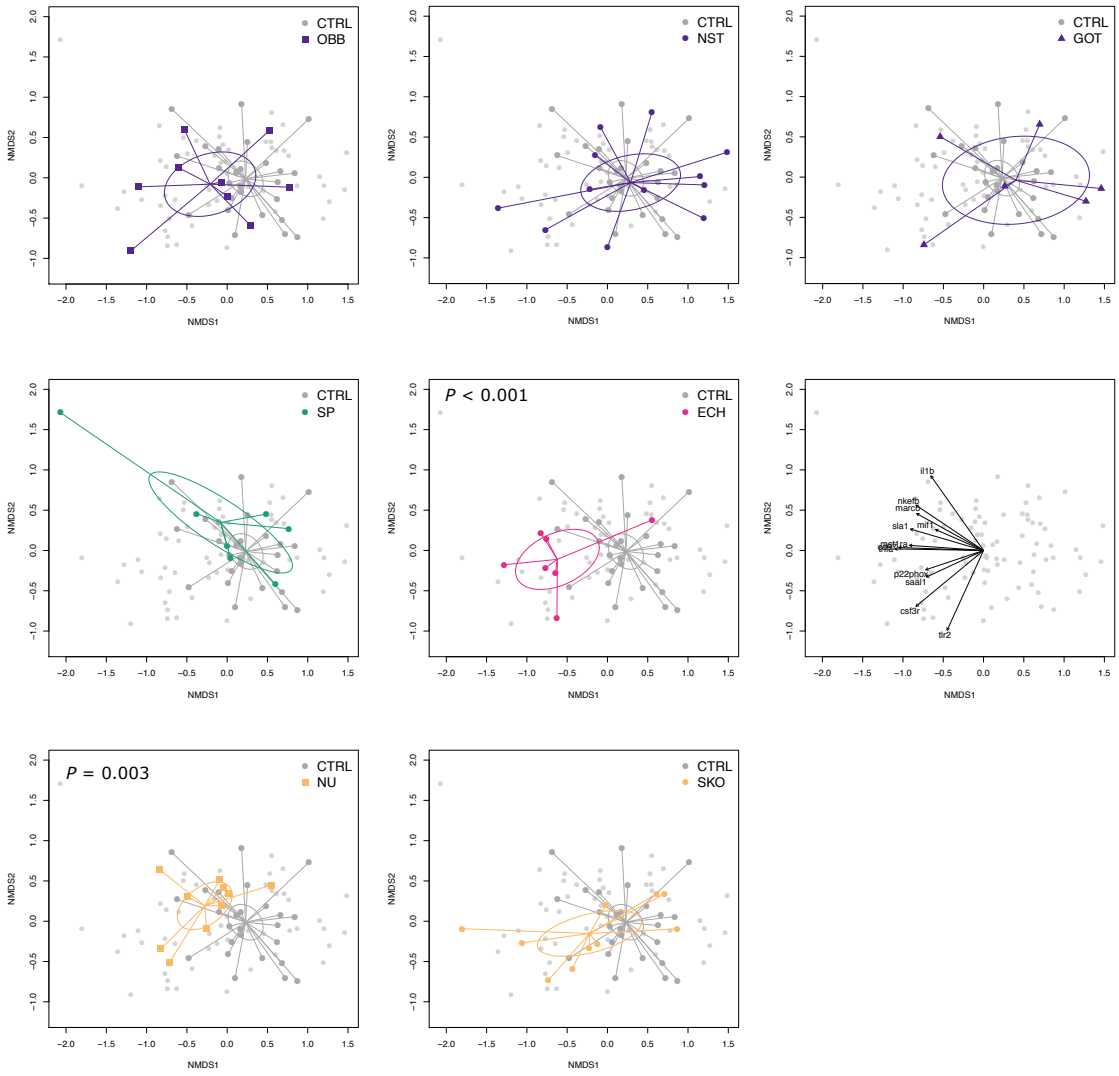


Figure S8. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions showing multivariate data from 12 innate immune genes of infected and sham-exposed (CTRL) DE sticklebacks (2014). Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

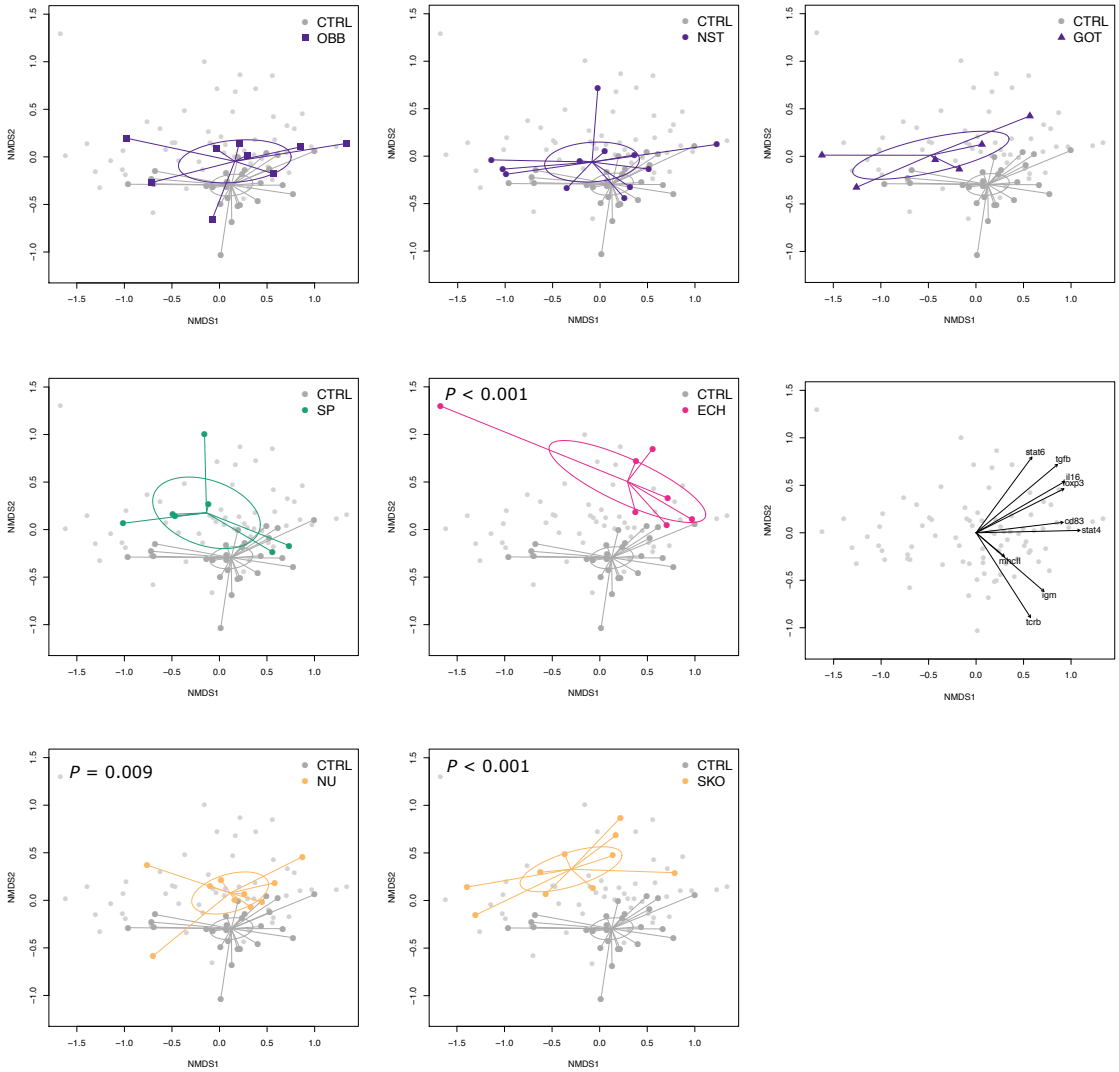


Figure S9. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions showing multivariate data from 9 genes of the adaptive immune system of infected and sham-exposed (CTRL) DE sticklebacks (2014). Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

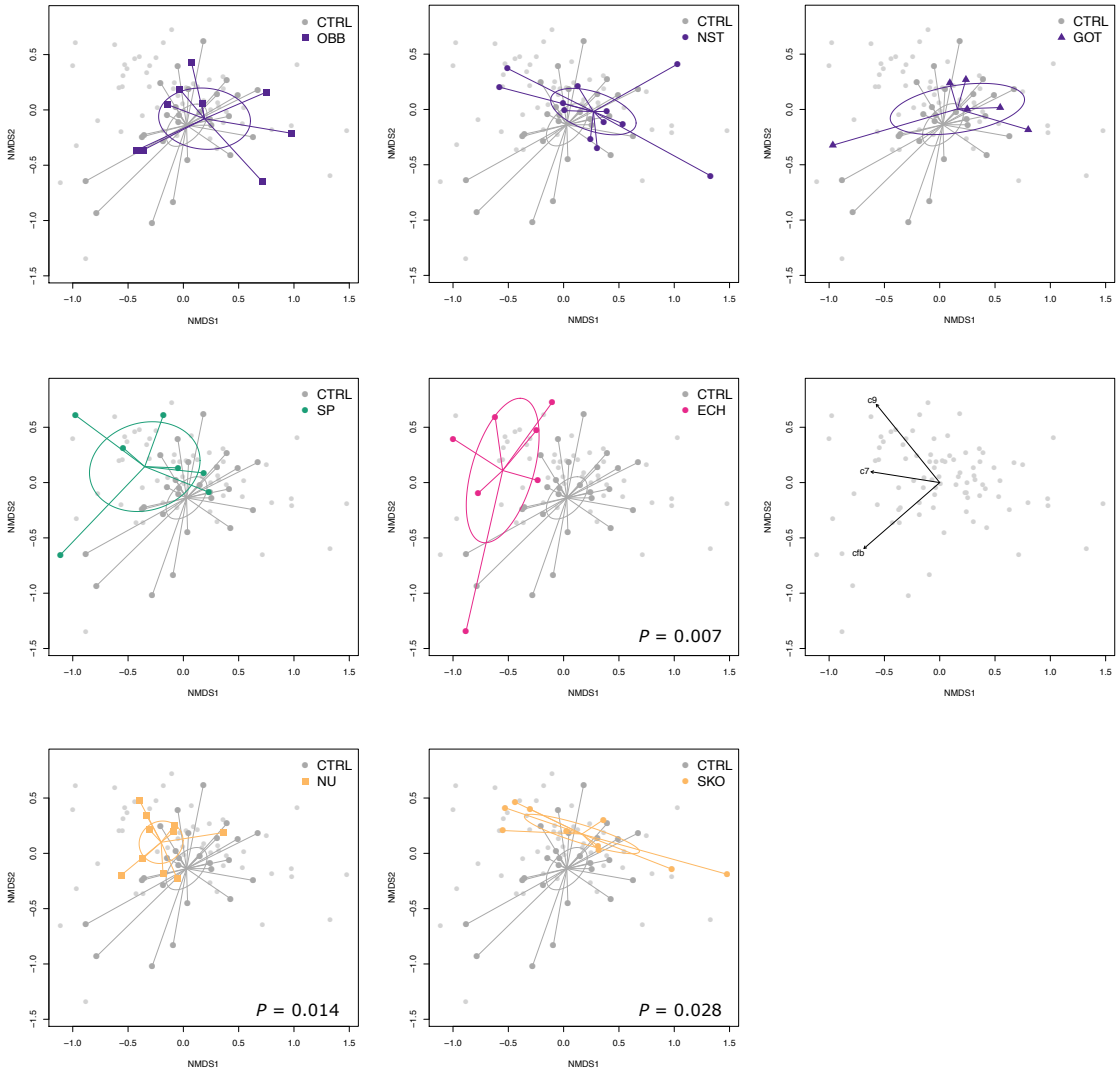


Figure S10. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions showing multivariate data from three genes of the complement system of infected and sham-exposed DE sticklebacks (2014). Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

SI.5.4 NMDS: infected versus control NO sticklebacks (contrast 3)

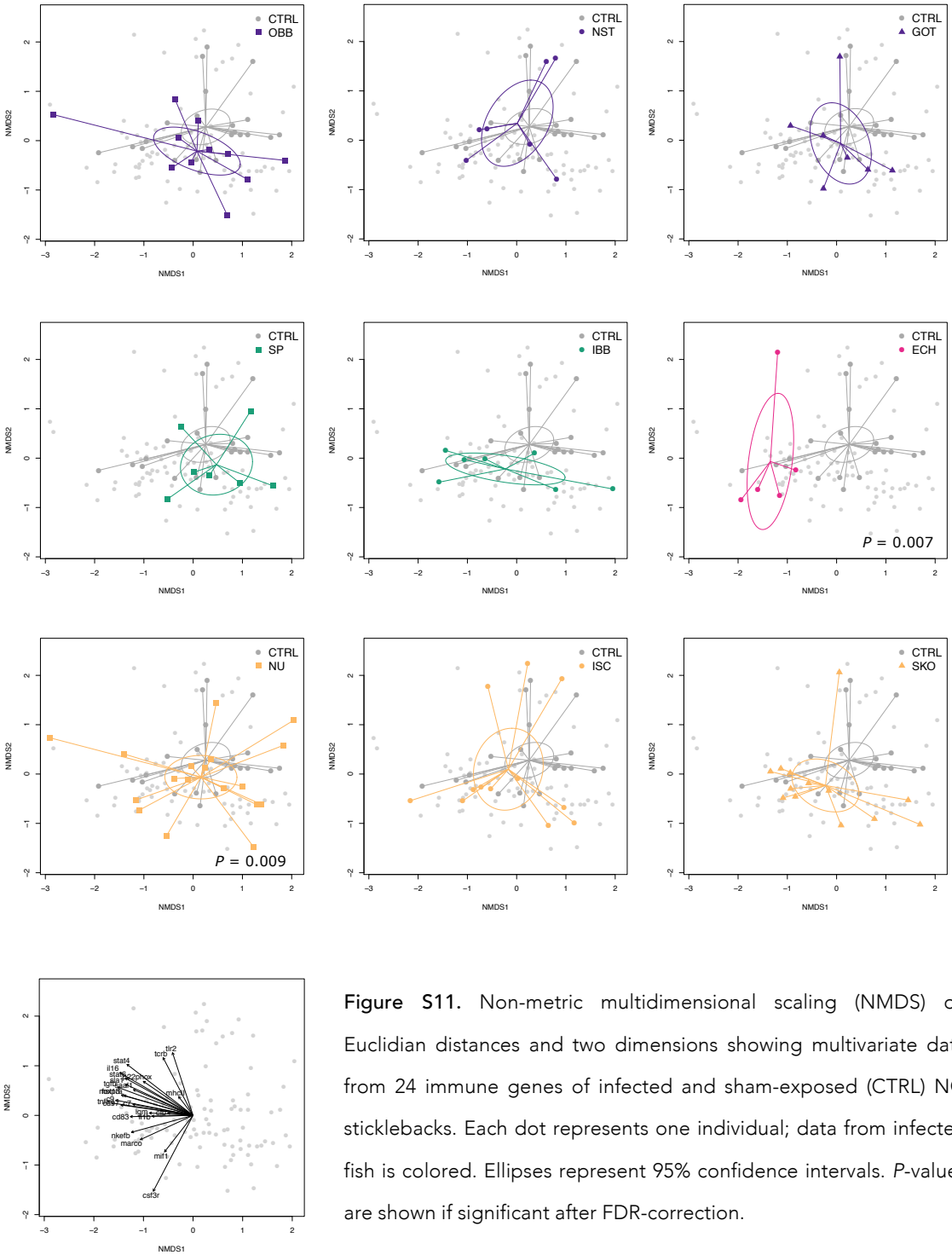
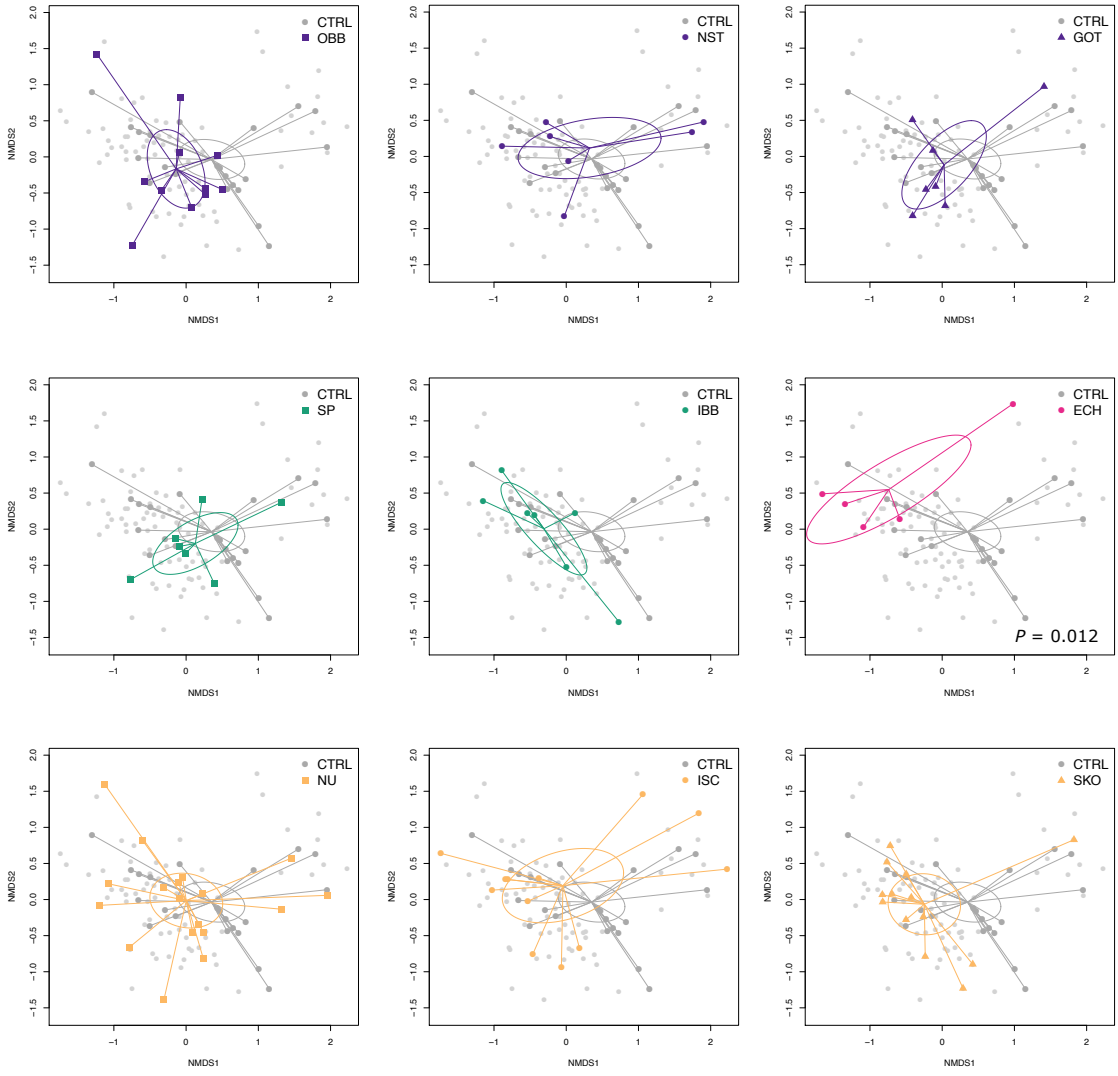


Figure S11. Non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions showing multivariate data from 24 immune genes of infected and sham-exposed (CTRL) NO sticklebacks. Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.



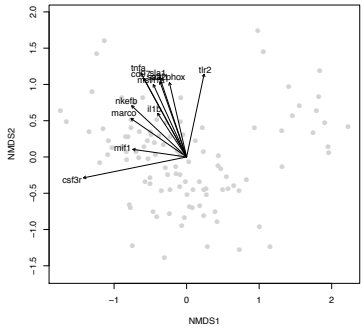
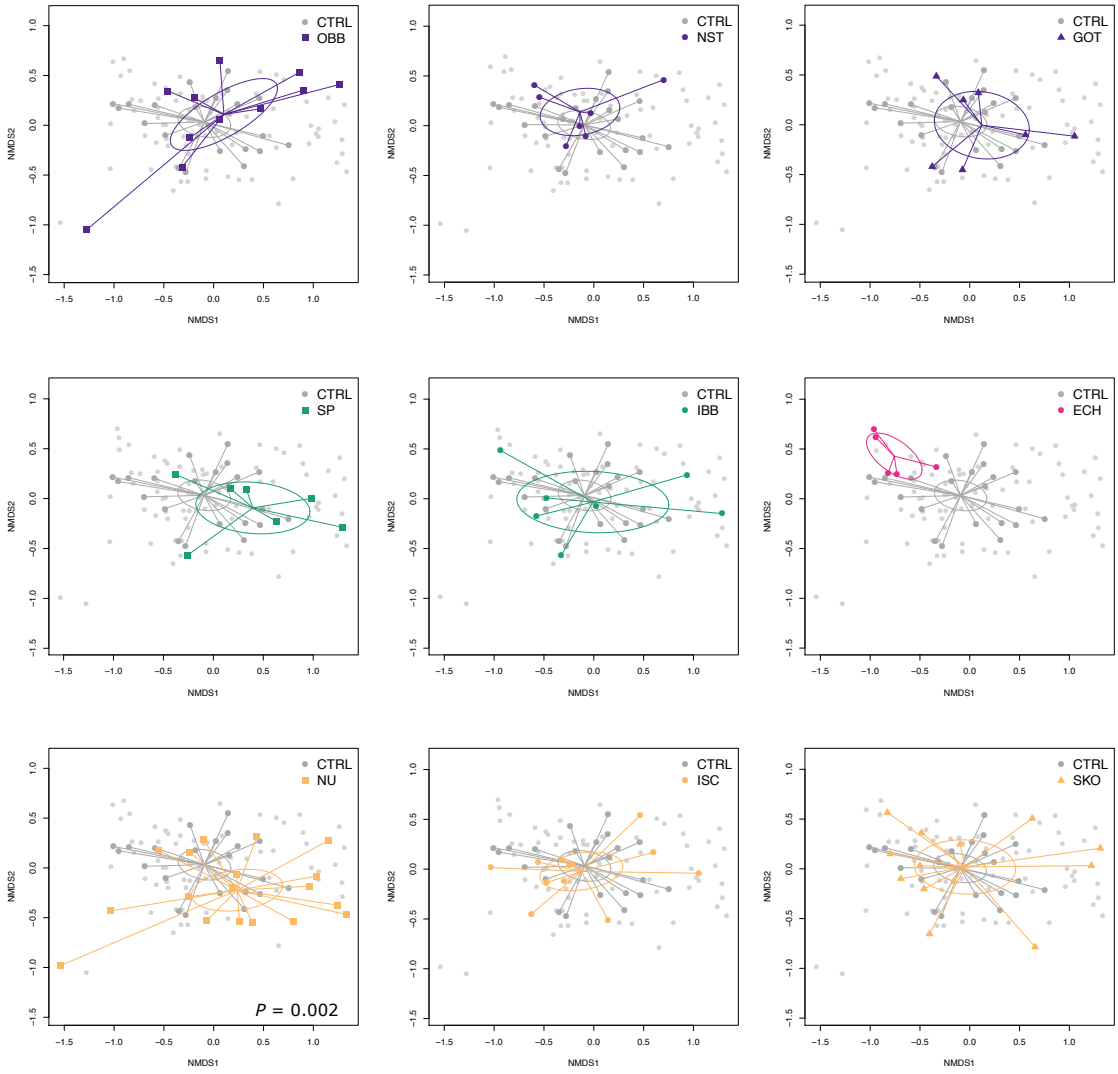


Figure S12. Non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions showing multivariate data from 12 innate immune genes of infected and sham-exposed (CTRL) NO sticklebacks. Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.



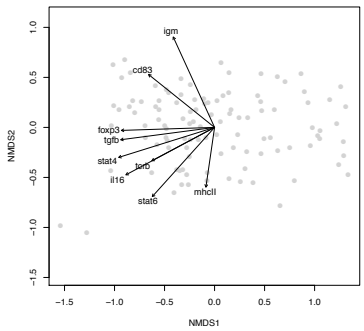


Figure S13. Non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions showing multivariate data from 9 adaptive immune genes of infected and sham-exposed (CTRL) NO sticklebacks. Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

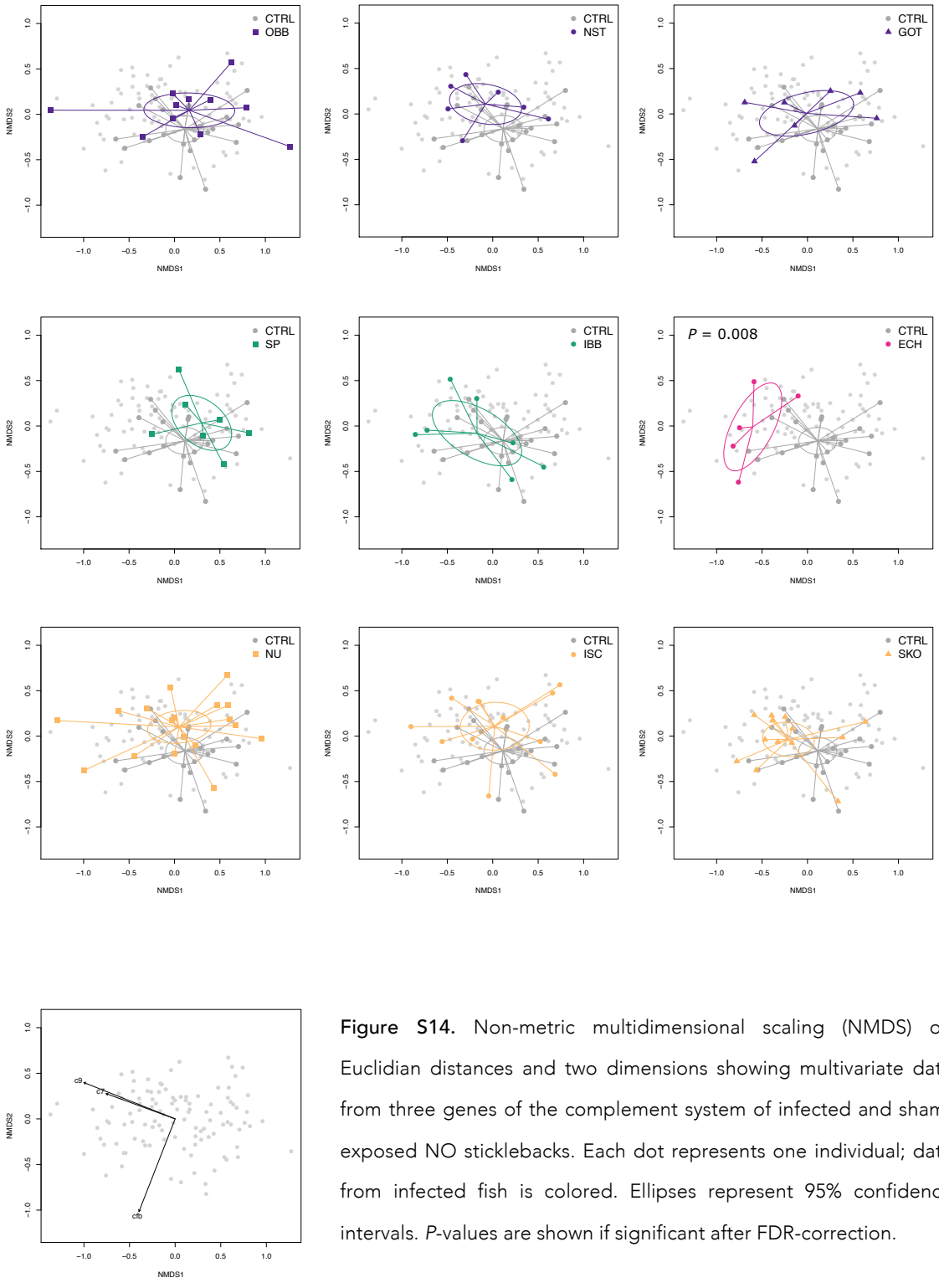


Figure S14. Non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions showing multivariate data from three genes of the complement system of infected and sham-exposed NO sticklebacks. Each dot represents one individual; data from infected fish is colored. Ellipses represent 95% confidence intervals. *P*-values are shown if significant after FDR-correction.

SI.5.5 NMDS: infected versus control DE sticklebacks (in contrast 1)

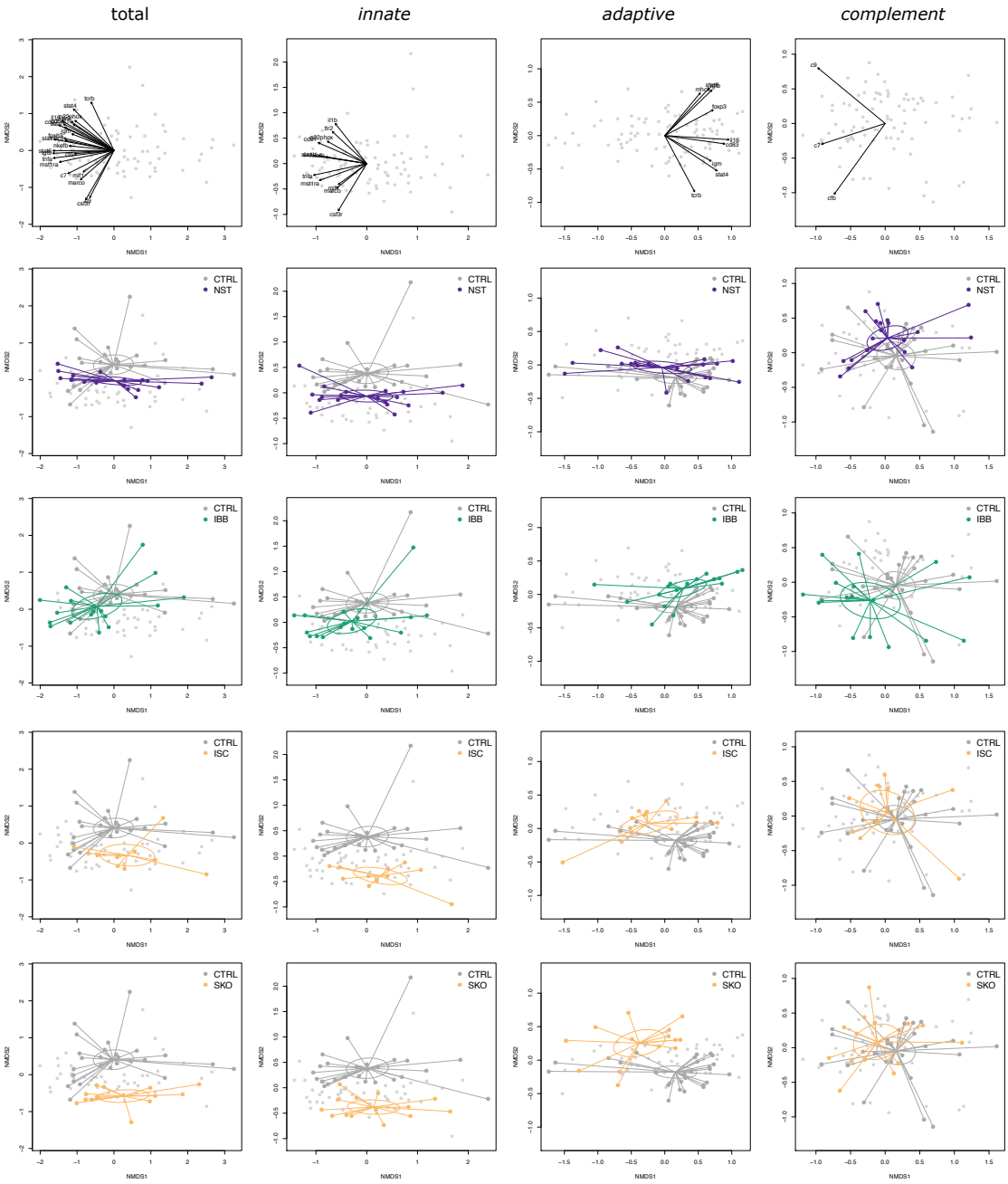


Figure S15. Non-metric multidimensional scaling (NMDS) plots on Euclidian distances and two dimensions showing multivariate data from infected and sham-exposed (CTRL) DE sticklebacks (2015). NMDS were based on log10-transformed CNRQ values of all 24 immune genes, 12 genes of the innate immune system (*marco*, *mst1ra*, *mif*, *il-1β*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), 9 genes of the adaptive immune system (*stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *tgf-β*, *il-16*, *mhcII*, *tcr-β*), or three genes of the complement system (*cfb*, *c7*, *c9*). Each dot represents one individual; colors refer to the origin of *S. solidus* in infected fish. Ellipses represent 95% confidence intervals. *P*-values are not indicated, because none were significant after FDR-correction.

REFERENCES

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4. ArXiv14065823 Stat.

Chellappa, S., Huntingford, F.A., Strang, R.H.C., and Thomson, R.Y. (1995). Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. J. Fish Biol. *47*, 775–787.

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

Hammerschmidt, K., and Kurtz, J. (2005). Evolutionary implications of the adaptation to different immune systems in a parasite with a complex life cycle. Proc. R. Soc. B Biol. Sci. *272*, 2511–2518.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. Biom. J. *50*, 346–363.

Johnson, P.C.D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods Ecol. Evol. *5*, 944–946.

Kurtz, J., Kalbe, M., Aeschlimann, P.B., Häberli, M.A., Wegner, K.M., Reusch, T.B.H., and Milinski, M. (2004). Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. Proc. R. Soc. Lond. B Biol. Sci. *271*, 197–204.

Lefcheck, J.S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. *7*, 573–579.

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. *4*, 133–142.

Pechhold, K., Pohl, T., and Kabelitz, D. (1994). Rapid quantification of lymphocyte subsets in heterogeneous cell populations by flow cytometry. Cytometry *16*, 152–159.

Scharsack, J.P., Kalbe, M., Derner, R., Kurtz, J., and Milinski, M. (2004). Modulation of granulocyte responses in three-spined sticklebacks Gasterosteus aculeatus infected with the tapeworm Schistocephalus solidus. Dis. Aquat. Organ. *59*, 141–150.

Scott, A.L., and Klesius, P.H. (1981). Chemiluminescence: A novel analysis of phagocytosis in fish. Dev. Biol. Stand. *49*, 243–254.

Seppänen, E., Kuukka, H., Voutilainen, A., Huuskonen, H., and Peuhkuri, N. (2009). Metabolic depression and spleen and liver enlargement in juvenile Arctic charr Salvelinus alpinus exposed to chronic parasite infection. J. Fish Biol. *74*, 553–561.

Wedekind, C., and Milinski, M. (1996). Do three-spined sticklebacks avoid consuming copepods, the first intermediate host of Schistocephalus solidus? — an experimental analysis of behavioural resistance. Parasitology *112*, 371–383.

SUPPLEMENTARY INFORMATION

Chapter 2

The right response at the right time: Exploring helminth immune modulation in sticklebacks by experimental co-infection

Agnes Piecyk, Marc Ritter, Martin Kalbe

SI.1 Experimental hosts and parasites

Table S1. Sampling sites

Type	*Gasterosteus aculeatus*		
LR	Lake Großer Plöner See	Germany	54°08'48" N, 10°24'30" E
HR	Lake Skogseidvatnet	Norway	60°14'44'' N, 5°55'03'' E
	Schistocephalus solidus		
LG	Lagoon Neustädter Binnenwasser	Germany	54°06'40'' N, 10°48'50'' E
HG	Lake Skogseidvatnet	Norway	60°14'44'' N, 5°55'03'' E
	Diplostomum pseudospathaceum		
-	Lake Kleiner Plöner See (1)	Germany	54°09'41.6" N 10°22'36.5" E
-	Lake Kleiner Plöner See (2)	Germany	54°09'46.2" N 10°24'05.2" E
-	Lake Bischhofsee	Germany	54°06'36.7" N 10°25'44.3" E

Information on Diplostomum pseudospathaceum sampling and use

Limnea stagnalis were sampled by hand or using a small dip net. In the laboratory, each snail was subsequently rinsed with filtered lake water and individually placed in a plastic cup (Bioware 200ml, Huhtamaki) with filtered lake water. After two hours of direct light exposure, snails were screened for trematode infections by inspecting the shed cercariae in the water of the cup. The snails were kept in 16L aquaria at 18°C, with 16 hours of light per day. We used clone mixes from a pool of snail hosts in every infection round in order to overcome strong influences of *D. pseudospathaceum* genotype specificities: following a recovery period of at least two weeks post sampling, *D. pseudospathaceum* positive snails were individually placed in plastic cups with filtered lake water and exposed to direct light for 60 minutes. After verification of infection status and snail viability, 10 snails shedding the largest number of cercariae of the day were transferred to new plastic cups with fresh lake water and exposed to direct light for another 60 minutes. Cercariae from this supernatant were used to create a pool of *D. pseudospathaceum* cercariae of similar age.

SI.2 Further information on reverse transcription

The reverse transcription protocol was modified by using 0.2 µL Qiagen RNAse inhibitor (instead of 1 µL). The manufacturer ensured that 0.2 µL is sufficient due to differences in effective inhibitor concentrations.

SI.3 Further information on direct sequencing

PCR conditions were the same in all sequencing attempts. All PCR products were checked on a gel for the right size and amplification specificity. 5 µL aliquot of the completed PCR reaction were mixed thoroughly. 2 µL of illustra ExoStar 1 Step were added to the reaction mix and incubated at 37°C for 15 minutes. Incubation at 80°C for 15 minutes inactivated the enzymes. Afterwards, the cycle sequencing was prepared as follows:

2 µL PCR/Product /ExoSAP + 2 µL Seqbuffer+1 µL Primer (forward or reverse of each PCR primer) + 4 µL HPLC H^2O + 1 µL BDT

Program: BDT 3.1

Cycle	Temp	Min
Pre-denaturation	96°C	01:00
Denaturation	96°C	00:10
Annealing	60°C	04:00
	4°C	∞

cleaned up with BigDye XTerminator® Purification Kit from Applied Biosystems

sequenced on 3130 XL Genetic Analyzer from Applied Biosystems

SI.4 Further information on gene expression analyses

Table S2. Excluded primers

Gene	Function	References	comment
tlr2	Toll-like receptor 2; Germline-encoded pattern-recognition receptor	(Zhu et al., 2012; Brunner et al., 2017)	Amplification efficiency of primer product was not within acceptable range
p40phox	Component of NADPH oxidases	(Stutz et al., 2015)	Product sequencing revealed amplification of unspecific target
vegfa1	Stimulates macrophage and monocyte migration	(Brunner et al., 2017)	Unspecific primer products, ambiguous PCR products
ly75	Reduces B-lymphocyte proliferation	(Brunner et al., 2017)	Unspecific primer products, ambiguous PCR products
cmip	Signaling protein in Th2 pathway	(Robertson et a., 2015)	Unspecific primer products, ambiguous PCR products

SI.5 Gene expression targets, gene references and primer sequences

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
Reference genes					
b2m	Beta-2-microglobulin	(Hibbeler et al., 2008)	ENSGACT00000025544	GAAGATGTGTTGAATAGAAGCTGG	GAAGATGTGTTGAATAGAAGCTGG
ef1a	Elongation factor 1 α	(Hibbeler et al., 2008)	ENSGACT00000002893	CCACCGTTGCCTTTGTCC	TGGGACTGTTCCAATACCTCC
rpl13a	L13A ribosomal binding protein	(Hibbeler et al., 2008)	ENSGACT00000012319	CACCTTGGTCAACTTGAACAGTG	TCCCTCCGCCCTACGAC
ubc	Ubiquitin	(Hibbeler et al., 2008)	ENSGACT00000010662	AGACGGGCATAGCACTTGC	CAGGACAAGGAAGGCATCC
Innate					
cd97	Promotor of granulocyte and neutrophil migration, required for activation of the innate immune response	(Leemans et al., 2004; Rhodes et al., 2009)	ENSGACT00000024871	CTCGTGGCACTCTACGACATGAAG	CAGCCCTATCTTGGTGACCAGTTG
csf3r	Granulocyte colony-stimulating factor 3 receptor; role in differentiation and proliferation of granulocytes	(Birrer et al., 2012; Brunner, 2016; Maxson et al., 2013; Tabbara, 1993)	ENSGACT00000018254	TCGGGATTCGTCCTCTTCTCAG	TGGGTCAAACTTGGCTGCAC
il-1β	Interleukin 1 β; cytokine with function in early response proinflammatory signaling	(Brunner et al., 2017; Zhu et al., 2013)	ENSGACT00000019325	TGACGATGAAGCAGGTGGTCAAC	ACAGCGTCACGATCTCCTCTTC
marco, RON	Macrophage receptor with collagenous structure; mediates macrophage recognition and clearance of pathogens	(Kissick et al., 2014; Kraal et al., 2000)	ENSGACT00000001965	CCCTTTCGACCTTCACTGCC	TGTTTACCCCAACCCCTCCA

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
Innate (continued)					
mif1	Macrophage migration inhibitory factor; stops random macrophage migration through tissue, proinflammatory mediator of the innate immune system	(Brunner et al., 2017; Calandra and Roger, 2003)	ENSGACT00000023656	ATCAGCGGAGCTCACAACAAGC	TCAGGAGAGATGCTCAGGTGTTTG
mst1ra	Macrophage stimulating 1 receptor a; plays an important role in macrophage regulation	(Huang et al., 2016; Wang et al., 2002)	ENSGACT00000013997	ATGGCCATCGAAAGCTTGCA	TGATGTCGTACGGGTCCACA
nkef-β, peroxiredoxin 1	Natural killer cell enhancing factor; enhances cytotoxicity of NK cells, also protects against oxidative damage	(Shau et al., 1993; Stutz et al., 2015)	ENSGACT00000021380	ACTTCTCCCACTTTGCATGG	CAATGCCTTCATCCTCCTTC
p22^{phox}	NADPH oxidase component p22phox; part of the reactive oxygen species production machinery	((Bedard and Krause, 2007; Mayumi et al., 2008)	ENSGACT00000021084	GCCTCGGGACTCATTCTCCT	TGGCCCTCTTGCTTCTTGGA
saal1	Serum amyloid A; acute phase protein during inflammation response, mediates release of TNF-α and IL-1β	(Brunner et al., 2017; Haarder et al., 2013; Kovacevic et al., 2015)	ENSGACT00000007599	TCGCAGTGAGGCCAAAGATGAG	AAATCTGCCACCGTGTCCTTGG
sla1	Src-like-adaptor; necessary for maturation and activation of monocytic and dendritic cells, functions in T-cell signaling and B-cell development and function	(Brunner et al., 2017; Marton et al., 2015)	ENSGACT00000007895	ACAGAGTCGGCTCCTTCATGATAC	TCACAGAGAGCGAATACAGACCTC

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
Innate (continued)					
tnfr1	Tumor necrosis factor receptor 1; functions in regulation of inflammation, mediates cellular apoptosis and differentiation	(Brunner et al., 2017; Zhu et al., 2013)	ENSGACT00000013502	AACTACTACAGAGCCAAGGGCAAG	ACGGCACTCAGCGGTACAATTC
Adaptive					
cd83	Marker for mature dendritic cells, expressed on activated B and T cells, costimulatory to activate naïve and memory T-cells	(Aerts-Toegaert et al., 2007; Stutz et al., 2015)	ENSGACT00000000428	AGGACCCAGCGTATAAATGG	CCCTGGTGATTTTCCTCATC
foxp3; forkhead box N2b	Transcription factor; regulates functions important for the establishment of the T-reg lineage, key mediator of T-cell activation	(Kasheta et al., 2017; Rao and Naqvi, 2011; Robertson et al., 2015)	ENSGACT00000007261	GTTGACCCATGCAATTCCGA	CTGCTGTAGTTGTGGTCCTG
igm	Immunoglobulin heavy constant mu (IgM); antibody molecule, part of the humoral immune response	(S. Hibbeler, unpublished; Rønneseth et al., 2015; Zhu et al., 2013)	ENSGACT00000016907	AAGGCAGGAGAATGAAACCTTGG	CCGAGTGAGCAGACAGGGACTGG
il-16	Interleukin 16; cytokine with function in T-cell migration and expansion, chemoattractant for monocytes and eosinophils	(Brunner et al., 2017; Wen et al., 2006; Zhu et al., 2013)	ENSGACT00000016499	CTGGTCTGGGCTTCAGTATTGC	CTGGGAAACACTCTGTGGACTG
mhcII	Major histocompatibility complex class IIb exon 2; pathogen recognizing protein of the adaptive immune response	(Lenz et al., 2009)	ENSGACT00000000425	GTCTTTAACTCCACGGAGCTGAAGG	ACTCACCGGACTTAGTCAG

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
Adaptive (continued)					
stat4	Signal transducer and activator of transcription 4; required for TH1-cell differentiation, opposes TH2 and TH17 like responses	(Kaplan, 2005; Premachandra et al., 2013; Wang and Secombes, 2013)	ENSGACT00000003538	CTCTCAGTTTCGAGGCTTGCTT	GGCAGTTGGCTCACATTGG
stat6	Signal transducer and activator of transcription 6; required for TH2-cell differentiation, regulates expression of TH2 relevant cytokine IL-4	(Robertson et al., 2015; Wang and Secombes, 2013)	ENSGACT00000011232	CTCAGCCACAGTTCCAACCGTTC	GTCGGATGTTCTGGACCTCGAGT
tcr-β	T-cell receptor β-chain; function in binding of MHC-peptide ligands to initiate adaptive immune response	(Smith-Garvin et al., 2009; Stutz et al., 2015; Yanagi et al., 1984)	ENSGACT00000016457	GAGGGCAAAAACTTCACCTG	TAGGAGAATCTGGCCGTTTG
tgf-β	Transforming growth factor β; cytokine with functions in cell growth, migration, differentiation and proliferation of T and B-cells	(Robertson et al., 2015; Zhu et al., 2013)	ENSGACT00000016968	TCCCGCTTCGTCACCAACCA	ACGTCTGTCTGGCCACATTCAC
Complement					
c7	Complement component 7; initializing function in the membrane attack complex of the complement system	(Brunner et al., 2017; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000009181	TGGCTCAAGCTCAGCACAACAG	AGCGACACGTGTTTGTTTGATCG
c9	Complement component 9; structural part of the membrane attack complex of the complement system,	(Brunner et al., 2017; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000020968	CCGTGACGAACAAAGACTCAGTTG	TCTGACCGATGTCAGCACCTTG

Gene	Function	References	ENSEMBL ID	Forward primer	Reverse primer
Complement (continued)					
cfb	Complement factor B; activating complement component of the alternative pathway	(Brunner, 2016; Haase et al., 2014; Zhu et al., 2013)	ENSGACT00000027346	GAGCGTCGCACAATACAGGTTG	TACCACCGGAAGCGCACAAATC

Table S3. Primer efficiencies

Primer ID	Efficiency	E (SE)	R^2
cfb	1.909	0.093	0.803
c7	2.089	0.015	0.995
c9	1.948	0.126	0.742
cd83	2.047	0.033	0.978
csf3r	2.024	0.085	0.885
foxp3	2.065	0.028	0.986
cd97	2.029	0.05	0.957
igm	2.087	0.017	0.994
mhcII	2.011	0.024	0.988
mif1	2.064	0.015	0.995
nkef-β	2.144	0.016	0.996
sla1	2.161	0.017	0.996
stat4	2.243	0.028	0.99
stat6	2.062	0.024	0.989
tcr-β	2.02	0.014	0.996
tgf-β	2.079	0.02	0.994
tnfr1	2.19	0.025	0.99
Il-16	2.163	0.018	0.994
Il-1β	2.016	0.101	0.852
marco	2.092	0.02	0.993
mst1ra	2.228	0.042	0.976
p22phox	2.006	0.012	0.996
rpl13a	2.048	0.022	0.992
saal1	2.025	0.034	0.974
ubc	2.116	0.014	0.996

SI.6 Pre-amplification of target cDNA for Fluidigm 96.96 Dynamic Array run

- Primer Mix: total 200 µL
- 1 µL of each 100µM primer (fwd and rev) or 2 µL of paired primer mix
- plus 136 µL DNA suspension buffer (10 mM Tris, pH 8.0, 0,1 mM EDTA)

Pre Mix - Prepared in a 1.5 ml tube: total 396 µL (includes overage)
- 264 µL 2X TaqMan PreAmp Master Mix (Applied Biosystems, PN 4391128)
- 52.8 µL Primer Mix
- 79.2 µL H_2O

We pipetted 3.7 µL Pre-Mix in each well of a 96 well plate and added 1.3 µL of cDNA. Negative controls (NTCs) were included by using 1.3 µL of ddH_2O instead of cDNA. The PCR protocol was the following:

Temp	Time	No. cycles
95 °C	10 min	
95 °C	15 sec	14
60 °C	4 min	
4 °C	∞	

SI.7 Fluidigm 96.96 Dynamic Array run using pre-amplified cDNA

Pre Mix – prepared in a 1.5 ml tube: total 406.6 µL (for 96 samples, includes overage)
- 369.6 µL SsoFast EvaGreen Supermix with Low ROX (BioRad, PN 172-5211)
- 37 µL 20X DNA Binding Dye Sample Loading Reagent (Fluidigm, PN 100-3738)

The following was pipetted into each well of a 96 well plate
- 3.9 µL Sample Pre Mix
- 3.1 µL sample (preamplified)
- vortexed 20s, spun down 30s

Assay Pre-mix – prepared in a 1.5 ml tube: total 665.3 µL (for 96 reactions, includes overage)
- 369.6 µL 2X Assay loading Reagent (Fluidigm, PN 85000736)
- 295.7 µL low TE buffer

The following was pipetted into each well of a 96 well plate (7 µL per well)
- 6.3 µL Assay Pre-mix
- 0.35 µL from each of the 100µM primers (fwd and rev) or 0.7 µL from the mix

After priming of the chip, Sample Pre Mix and Assay pre Mix were loaded according to the manufacturer's instructions and the chip was run under cycler protocol: "GE Fast 96x96 PCR+Melt v2".

Temp	Time	No. Cycles
70 °C	40 min	
60 °C	30 sec	
95 °C	1 min	
96 °C	5 sec	30
60 °C	20 sec	
60 - 95 °C	+ 1°C/3s	

SI.8 Further information on S. solidus infection rates

The GLMM using the origin of the host and the origin of the parasite as well as their interaction as fixed effects and fish family as random term did not differ significantly from the Nullmodel (likelihood ratio test: $X^2_3 = 4.2365$, $p = 0.237$).

SI.9 Further information on S. solidus growth and parasite index

S. solidus growth differed significantly between the two parasite populations. HG parasites grew faster and larger than LG parasites (Type III Wald chisquare tests: H:P:T three-way interaction: $X^2_4 = 24.8413$, $p < 0.0001$).

Table S4. Differences in *S. solidus* parasite indices according to host types

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
3	LG:HR - LR	-0.04425	0.06905	-0.641	0.98779
3	HG:HR - LR	0.03158	0.06729	0.469	0.99773
6	LG:HR - LR	-2.52061	0.77996	-3.232	0.00736
6	HG:HR - LR	-2.74607	0.89502	-3.068	0.01284
9	LG:HR - LR	-7.48326	1.34521	-5.563	< 1e-05
9	HG:HR - LR	-6.00136	1.15927	-5.177	< 1e-05

Table S5. Differences in *S. solidus* parasite indices according to *S. solidus* types

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
3	HG - LG:LR	0.27701	0.05873	4.717	1.44E-05
3	HG - LG:HR	0.35284	0.06169	5.72	6.40E-08
6	HG - LG:LR	8.96403	0.8441	10.62	< 1e-10
6	HG - LG:HR	8.73858	0.83369	10.482	< 1e-10
9	HG - LG:LR	13.17036	1.18776	11.088	< 1e-10
9	HG - LG:HR	14.65227	1.31937	11.105	< 1e-10

Table S6. Differences in parasite indices over time

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
6 - 3	LG:LR	3.4222	0.541	6.325	< 1e-04
6 - 3	LG:HR	0.9459	0.5643	1.676	0.593759
6 - 3	HG:LR	12.1092	0.6505	18.614	< 1e-04
6 - 3	HG:HR	9.3316	0.6168	15.129	< 1e-04
9 - 3	LG:LR	11.3698	0.9028	12.594	< 1e-04
9 - 3	LG:HR	3.9308	0.9986	3.936	0.000904
9 - 3	HG:LR	24.2632	0.7739	31.35	< 1e-04
9 - 3	HG:HR	18.2303	0.8645	21.087	< 1e-04
9 - 6	LG:LR	7.9476	1.0514	7.559	< 1e-04
9 - 6	LG:HR	2.985	1.1448	2.607	0.087956
9 - 6	HG:LR	12.1539	1.0089	12.047	< 1e-04
9 - 6	HG:HR	8.8987	1.0606	8.39	< 1e-04

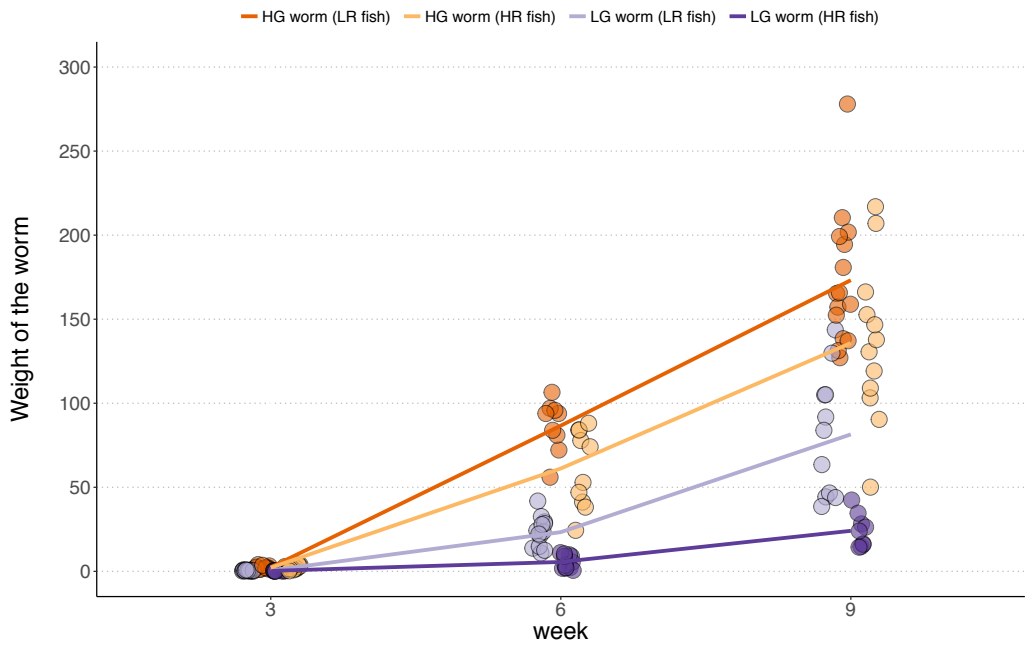


Fig. S1. *Schistocephalus solidus* growth over time. Plerocercoids were weighed 3, 6, or 9 weeks after infection. Each dot represents one *S. solidus* individual; color coding follows Fig. 1. Weights were measured in mg. HG: high gowth *S. solidus*; LR: low resistance stickleback; LG: low growth *S. solidus*; HR: high resistance stickleback.

SI.10 Further information on D. pseudospathaceum infection rates

Table S7. *D. pseudospathaceum* infection rate differences between host types

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
3	CTRL:HR - LR	-0.5708	0.516	-1.106	0.5222
3	LG:HR - LR	0.4501	0.52	0.866	0.696
3	HG:HR - LR	-0.124	0.5099	-0.243	0.9994
6	CTRL:HR - LR	1.2871	0.5485	2.347	0.0541
6	LG:HR - LR	0.6251	0.5226	1.196	0.4638
6	HG:HR - LR	0.422	0.5131	0.822	0.7289
9	CTRL:HR - LR	0.2924	0.5298	0.552	0.9212
9	LG:HR - LR	0.1643	0.5221	0.315	0.9962
9	HG:HR - LR	-0.1888	0.5022	-0.376	0.9883

Table S8. *D. pseudospathaceum* infection rate differences over time

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
6 - 3	CTRL:LR	-2.03113	0.23082	-8.799	< 1e-05
6 - 3	CTRL:HR	-0.17322	0.17765	-0.975	0.99532
6 - 3	LG:LR	-0.24223	0.1645	-1.473	0.88485
6 - 3	LG:HR	-0.06721	0.19189	-0.35	1
6 - 3	HG:LR	-0.29627	0.14855	-1.994	0.512
6 - 3	HG:HR	0.24966	0.13211	1.89	0.59702
9 - 3	CTRL:LR	-1.15953	0.17763	-6.528	< 1e-05
9 - 3	CTRL:HR	-0.2964	0.18226	-1.626	0.79653
9 - 3	LG:LR	-0.09894	0.16859	-0.587	0.99997
9 - 3	LG:HR	-0.38468	0.17346	-2.218	0.34321
9 - 3	HG:LR	0.78552	0.12199	6.439	< 1e-05
9 - 3	HG:HR	0.72067	0.12039	5.986	< 1e-05
9 - 6	CTRL:LR	0.8716	0.25233	3.454	0.00949
9 - 6	CTRL:HR	-0.12318	0.18768	-0.656	0.99991
9 - 6	LG:LR	0.14329	0.16962	0.845	0.99879
9 - 6	LG:HR	-0.31746	0.18746	-1.694	0.74999
9 - 6	HG:LR	1.08179	0.12868	8.407	< 1e-05
9 - 6	HG:HR	0.47101	0.12281	3.835	0.0022

Table S9. *D. pseudospathaceum* infection rate differences between *S. solidus* types

	Contrast				
T	P:H	Estimate	Std. Error	z value	Pr(>|z|)
3	LG - CTRL:LR	-0.98089	0.15285	-6.417	< 1e-05
3	LG - CTRL:HR	0.03994	0.17581	0.227	1
3	HG - CTRL:LR	-0.29524	0.14467	-2.041	0.472444
3	HG - CTRL:HR	0.15155	0.15047	1.007	0.993599
3	HG - LG:LR	0.68564	0.15291	4.484	0.000134
3	HG - LG:HR	0.11161	0.15733	0.709	0.999787
6	LG - CTRL:LR	0.80801	0.23732	3.405	0.011265
6	LG - CTRL:HR	0.14595	0.18638	0.783	0.999416
6	HG - CTRL:LR	1.43961	0.23358	6.163	< 1e-05
6	HG - CTRL:HR	0.57443	0.16127	3.562	0.006328
6	HG - LG:LR	0.63161	0.1596	3.957	0.001328
6	HG - LG:HR	0.42848	0.17063	2.511	0.175896
9	LG - CTRL:LR	0.0797	0.19027	0.419	0.999999
9	LG - CTRL:HR	-0.04833	0.18436	-0.262	1
9	HG - CTRL:LR	1.64981	0.15982	10.323	< 1e-05
9	HG - CTRL:HR	1.16862	0.15769	7.411	< 1e-05
9	HG - LG:LR	1.57011	0.14074	11.156	< 1e-05
9	HG - LG:HR	1.21696	0.14675	8.293	< 1e-05

SI.11 The effect of S. solidus weight on D. pseudospathaceum infection rates

Using *S. solidus* weight as a covariate in the statistical model did not improve the model fit in week 3, but did so at later time points, namely for data from LR fish in week 6 (likelihood ratio test: $X^2_2 = 10.01$, $p = 0.0067$) and data from both fish origins in week 9 (likelihood ratio test: $X^2_2 = 13.37$, $p = 0.0013$). The model fit for HR data of week 6 was not improved (likelihood ratio test: $X^2_2 = 4.82$, $p = 0.0897$). Due to very large eigenvalues, we z-transformed the weight of the worm in week 6 and week 9.

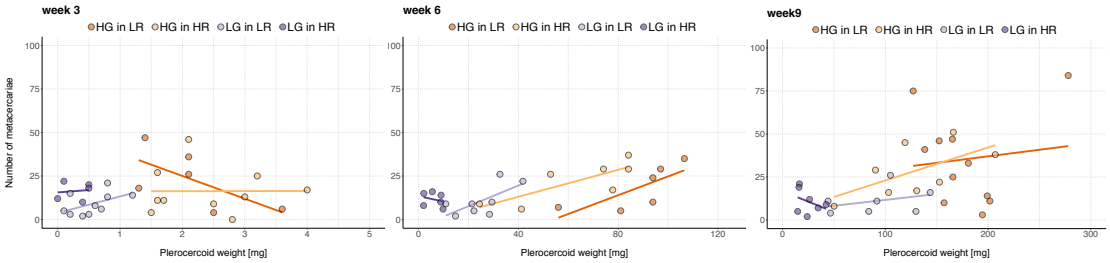


Figure S2. The relationship between *S. solidus* weight and *D. pseudospathaceum* infection rates. *S. solidus* infected sticklebacks were exposed to 100 *D. pseudospathaceum* cercariae at three different time points. Each dot represents one *S. solidus* individual; lines represent linear model fits; color coding follows Fig. 1.

SI.12 Further information on host condition and immunological parameters

The condition factor (CF) differed significantly between host populations ($F_{1,4} = 25.027$, $p = 0.0075$) and according to an interaction between time point and treatment ($F_{10,170} = 2.543$, $p = 0.007$). FDR-corrected post hoc comparisons confirmed significantly higher condition of HR than LR hosts at all time points (LMMs; each $p < 0.0001$). Treatment had no significant influence; the CF increased between week 3 and week 9 in *D. pseudospathaceum* infected HR fish. The hepatosomatic index (HSI) was significantly affected by an interaction between treatment and time point ($F_{10,170} = 4.102$, $p < 0.0001$). LR controls had higher HSIs than HR controls in week 9; the HSI increased between week 3 and 9 in LR controls (LMMs; each $p < 0.001$). In week 9, LG infection was associated with a smaller HSI than in controls in LR fish; infection with *D. pseudospathaceum* correlated with significantly higher HSI in comparison to co-infection with HG *S. solidus* in LR sticklebacks (LMMs; each $p < 0.001$). Splenosomatic indices (SSI) and head kidney indices (HKI) were not affected by experimental factors.

SI.13 Detailed results of gene expression analyses

Table S10. Multivariate statistics (PERMANOVA results) of *S. solidus* infection effect on stickleback immune gene expression

T	contrast P:H		Df	SumsOfSqs	F.Model	Pr(<F)	R2
3	LG - CTRL:HR&LR	all genes	1	2.720	1.27410	0.3787	0.06220
3	HG - CTRL:HR&LR	all genes	1	1.813	0.6536	0.5375	0.03490
3	HG - LG:HR&LR	all genes	1	1.180	0.6698	0.6554	0.03258
6	LG - CTRL:HR&LR	all genes	1	0.683	0.3826	0.8597	0.01536
6	**HG - CTRL:HR&LR**	all genes	1	6.037	3.08185	0.0081	0.15855
		innate	1	4.7112	4.9997	**0.0023**	0.23481
		adaptive	1	0.9015	3.9718	0.0176	0.19101
		complement	1	0.4241	0.53716	0.5240	0.03191
		Th1	1	0.27172	6.3830	0.0281	0.28856
		Th2	1	0.21162	1.41429	0.2842	0.08102
		Treg (P effect)	1	0.2842	11.4826	0.0003	0.32809
		Treg (H effect)	1	0.09366	4.3531	0.0222	0.12438
		Treg (P:H interaction)	1	0.09119	4.2384	0.0240	0.12110
6	HG - CTRL:LR	*Treg*	1	0.035747	1.11233	0.3463	0.13857
6	**HG - CTRL:HR**	***Treg***	1	0.28071	20.1432	**0.0105**	0.72529
6	HG - LG:HR&LR	all genes	1	0.857	0.45844	0.79982	0.02550
9	LG - CTRL:HR&LR	all genes (P effect)	1	1.979	1.4393	0.05411	0.06145
		all genes (H effect)	1	5.580	4.0580	0.01128	0.17326
9	LG - CTRL:LR	all genes	1	2.6294	1.7392	0.07292	0.18035
9	LG - CTRL:HR	all genes	1	1.8375	1.5914	0.2361	0.15425
9	HG - CTRL:HR&LR	all genes	1	16.972	5.8497	0.00340	0.27223
		innate	1	2.9039	2.16177	0.07469	0.11711
		adaptive	1	0.7096	3.4025	0.13248	0.0393
		complement	1	13.358	9.8992	**0.0082**	0.41497
		Th1	1	0.14091	1.90958	0.1356	0.11422
		Th2	1	0.17302	1.9625	0.1893	0.06081
		Treg	1	0.10111	2.99380	0.07879	0.15223
9	HG - LG:HR&LR	all genes	1	11.363	3.4461	0.07465	0.21681

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations that were constrained within fish family. PERMANOVA results were FDR corrected. If significant (marked in bold letters), single genes were analysed with linear mixed models (LMMs). Statistics for differences between host types or interactions are mentioned whenever significant. T: time point (week 3, 6, or 9); P: parasite type (low growth, LG; high growth, HG); H: host type (low resistance, LR; high resistance, HR); *all genes*: data from all 23 genes; *innate*: 11 genes (*cd97*, *csf3r*, *il-1β*, *marco*, *mif1*, *mst1ra*, *nkef-β*, *p22^{phox}*, *saal1*, *sla1*, *tnfr1*); adaptive: nine genes (*stat4*, *cd83*, *igm*, *stat6*, *foxp3*, *il-16*, *tgf-β*, *mhcII*, *tcr-β*); *complement*: three genes (*cfb*, *c7*, *c9*); *Th1*: two genes (*stat4*, *tnfr1*), *Th2* covers three genes (*stat6*, *cd83*, *igm*); *Treg* covers three genes (*il-16*, *foxp3*, *tgf-β*).

Table S11. Differential innate immune gene expression between HG-*S. solidus* infected and control (HR and LR) stickleback in week 6

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	9	0.193756	0.6702	0.4923706
mst1ra	1	9	1.88193	0.2033	0.9672328
mif1	1	9	3.305022	0.1024	0.4501657
il-1β	1	9	0.400218	0.5427	0.4503203
tnfr1	1	9	6.234099	0.0340	1
saal1	1	9	6.068530	0.0360	0.5988541
csf3r	1	9	2.0903358	0.1821	0.427818
p22^{phox}	1	9	1.250352	0.2924	0.512474
nkef-β	1	9	0.0634747	0.8067	0.09538045
sla1	1	9	1.2502136	0.2925	0.4952347
cd97	1	9	0.347441	0.5701	0.3215247

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Data from genes from significantly differentially expressed functional gene groups was analysed with linear mixed models (LMMs; function lme() from *nlme*) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with sem.model.fits() from *piecewiseSEM* (Lefcheck, 2015). No gene was significantly differentially expressed after FDR correction..

Table S12. Differential expression of T regulatory genes between HG-*S. solidus* infected and control HR stickleback in week 6

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
il-16	1	4	2.7784351	0.1709	0.3274503
foxp3	1	4	12.615158	0.0238	0.677801
tgf-β	1	4	63.60417	**0.0013**	0.8974288

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Data from genes from significantly differentially expressed functional gene groups was analysed with linear mixed models (LMMs; function lme() from *nlme*) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with sem.model.fits() from *piecewiseSEM* (Lefcheck, 2015). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S13. Differential expression of complement genes between HG-*S. solidus* infected and control (HR and LR) stickleback in week 9

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
cfb	1	10	10.051180	**0.0100**	0.7509158
c7	1	10	0.000858	0.9772	0.1542638
c9	1	10	5.861681	0.0360	0.9810338

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Data from genes from significantly differentially expressed functional gene groups was analysed with linear mixed models (LMMs; function lme() from *nlme*) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with sem.model.fits() from *piecewiseSEM* (Lefcheck, 2015). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S14. Differential immune gene expression between *D. pseudospathaceum* infected and control stickleback

contrast						
P:H		Df	SumsOfSqs	F.Model	Pr(<F)	R2
CTRL - D:LR&HR	all genes (D effect)	1	3.913	1.53871	0.12119	0.02230
	all genes (H:T interaction)	2	11.820	2.32405	0.01040	0.06737

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations that were constrained within fish family. The weight of the fish was included as covariate to account for size related effects. D effect: effect of *D. pseudospathaceum* infection. In this case, gene expression was only affected by an interaction between host type and time.

Table S15. Multivariate statistics (PERMANOVA results) of the effect of *S. solidus – D. pseudo-spathaceum* co-infection on stickleback immune gene expression

	contrast						
T	P:H		Df	SumsOfSqs	F.Model	Pr(<F)	R2
3	Co-LG - CTRL:LR&HR	all genes	1	3.830	1.39407	0.2198	0.05774
3	Co-HG - CTRL:LR&HR	all genes	1	6.807	1.88755	0.1499	0.07230
3	Co-HG – Co-LG:LR&HR	all genes	1	4.138	1.22793	0.5500	0.04170
6	Co-LG - CTRL:LR&HR	all genes	1	4.754	1.82810	0.1263	0.06671
6	Co-HG - CTRL:LR&HR	all genes	1	5.814	2.43653	0.05399	0.09334
6	Co-HG – Co-LG:LR&HR	all genes	1	3.696	1.3185	0.2642	0.04603
9	Co-LG - CTRL:LR&HR	all genes	1	4.926	2.02926	0.05299	0.08354
9	Co-HG - CTRL:LR&HR	all genes (P effect)	1	3.169	1.5420	0.1608	0.04346
		all genes (H effect)	1	10.510	5.1148	0.0216	0.14414
9	Co-HG – CTRL:HR	all genes	1	1.348	0.6052	0.6902	0.04097
9	Co-HG – CTRL:LR	all genes	1	6.6020	3.8846	0.0178	0.22315
		innate	1	5.1340	5.4308	**0.0195**	0.28195
		adaptive	1	1.1485	5.2576	**0.0122**	0.25374
		complement	1	0.3195	0.59627	0.4433	0.04664
		Th1	1	0.40863	4.8038	**0.0232**	0.26610
		Th2	1	0.42719	4.9585	**0.0226**	0.23610
		Treg	1	0.59940	11.6801	**0.0074**	0.47104
9	Co-HG – Co-LG:LR&HR	all genes	1	1.210	0.4679	0.7198	0.01348

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations that were constrained within fish family. PERMANOVA results were FDR corrected. If significant (marked in bold letters), single genes were analysed with linear mixed models (LMMs). Statistics for differences between host types or interactions are mentioned whenever significant.

Table S16. Differential immune gene expression between HG-*S. solidus* - *D. pseudospathaceum* co-infected and control LR stickleback in week 9

ANOVA results	numDF	denDF	F-value	p-value	pseudo R2
marco	1	10	5.880190	0.0358	0.3029049
mst1ra	1	10	1.0458040	0.3306	0.7409858
mif1	1	10	8.678699	0.0146	0.6739742
il-1β	1	10	2793093.4	**< 0.0001**	0.9999997
tnfr1	1	10	5.668682	0.0386	0.5735934
saal1	1	10	1.5908441	0.2358	0.1020698
csf3r	1	10	4.343719	0.0638	0.2487352
p22^{phox}	1	10	4.312283	0.0646	0.4603152
nkef-β	1	10	7.287676	0.0223	0.2660523
sla1	1	10	5.184409	0.0460	0.2948069
cd97	1	10	5.056703	0.0483	0.286804
stat4	1	10	1.985945	0.1891	0.4252173
stat6	1	10	7.755325	0.0193	0.4208772
igm	1	10	5.580071	0.0398	0.7597721
cd83	1	10	0.1557763	0.7014	0.0529079
foxp3	1	10	17.383392	**0.0019**	0.7420727
tgf-β	1	10	10.890636	**0.0080**	0.4390618
il-16	1	10	10.284613	**0.0094**	0.4926989
mhcII	1	10	0.457965	0.5139	0.5255997
tcr-β	1	10	0.233701	0.6392	0.5590239

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Data from genes from significantly differentially expressed functional gene groups was analysed with linear mixed models (LMMs; function lme() from *nlme*) and analyses of variance (ANOVAs). Conditional pseudo R^2 values (Nakagawa and Schielzeth, 2013; Johnson, 2014) were calculated with sem.model.fits() from *piecewiseSEM* (Lefcheck, 2015). Differentially expressed genes are marked in bold letters if significant after FDR correction.

Table S17. Differential immune gene expression between *S. solidus* infected and co-infected stickleback

T	contrast P:H		Df	SumsOfSqs	F.Model	Pr(<F)	R2
3	Co-LG - LG:LR&HR	all genes	1	1.979	0.96971	0.4699	0.04058
3	Co-HG - HG:LR&HR	all genes	1	2.275	0.63169	0.5374	0.02671
6	Co-LG - LG:LR&HR	all genes	1	3.354	1.3296	0.1487	0.04992
6	Co-HG - HG:LR&HR	all genes	1	3.566	1.37490	0.2674	0.07615
9	Co-LG -LG:LR&HR	all genes	1	1.156	0.46060	0.75532	0.02303
9	Co-HG -HG:LR&HR	all genes	1	18.471	6.4611	0.005899	0.18743
		innate	1	0.433	0.4466	0.60324	0.01343
		adaptive	1	0.1859	0.6555	0.56034	0.02125
		complement	1	17.852	11.1198	0.005799	0.31020
		Th1 (P effect)	1	0.02807	0.7082	0.4527	0.02336
		Th1 (H effect)	1	0.02855	0.7205	0.0268	0.02377
		Th2 (P:H interaction)	1	0.5031	3.2453	0.0208	0.10287
		Treg	1	0.05298	1.3689	0.26737	0.03918
9	Co-HG -HG:LR	*Th1*	1	0.03520	0.6851	0.45035	0.03320
		Th2	1	0.27530	3.5893	0.06289	0.13303
9	Co-HG -HG:HR	*Th1*	1	0.034140	3.3354	0.07979	0.26900
		Th2	1	0.24033	1.27777	0.1914	0.11415

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations that were constrained within fish family. Statistics for differences between host types or interactions are mentioned whenever significant. PERMANOVA results were FDR corrected. In this case, no result remained significant after FDR correction.

Table S18. Differential immune gene expression between *D. pseudospathaceum* infected and co-infected stickleback

T	contrast P:H		Df	SumsOfSqs	F.Model	Pr(<F)	R2
3	Co-LG - D:LR&HR	all genes	1	1.448	0.61862	0.6900	0.02419
3	Co-HG - D:LR&HR	all genes	1	4.478	1.32821	0.2193	0.05041
6	Co-LG - D:LR&HR	all genes	1	2.580	0.79285	0.65243	0.03015
6	Co-HG - D:LR&HR	all genes	1	6.998	2.29249	0.06219	0.08835
9	Co-LG -D:LR&HR	all genes	1	2.249	0.79498	0.4033	0.03304
9	Co-HG -D:LR&HR	all genes (P effect)	1	2.052	0.8567	0.56224	0.02515
9	Co-HG -D:LR&HR	all genes (H effect)	1	9.948	4.1524	0.04440	0.12191
9	Co-HG -D:LR	all genes	1	4.893	2.4794	0.03130	0.14528
		innate	1	1.4688	1.4344	0.1450	0.08567
		adaptive	1	1.8106	8.7102	0.03170	0.35095
		complement	1	1.6133	2.17597	0.06329	0.14185
		Th1	1	0.71763	12.6316	0.0354	0.47246
		Th2	1	0.91437	8.8065	0.0478	0.37766
		Treg	1	0.54614	11.2381	0.0329	0.43678
9	Co-HG -D:HR	all genes		2.940	1.00147	0.4807	0.07760

The statistical models were based on log10-transformed calibrated normalized relative quantities (CNRQ values). The weight of the fish was included as covariate to account for size related effects. Non-parametric permutational multivariate analyses of variance (PERMANOVA) were calculated on Euclidian distances and 10,000 permutations that were constrained within fish family. Statistics for differences between host types are mentioned when significant and infection effects where then tested for HR and LR host types separately. PERMANOVA results were FDR corrected. In this case, no results remained significant after FDR correction. D: *Diplostomum pseudospathaceum* infection.

REFERENCES

Aerts-Toegaert, C., Heirman, C., Tuyaerts, S., Corthals, J., Aerts, J.L., Bonehill, A., Thielemans, K., and Breckpot, K. (2007). CD83 expression on dendritic cells and T cells: Correlation with effective immune responses. Eur. J. Immunol. *37*, 686–695.

Bedard, K., and Krause, K.-H. (2007). The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiol. Rev. *87*, 245–313.

Birrer, S.C., Reusch, T.B.H., and Roth, O. (2012). Salinity change impairs pipefish immune defence. Fish Shellfish Immunol. *33*, 1238–1248.

Brunner, F.S. (2016). Parasitism along the speciation continuum - Variation of parasite effects across speciation stages of Gasterosteus aculeatus and their modification by environmental changes. Queen Mary University of London.

Brunner, F.S., Anaya-Rojas, J.M., Matthews, B., and Eizaguirre, C. (2017). Experimental evidence that parasites drive eco-evolutionary feedbacks. Proc. Natl. Acad. Sci. 201619147.

Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. *3*, 791–800.

Haarder, S., Kania, P.W., Bahlool, Q.Z.M., and Buchmann, K. (2013). Expression of immune relevant genes in rainbow trout following exposure to live Anisakis simplex larvae. Exp. Parasitol. *135*, 564–569.

Haase, D., Rieger, J.K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., and Reusch, T.B.H. (2014). Specific Gene Expression Responses to Parasite Genotypes Reveal Redundancy of Innate Immunity in Vertebrates. PLoS ONE *9*, e108001.

Haase, D., Rieger, J.K., Witten, A., Stoll, M., Bornberg-Bauer, E., Kalbe, M., and Reusch, T.B.H. (2016). Immunity comes first: The effect of parasite genotypes on adaptive immunity and immunization in three-spined sticklebacks. Dev. Comp. Immunol. *54*, 137–144.

Hibbeler, S., Scharsack, J.P., and Becker, S. (2008). Housekeeping genes for quantitative expression studies in the three-spined stickleback Gasterosteus aculeatus. BMC Mol. Biol. *9*, 18.

Huang, Y., Chain, F.J.J., Panchal, M., Eizaguirre, C., Kalbe, M., Lenz, T.L., Samonte, I.E., Stoll, M., Bornberg-Bauer, E., Reusch, T.B.H., et al. (2016). Transcriptome profiling of immune tissues reveals habitat-specific gene expression between lake and river sticklebacks. Mol. Ecol. n/a-n/a.

Johnson, P.C.D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods Ecol. Evol. *5*, 944–946.

Kaplan, M.H. (2005). STAT4: A Critical Regulator of Inflammation In Vivo. Immunol. Res. *31*, 231–242.

Kasheta, M., Painter, C.A., Moore, F.E., Lobbardi, R., Bryll, A., Freiman, E., Stachura, D., Rogers, A.B., Houvras, Y., Langenau, D.M., et al. (2017). Identification and characterization of T reg–like cells in zebrafish. J. Exp. Med. *214*, 3519–3530.

Kissick, H.T., Dunn, L.K., Ghosh, S., Nechama, M., Kobzik, L., and Arredouani, M.S. (2014). The Scavenger Receptor MARCO Modulates TLR-Induced Responses in Dendritic Cells. PLoS ONE *9*, e104148.

Kovacevic, N., Hagen, M.O., Xie, J., and Belosevic, M. (2015). The analysis of the acute phase response during the course of Trypanosoma carassii infection in the goldfish (Carassius auratus L.). Dev. Comp. Immunol. *53*, 112–122.

Kraal, G., van der Laan, L.J.W., Elomaa, O., and Tryggvason, K. (2000). The macrophage receptor MARCO. Microbes Infect. *2*, 313–316.

Leemans, J.C., te Velde, A.A., Florquin, S., Bennink, R.J., de Bruin, K., van Lier, R.A.W., van der Poll, T., and Hamann, J. (2004). The Epidermal Growth Factor-Seven Transmembrane (EGF-TM7) Receptor CD97 Is Required for Neutrophil Migration and Host Defense. J. Immunol. *172*, 1125–1131.

Lefcheck, J.S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. *7*, 573–579.

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

Marton, N., Baricza, E., &#xc9, Rsek, B., Buz&#xe1, S, E.I., Nagy, G., and rgy (2015). The Emerging and Diverse Roles of Src-Like Adaptor Proteins in Health and Disease. Mediators Inflamm. *2015*, e952536.

Maxson, J.E., Gotlib, J., Pollyea, D.A., Fleischman, A.G., Agarwal, A., Eide, C.A., Bottomly, D., Wilmot, B., McWeeney, S.K., Tognon, C.E., et al. (2013). Oncogenic CSF3R Mutations in Chronic Neutrophilic Leukemia and Atypical CML. N. Engl. J. Med. *368*, 1781–1790.

Mayumi, M., Takeda, Y., Hoshiko, M., Serada, K., Murata, M., Moritomo, T., Takizawa, F., Kobayashi, I., Araki, K., Nakanishi, T., et al. (2008). Characterization of teleost phagocyte NADPH oxidase: Molecular cloning and expression analysis of carp (Cyprinus carpio) phagocyte NADPH oxidase. Mol. Immunol. *45*, 1720–1731.

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. *4*, 133–142.

Premachandra, H.K.A., Elvitigala, D.A.S., Bathige, S.D.N.K., Whang, I., Lee, Y., De Zoysa, M., and Lee, J. (2013). Genomic structure and immunological response of an STAT4 family member from rock bream (Oplegnathus fasciatus). Fish Shellfish Immunol. *35*, 1829–1837.

Rao, D.N., and Naqvi, R.A. (2011). FoxP3: A Key Player in T Regulatory Biology. Indian J. Clin. Biochem. *26*, 1–2.

Rauch, G., Kalbe, M., and Reusch, T.B.H. (2006). One day is enough: rapid and specific host–parasite interactions in a stickleback-trematode system. Biol. Lett. *2*, 382–384.

Rhodes, L.D., Wallis, S., and Demlow, S.E. (2009). Genes associated with an effective host response by Chinook salmon to Renibacterium salmoninarum. Dev. Comp. Immunol. *33*, 176–186.

Robertson, S., Bradley, J.E., and MacColl, A.D.C. (2015). Measuring the immune system of the three-spined stickleback – investigating natural variation by quantifying immune expression in the laboratory and the wild. Mol. Ecol. Resour. n/a-n/a.

Rønneseth, A., Ghebretnsae, D.B., Wergeland, H.I., and Haugland, G.T. (2015). Functional characterization of IgM+ B cells and adaptive immunity in lumpfish (Cyclopterus lumpus L.). Dev. Comp. Immunol. *52*, 132–143.

Shau, H., Gupta, R.K., and Golub, S.H. (1993). Identification of a Natural Killer Enhancing Factor (NKEF) from Human Erythroid Cells. Cell. Immunol. *147*, 1–11.

Smith-Garvin, J.E., Koretzky, G.A., and Jordan, M.S. (2009). T Cell Activation. Annu. Rev. Immunol. *27*, 591–619.

Stutz, W.E., Schmerer, M., Coates, J.L., and Bolnick, D.I. (2015). Among-lake reciprocal transplants induce convergent expression of immune genes in threespine stickleback. Mol. Ecol. *24*, 4629–4646.

Tabbara, I.A. (1993). Granulocyte colony-stimulating factor. South. Med. J. *86*, 350–355.

Wang, T., and Secombes, C.J. (2013). The cytokine networks of adaptive immunity in fish. Fish Shellfish Immunol. *35*, 1703–1718.

Wang, M.-H., Zhou, Y.-Q., and Chen, Y.-Q. (2002). Macrophage-Stimulating Protein and RON Receptor Tyrosine Kinase: Potential Regulators of Macrophage Inflammatory Activities. Scand. J. Immunol. *56*, 545–553.

Wen, Y., Shao, J.-Z., Xiang, L.-X., and Fang, W. (2006). Cloning, characterization and expression analysis of two Tetraodon nigroviridis interleukin-16 isoform genes. Comp. Biochem. Physiol. B Biochem. Mol. Biol. *144*, 159–166.

Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S.P., Aleksander, I., and Mak, T.W. (1984). A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature *308*, 145–149.

Zhu, L., Nie, L., Zhu, G., Xiang, L., and Shao, J. (2013). Advances in research of fish immune-relevant genes: A comparative overview of innate and adaptive immunity in teleosts. Dev. Comp. Immunol. *39*, 39–62.

SUPPLEMENTARY INFORMATION

Chapter 3

Experimental evidence that divergent evolution is linked to distinct defence mechanisms

Agnes Piecyk, Megan Hahn, Michael A. Bell, David Heins, Tina Henrich, Olivia Roth, Nolwenn Deilly, Martin Kalbe

SI.1. Further information on infection rates

Table S1. Host and parasite population effects on infection rates. Results from Type III chisquare tests of generalized linear mixed effect models.

In copepods (first intermediate host)	Chisq	Df	Pr(>Chisq)
(Intercept)	11.6737	1	0.0006339
Parasite population	2.9572	2	0.2279
Round	3.8827	2	0.1435
Parasite population : round	7.2386	4	0.1238
In sticklebacks	**Chisq**	**Df**	**Pr(>Chisq)**
(Intercept)	2.1497	1	0.1426
Parasite population	2.7889	2	0.2480
Host population	7.7672	2	0.0206
Parasite : host population	14.4449	4	**0.0060**

SI.2. Further information on parasite indices

Linear mixed models (LMMs) with parasite origin as fixed effect did not differ significantly from the respective Nullmodels.

Table S2. Effect of host population on parasite indices. Linear mixed models (LMMs) included host population as fixed effect and fish sex and tank as crossed random terms. Bold numbers indicate significance post fdr correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Parasite	Contrast (Host)			Estimate	Std. Error	z value	Pr(>|z|)	
SKO	GPS	-	ALO	8.924	1.592	5.607	**<0.0001**	***
ALO	ALX	-	ALO	-3.072	2.459	-1.249	0.424	
	GPS	-	ALO	11.778	2.378	4.954	**<0.0001**	***
	GPS	-	ALX	14.850	2.631	5.643	**<0.0001**	***
ALX	ALX	-	ALO	-2.199	2.020	-1.089	0.51712	
	GPS	-	ALO	6.816	2.774	2.457	0.0363	*
	GPS	-	ALX	9.015	2.624	3.436	**0.0018**	**

SI.3. Further information on host condition and immunological parameters

Table S3. Effect of host population on condition and immunological indices in controls. Response variables were the condition factor (CF) and hepatosomatic index (HSI) as well as the splenosomatic index (SSI) and head kidney index (HKI). LMMs included host population as fixed effect and fish sex and tank as crossed random terms. Bold numbers indicate significance post fdr correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Index	Contrast (Host)			Estimate	Std. Error	z value	Pr(>|z|)	
CF	ALX	-	ALO	-0.362	0.059	-6.187	**<0.0001**	***
	GPS	-	ALO	1.024	0.058	17.777	**<0.0001**	***
	GPS	-	ALX	1.386	0.057	24.200	**<0.0001**	***
HSI	ALX	-	ALO	-1.293	0.274	-4.717	**< 0.001**	***
	GPS	-	ALO	-0.924	0.278	-3.330	**0.002**	**
	GPS	-	ALX	0.369	0.277	1.331	0.378	
SSI	ALX	-	ALO	-0.013	0.008	-1.581	0.254	
	GPS	-	ALO	0.016	0.008	1.873	0.147	
	GPS	-	ALX	0.029	0.008	3.411	**0.002**	**
HKI	ALX	-	ALO	-0.036	0.021	-1.705	0.203	
	GPS	-	ALO	0.084	0.021	3.937	**<0.0001**	***
	GPS	-	ALX	0.120	0.021	5.667	**<0.0001**	***

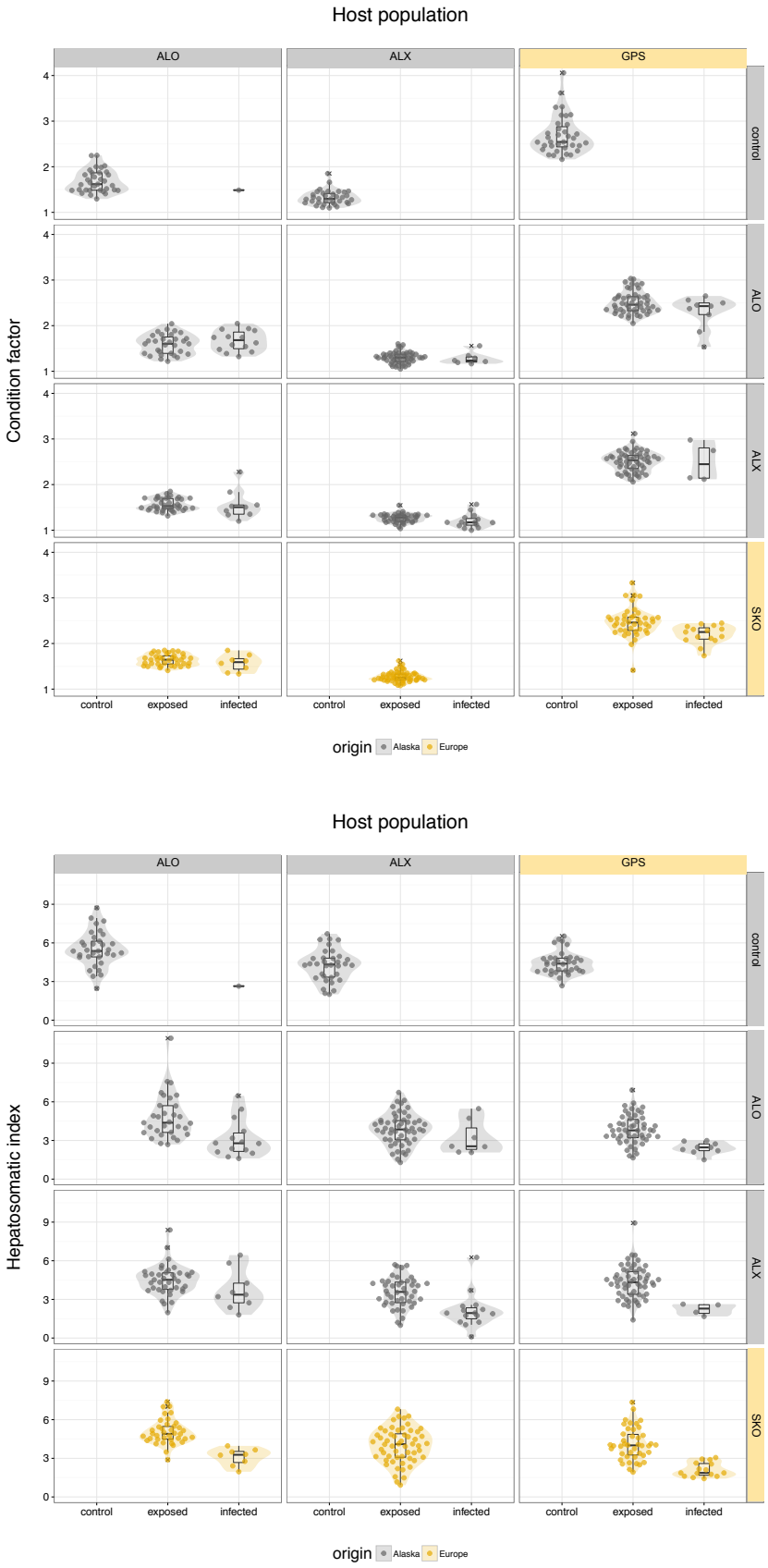


Figure S2. Stickleback condition (condition factor, CF, and hepatosomatic index, HSI). Fish originated from Walby Lake (ALO), Wolf Lake (ALX), and Großer Plöner See (GPS); *S. solidus* came from ALO, ALX, and Lake Skogseidvatnet (SKO); controls were sham-exposed; 'exposed' fish were exposed but uninfected.

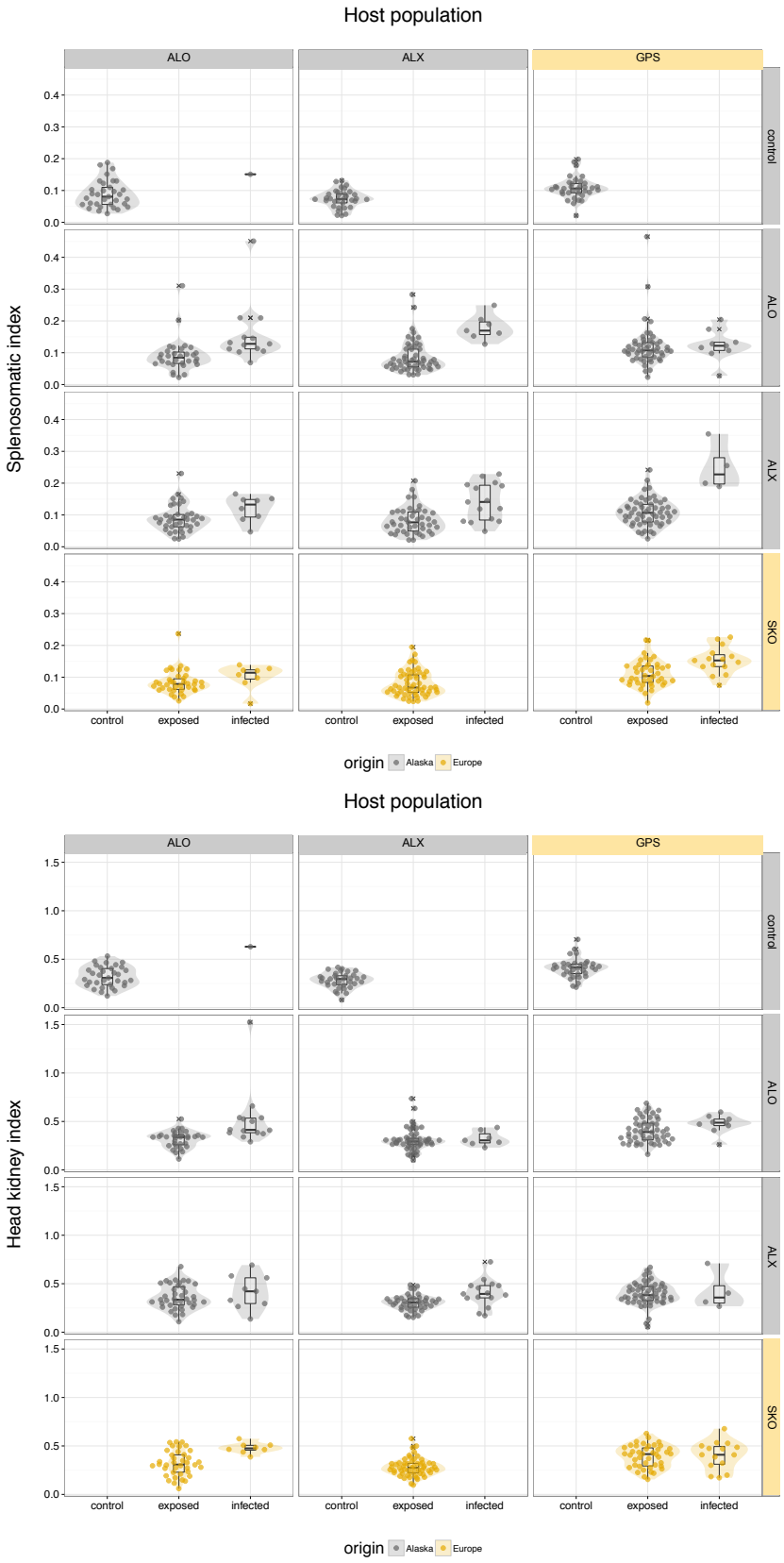


Figure S3. Stickleback immunological parameters (splenosomatic index, SSI, and head kidney index, HKI). Fish originated from Walby Lake (ALO), Wolf Lake (ALX), and Großer Plöner See (GPS); *S. solidus* came from ALO, ALX, and Lake Skogseidvatnet (SKO); controls were sham-exposed; 'uninfected' fish were exposed but uninfected.

Table S4. Effect of host population on condition and immunological indices in *S. solidus* exposed but uninfected stickleback. Response variables were the condition factor (CF) and hepatosomatic index (HSI) as well as the splenosomatic index (SSI) and head kidney index (HKI). LMMs included host population as fixed effect and fish sex and tank as crossed random effects. Bold numbers indicate significance post FDR correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Index	Contrast (Host)			Estimate	Std. Error	z value	Pr(>|z|)	
CF	ALX	-	ALO	-0.329	0.021	-15.300	**<0.0001**	***
	GPS	-	ALO	0.899	0.022	41.600	**<0.0001**	***
	GPS	-	ALX	1.227	0.020	62.100	**<0.0001**	***
HSI	ALX	-	ALO	-0.979	0.140	-7.003	**<0.001**	***
	GPS	-	ALO	-0.635	0.141	-4.512	**<0.001**	***
	GPS	-	ALX	0.344	0.129	2.674	**0.020**	*
SSI	ALX	-	ALO	-0.005	0.006	-0.911	0.633	
	GPS	-	ALO	0.026	0.006	4.521	**<0.0001**	***
	GPS	-	ALX	0.031	0.005	5.927	**<0.0001**	***
HKI	ALX	-	ALO	-0.037	0.014	-2.658	**0.021**	*
	GPS	-	ALO	0.064	0.014	4.578	**<0.0001**	***
	GPS	-	ALX	0.101	0.013	7.905	**<0.0001**	***

Table S5. Effect of host population on condition and immunological indices in *S. solidus* infected stickleback. Response variables were the condition factor (CF) and hepatosomatic index (HSI) as well as the splenosomatic index (SSI) and head kidney index (HKI). LMMs included host population as fixed effect and fish sex and tank as crossed random terms. Bold numbers indicate significance post fdr correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Index	Contrast (Host)			Estimate	Std. Error	z value	Pr(>|z|)	
CF	ALX	-	ALO	-0.386	0.059	-6.585	**<0.0001**	***
	GPS	-	ALO	0.651	0.053	12.300	**<0.0001**	***
	GPS	-	ALX	1.037	0.062	16.658	**<0.0001**	***
HSI	ALX	-	ALO	-0.780	0.261	-2.986	**0.008**	**
	GPS	-	ALO	-1.165	0.235	-4.957	**< 0.001**	***
	GPS	-	ALX	-0.385	0.282	-1.366	0.357	
SSI	ALX	-	ALO	0.005	0.039	0.139	0.989	
	GPS	-	ALO	0.012	0.036	0.338	0.939	
	GPS	-	ALX	0.007	0.040	0.165	0.985	
HKI	ALX	-	ALO	-0.093	0.048	-1.922	0.132	
	GPS	-	ALO	-0.043	0.045	-0.975	0.592	
	GPS	-	ALX	0.049	0.050	0.980	0.589	

Table S6. Effect of *S. solidus* exposure and infection on host condition (condition factor, CF). Sticklebacks were either sham-exposed (control), *S. solidus* exposed but uninfected (exposed), or *S. solidus* infected (infected). We further tested the effect of parasite origin in infected versus control stickleback. LMMs included either infection status or parasite origin as fixed effects and fish sex and tank as crossed random terms. Bold numbers indicate significance post FDR correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Host	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	infected	-	control	-0.462	0.068	-6.818	**<0.001**	***
	exposed	-	control	-0.205	0.048	-4.233	**<0.001**	***
	exposed	-	infected	0.258	0.058	4.457	**<0.001**	***
ALX	infected	-	control	-0.087	0.031	-2.781	**0.0143**	*
	exposed	-	control	-0.040	0.020	-1.983	0.1119	
	exposed	-	infected	0.047	0.027	1.728	0.1881	
ALO	infected	-	control	-0.144	0.035	-4.140	**< 0.001**	***
	exposed	-	control	-0.055	0.026	-2.072	0.094	
	exposed	-	infected	0.089	0.031	2.838	**0.013**	*
Parasite	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
SKO	exposed	-	infected	-0.234	0.119	-1.972	0.0486	*
ALO	exposed	-	infected	0.055	0.113	0.486	0.627	
ALX	exposed	-	infected	0.345	0.118	2.937	**0.0033**	**
Host	Contrast (Parasite)			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	ALO	-	CTRL	-0.461	0.126	-3.671	**0.001**	**
	ALX	-	CTRL	-0.298	0.161	-1.856	0.231	
	SKO	-	CTRL	-0.424	0.104	-4.059	**< 0.001**	***
	ALX	-	ALO	0.163	0.200	0.814	0.836	
	SKO	-	ALO	0.037	0.158	0.237	0.995	
	SKO	-	ALX	-0.126	0.187	-0.671	0.900	
ALX	ALO	-	CTRL	-0.049	0.061	-0.803	0.693	
	ALX	-	CTRL	-0.116	0.046	-2.502	0.031	*
	ALX	-	ALO	-0.067	0.073	-0.930	0.612	
ALO	ALO	-	CTRL	-0.113	0.066	-1.716	0.298	
	ALX	-	CTRL	-0.213	0.079	-2.688	0.034	*
	SKO	-	CTRL	-0.149	0.081	-1.849	0.236	
	ALX	-	ALO	-0.099	0.100	-0.992	0.739	
	SKO	-	ALO	-0.036	0.101	-0.353	0.984	
	SKO	-	ALX	0.064	0.110	0.576	0.934	

Table S7. Effect of *S. solidus* exposure and infection on host condition (hepatosomatic index, HSI). Sticklebacks were either sham-exposed (control), *S. solidus* exposed but uninfected (exposed), or *S. solidus* infected (infected). We further tested the effect of parasite origin in infected versus control stickleback. LMMs included either infection status or parasite origin as fixed effects and fish sex and tank as crossed random terms. Bold numbers indicate significance post FDR correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Host	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	infected	-	control	-2.119	0.245	-8.642	**<0.001**	***
	exposed	-	control	-0.425	0.174	-2.438	0.038	*
	exposed	-	infected	1.695	0.210	8.073	**<0.001**	***
ALO	infected	-	control	-2.592	0.272	-9.541	**<0.001**	***
	exposed	-	control	-0.626	0.209	-2.995	**0.008**	**
	exposed	-	infected	1.966	0.240	8.194	**<0.001**	***
ALX	infected	-	control	-1.651	0.269	-6.129	**<0.001**	***
	exposed	-	control	-0.292	0.172	-1.700	0.181	
	exposed	-	infected	1.360	0.235	5.780	**<0.001**	***
Parasite	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
SKO	exposed	-	infected	1.875	0.265	7.081	**<0.0001**	***
ALO	exposed	-	infected	1.473	0.257	5.723	**<0.0001**	***
ALX	exposed	-	infected	1.386	0.256	5.421	**<0.0001**	***
Host	Contrast (Parasite)			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	ALO	-	CTRL	-2.018	0.284	-7.109	**<0.0001**	***
	ALX	-	CTRL	-2.047	0.375	-5.459	**<0.0001**	***
	SKO	-	CTRL	-2.265	0.234	-9.678	**<0.0001**	***
	ALX	-	ALO	-0.029	0.454	-0.064	1.000	
	SKO	-	ALO	-0.247	0.346	-0.714	0.884	
	SKO	-	ALX	-0.218	0.424	-0.513	0.953	
ALX	ALO	-	CTRL	-1.128	0.458	-2.465	0.034	**
	ALX	-	CTRL	-2.303	0.345	-6.677	**<0.001**	***
	ALX	-	ALO	-1.175	0.556	-2.112	0.082	
ALO	ALO	-	CTRL	-2.620	0.408	-6.427	**<0.001**	***
	ALX	-	CTRL	-1.597	0.485	-3.291	**0.005**	**
	SKO	-	CTRL	-2.411	0.497	-4.852	**<0.001**	***
	ALX	-	ALO	1.023	0.598	1.711	0.304	
	SKO	-	ALO	0.209	0.607	0.345	0.985	
	SKO	-	ALX	-0.814	0.660	-1.233	0.591	

Table S8. Effect of *S. solidus* exposure and infection on host immunity (splenosomatic index, SSI). Sticklebacks were either sham-exposed (control), *S. solidus* exposed but uninfected (exposed), or *S. solidus* infected (infected). We further tested the effect of parasite origin in infected versus control stickleback. LMMs included either infection status or parasite origin as fixed effects and fish sex and tank as crossed random terms. Bold numbers indicate significance post fdr correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Host	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	infected	-	control	0.048	0.013	3.763	**<0.001**	***
	exposed	-	control	0.007	0.009	0.720	0.747	
	exposed	-	infected	-0.041	0.011	-3.895	**<0.001**	***
ALO	infected	-	control	0.072	0.024	3.041	**0.007**	**
	exposed	-	control	0.002	0.019	0.113	0.993	
	exposed	-	infected	-0.070	0.020	-3.468	**0.001**	**
ALX	infected	-	control	0.083	0.011	7.472	**<0.0001**	***
	exposed	-	control	0.009	0.007	1.163	0.467	
	exposed	-	infected	-0.075	0.010	-7.791	**<0.0001**	***
Parasite	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
SKO	exposed	-	infected	-0.044	0.010	-4.652	**<0.0001**	***
ALO	exposed	-	infected	-0.087	0.021	4.083	**<0.0001**	***
ALX	exposed	-	infected	-0.051	0.010	-5.148	**<0.0001**	***
Host	Contrast (Parasite)			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	ALO	-	CTRL	0.014	0.016	0.851	0.821	
	ALX	-	CTRL	0.140	0.022	6.412	**<0.001**	***
	SKO	-	CTRL	0.043	0.013	3.275	**0.005**	**
	ALX	-	ALO	0.127	0.025	4.981	**<0.001**	***
	SKO	-	ALO	0.029	0.018	1.594	0.367	
	SKO	-	ALX	-0.097	0.024	-4.101	**<0.001**	***
ALX	ALO	-	CTRL	0.108	0.017	6.537	**<0.001**	***
	ALX	-	CTRL	0.069	0.012	5.592	**<0.001**	***
	ALX	-	ALO	-0.039	0.019	-2.044	0.098	
ALO	ALO	-	CTRL	0.140	0.047	2.947	**0.016**	*
	ALX	-	CTRL	0.029	0.058	0.505	0.956	
	SKO	-	CTRL	0.013	0.058	0.215	0.996	
	ALX	-	ALO	-0.110	0.067	-1.646	0.344	
	SKO	-	ALO	-0.127	0.067	-1.898	0.222	
	SKO	-	ALX	-0.017	0.075	-0.225	0.996	

Table S9. Effect of *S. solidus* exposure and infection on host immunity (head kidney index, HKI). Sticklebacks were either sham-exposed (control), *S. solidus* exposed but uninfected (exposed), or *S. solidus* infected (infected). We further tested the effect of parasite origin in infected versus control stickleback. LMMs included either infection status or parasite origin as fixed effects and fish sex and tank as crossed random terms. Bold numbers indicate significance post fdr correction according to Benjamini and Hochberg (1995) with an alpha of 0.05.

Host	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	infected	-	control	0.011	0.030	0.370	0.926	
	exposed	-	control	-0.008	0.022	-0.390	0.918	
	exposed	-	infected	-0.019	0.025	-0.783	0.709	
ALO	infected	-	control	0.150	0.033	4.513	**<0.0001**	***
	exposed	-	control	0.015	0.026	0.588	0.825	
	exposed	-	infected	-0.135	0.029	-4.715	**<0.0001**	***
ALX	infected	-	control	0.095	0.024	3.915	**<0.001**	***
	exposed	-	control	0.018	0.016	1.143	0.497	
	exposed	-	infected	-0.077	0.021	-3.674	**<0.001**	***
Host	Contrast			Estimate	Std. Error	z value	Pr(>|z|)	
SKO	exposed	-	infected	-0.105	0.028	-3.851	**0.0001**	***
ALO	exposed	-	infected	-0.111	0.030	-3.748	**0.0002**	***
ALX	exposed	-	infected	-0.052	0.026	-2.028	0.0425	
Host	Contrast (Parasite)			Estimate	Std. Error	z value	Pr(>|z|)	
GPS	ALO	-	CTRL	0.044	0.044	0.990	0.742	
	ALX	-	CTRL	0.003	0.060	0.047	1.000	
	SKO	-	CTRL	-0.012	0.036	-0.335	0.986	
	ALX	-	ALO	-0.041	0.071	-0.576	0.935	
	SKO	-	ALO	-0.056	0.053	-1.052	0.704	
	SKO	-	ALX	-0.015	0.067	-0.224	0.996	
ALX	ALO	-	CTRL	0.041	0.041	0.989	0.563	
	ALX	-	CTRL	0.120	0.031	3.843	**<0.001**	***
	ALX	-	ALO	0.079	0.048	1.662	0.213	
ALO	ALO	-	CTRL	0.177	0.057	3.110	**0.009**	**
	ALX	-	CTRL	0.096	0.067	1.427	0.471	
	SKO	-	CTRL	0.145	0.070	2.087	0.151	
	ALX	-	ALO	-0.081	0.080	-1.012	0.734	
	SKO	-	ALO	-0.032	0.082	-0.385	0.980	
	SKO	-	ALX	0.049	0.090	0.552	0.944	

SI.4. Further information on stickleback gene expression

Non-parametric permutational multivariate analyses of variance (PERMANOVA (Anderson, 2001) using the function adonis() from the *vegan* package (Oksanen et al., 2015)) were based on Euclidian distance matrices and 10,000 permutations. The weight of the fish was included as covariate to account for size-related effects. Permutations were constrained within tank. Pairwise PERMANOVAs were used *a posteriori* to identify significantly different groups (Anderson, 2001). The False Discovery Rate (FDR) (Benjamini and Hochberg, 1995) was used to account for multiple testing.

Multivariate patterns in gene expression were visualized by non-metric multidimensional scaling (NMDS) on Euclidian distances and two dimensions (function metaMDS()); the contribution of each gene was plotted by use of the envfit() function (both implemented in *vegan*).

Linear mixed models to compare expression levels of single genes were fit with lmer() from *lme4* (Bates et al., 2014); pseudo R^2 were calculated with sem.model.fits() from the R package *piecewiseSEM* (Lefcheck, 2016); the glht() function from *multcomp* (Hothorn et al., 2008) was used for post hoc pairwise comparisons. The R^2 includes the effect of the random term and was calculated according to (Nakagawa and Schielzeth, 2013; Johnson, 2014).

Table S10. Effects of host and parasite population and infection status on stickleback gene expression profiles. The PERMANOVA included host (H) and parasite (P) population and infection status (group: control, exposed, infected) and all interactions as explanatories and the weight of the fish as covariate. Permutations (10,000) were constrained within tank.

Explanatories	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Fish weight	1	25.92	25.9154	7.9633	0.02762	0.0542	.
Stickleback population (H)	2	38.77	19.3855	5.9568	0.04131	**0.0005**	***
S. solidus population (P)	3	11.79	3.9293	1.2074	0.01256	0.3596	
group	2	22.22	11.1076	3.4132	0.02367	**0.0004**	***
H:P interaction	6	22.65	3.7746	1.1599	0.02413	0.3885	
H:group interaction	2	9.08	4.5407	1.3953	0.00968	0.1048	
P:group interaction	2	9.63	4.8172	1.4802	0.01027	0.2120	
H:P:group interaction	3	7.59	2.5284	0.7769	0.00808	0.4672	
Residuals	243	790.81	3.2544		0.84268		
Total	264	938.45			1		

SI.4.1. Baseline gene expression profiles of the host populations (n = 84)

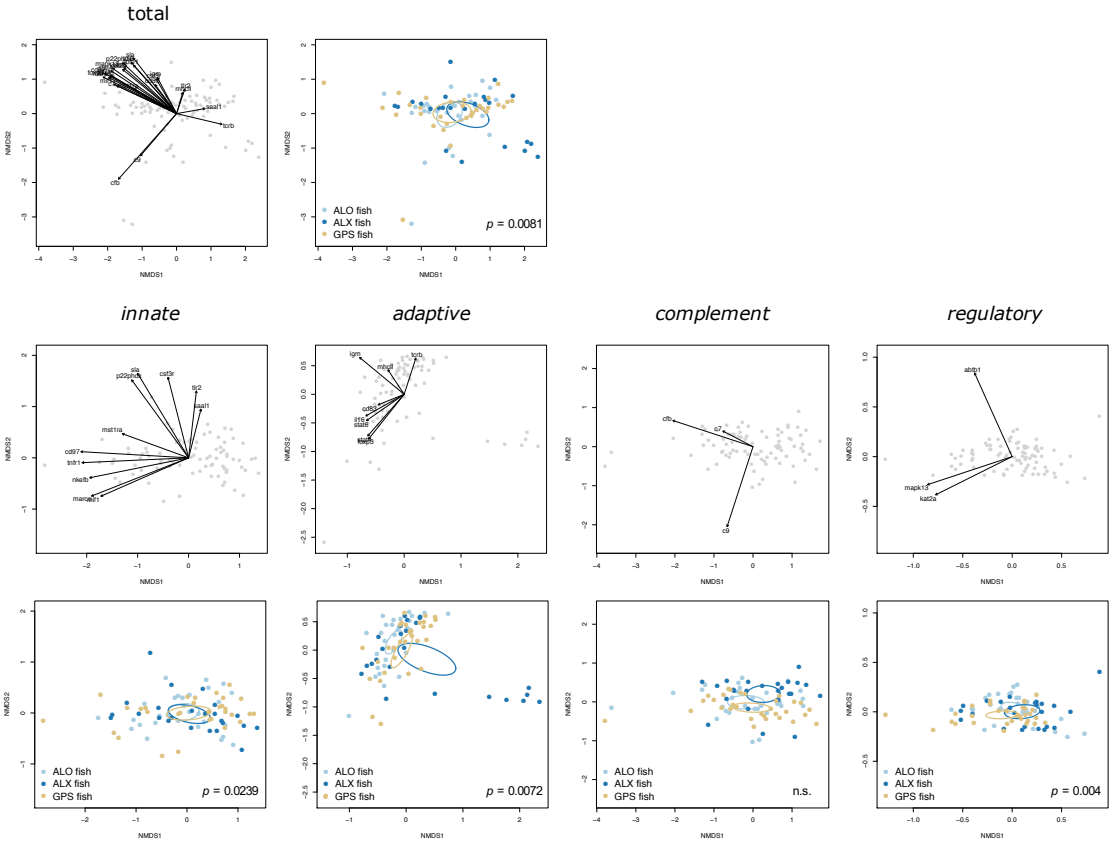


Figure S4. Gene expression profiles of sham-exposed stickleback. Sticklebacks originated from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Großer Plöner See (GPS; Germany) and were 10 months old. Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Table S10.

Table S11. Population effect on gene expression profiles of sham-exposed stickleback. Sticklebacks originated from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Großer Plöner See (GPS; Germany). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	5.344	5.3438	1.6574	0.0192	0.0330	*
	fish_origin	2	14.468	7.2342	2.2437	0.0521	**0.0081**	**
	Residuals	80	257.944	3.2243		0.9287		
	Total	83	277.757			1		
innate	fish_weight	1	2.961	2.96106	3.0167	0.0350	0.0005	***
	fish_origin	2	3.03	1.51516	1.5436	0.0359	**0.0239**	*
	Residuals	80	78.525	0.98156		0.9291		
	Total	83	84.516			1		
adaptive	fish_weight	1	2.107	2.1066	2.3098	0.0262	0.0334	*
	fish_origin	2	5.437	2.7187	2.981	0.0675	**0.0072**	**
	Residuals	80	72.96	0.912		0.9063		
	Total	83	80.504			1		
complement	fish_weight	1	0.237	0.23698	0.19546	0.0023	0.7881	
	fish_origin	2	5.219	2.60955	2.15231	0.0509	0.1398	
	Residuals	80	96.995	1.21244		0.9467		
	Total	83	102.451			1		
regulatory	fish_weight	1	0.0514	0.05138	0.33116	0.0039	0.6183	
	fish_origin	2	0.8496	0.42479	2.73814	0.0638	**0.0040**	**
	Residuals	80	12.4112	0.15514		0.9323		
	Total	83	13.3122			1		

Table S12. Population effect on gene expression profiles of sham-exposed Alaskan stickleback. Bold numbers indicate significance post FDR correction.

ALO vs ALX		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	6.43	6.4301	2.1272	0.0389	0.0524	.
	fish_origin	1	7.863	7.8629	2.6012	0.0475	**0.0034**	**
	Residuals	50	151.142	3.0228		0.9136		
	Total	52	165.435			1		
innate	fish_weight	1	0.867	0.86735	0.9995	0.0195	0.1886	
	fish_origin	1	0.27	0.26998	0.3111	0.0061	0.6797	
	Residuals	50	43.388	0.86776		0.9745		
	Total	52	44.525			1		
adaptive	fish_weight	1	4.124	4.1243	4.2979	0.0758	0.0958	.
	fish_origin	1	2.281	2.2806	2.3766	0.0419	0.042	*
	Residuals	50	47.98	0.9596		0.8822		
	Total	52	54.385			1		
complement	fish_weight	1	1.435	1.4355	1.3204	0.0235	0.2328	
	fish_origin	1	5.23	5.2299	4.8107	0.0857	**0.0072**	**
	Residuals	50	54.357	1.0871		0.8908		
	Total	52	61.022			1		
regulatory	fish_weight	1	0.0485	0.04846	0.3212	0.0063	0.555	
	fish_origin	1	0.1157	0.11574	0.7669	0.0150	0.1643	
	Residuals	50	7.5455	0.15091		0.9787		
	Total	52	7.7097			1		

Table S13. Population effect on gene expression profiles of sham-exposed Alaskan (ALO) and European stickleback. Bold numbers indicate significance post FDR correction.

ALO vs GPS		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	3.7390	3.7389	1.2052	0.0203	0.1892	
	fish_origin	1	4.0240	4.0237	1.2970	0.0218	0.1502	
	Residuals	57	176.84	3.1024		0.9580		
	Total	59	184.60			1		
innate	fish_weight	1	2.5680	2.5683	2.5805	0.0423	0.0016	**
	fish_origin	1	1.4540	1.4539	1.4608	0.0239	0.0456	*
	Residuals	57	56.732	0.9953		0.9338		
	Total	59	60.754			1		
adaptive	fish_weight	1	0.6740	0.6739	0.9776	0.0165	0.0647	.
	fish_origin	1	0.9030	0.9035	1.3107	0.0221	0.0555	.
	Residuals	57	39.292	0.6893		0.9614		
	Total	59	40.869			1		
complement	fish_weight	1	0.3800	0.3801	0.2911	0.0050	0.7961	
	fish_origin	1	1.2940	1.2940	0.9909	0.0170	0.3490	
	Residuals	57	74.434	1.3059		0.9780		
	Total	59	76.108			1		
regulatory	fish_weight	1	0.1224	0.1224	0.8214	0.0136	0.2395	
	fish_origin	1	0.3833	0.3833	2.5726	0.0426	**0.0117**	*
	Residuals	57	8.4918	0.1490		0.9438		
	Total	59	8.9974			1		

Table S14. Population effect on gene expression profiles of sham-exposed Alaskan (ALX) and European stickleback. Bold numbers indicate significance post FDR correction.

ALX vs GPS		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	5.129	5.1287	1.5268	0.0267	0.1318	
	fish_origin	1	12.005	12.0048	3.5738	0.0626	**0.0070**	**
	Residuals	52	174.674	3.3591		0.9107		
	Total	54	191.808			1		
innate	fish_weight	1	3.244	3.2435	3.0500	0.0528	0.0024	**
	fish_origin	1	2.893	2.8934	2.7208	0.0471	**0.0260**	*
	Residuals	52	55.300	1.0635		0.9001		
	Total	54	61.437			1		
adaptive	fish_weight	1	1.371	1.3709	1.2700	0.0218	0.2930	
	fish_origin	1	5.483	5.4827	5.0790	0.0871	0.0352	*
	Residuals	52	56.133	1.0795		0.8912		
	Total	54	62.987			1		
complement	fish_weight	1	0.764	0.7641	0.6447	0.0116	0.6050	
	fish_origin	1	3.280	3.2797	2.7670	0.0499	**0.0229**	*
	Residuals	52	61.635	1.1853		0.9384		
	Total	54	65.679			1		
regulatory	fish_weight	1	0.092	0.0924	0.5686	0.0098	0.2769	
	fish_origin	1	0.938	0.9381	5.7720	0.0989	**0.0132**	*
	Residuals	52	8.451	0.1625		0.8913		
	Total	54	9.482			1		

Table S15. Differentially expressed genes between sham-exposed controls. LMMs included the origin of the fish as fixed effect, the weight of the fish as covariate and tank as random term. Bold letters indicate significance.

	contrast			Estimate	Std. Error	z value	Pr(>|z|)		R2
regulatory									
abtb1	ALX	-	ALO	-0.0496	0.0397	-1.249	0.423		0.579
	GPS	-	ALO	0.0089	0.0437	0.205	0.977		
	GPS	-	ALX	0.0585	0.0379	1.543	0.27		
kat2a	ALX	-	ALO	-0.0754	0.0524	-1.4410	0.319		0.381
	GPS	-	ALO	0.0263	0.0569	0.4630	0.888		
	GPS	-	ALX	0.1018	0.0502	2.0270	0.105		
mapk13	ALX	-	ALO	0.0060	0.0596	0.1010	0.994		0.535
	GPS	-	ALO	0.2000	0.0653	3.0640	0.006	**	
	GPS	-	ALX	0.1940	0.0570	3.4040	0.002	**	
innate									
cd97	ALX	-	ALO	-0.03379	0.08173	-0.413	0.91		0.783
	GPS	-	ALO	0.11055	0.09082	1.217	0.441		
	GPS	-	ALX	0.14434	0.07783	1.855	0.151		
csf3r	ALX	-	ALO	-0.0197	0.0593	-0.3320	0.941		0.308
	GPS	-	ALO	-0.1393	0.0635	-2.1930	0.072	.	
	GPS	-	ALX	-0.1197	0.0571	-2.0950	0.091	.	
marco	ALX	-	ALO	-0.1026	0.0698	-1.4700	0.304		0.675
	GPS	-	ALO	-0.0889	0.0771	-1.1540	0.480		
	GPS	-	ALX	0.0137	0.0666	0.2060	0.977		
mif1	ALX	-	ALO	0.0150	0.0337	0.4460	0.896		0.582
	GPS	-	ALO	0.0557	0.0371	1.5020	0.289		
	GPS	-	ALX	0.0406	0.0322	1.2630	0.415		
mst1ra	ALX	-	ALO	-0.0361	0.0478	-0.7560	0.729		0.399
	GPS	-	ALO	-0.0978	0.0517	-1.8900	0.141		
	GPS	-	ALX	-0.0616	0.0459	-1.3430	0.371		
nkefb	ALX	-	ALO	0.0120	0.0718	0.1670	0.985		0.523
	GPS	-	ALO	0.0664	0.0788	0.8430	0.675		
	GPS	-	ALX	0.0544	0.0687	0.7920	0.707		
p22phox	ALX	-	ALO	-0.0444	0.0443	-1.0040	0.574		0.306
	GPS	-	ALO	0.1072	0.0477	2.2490	0.063	.	
	GPS	-	ALX	0.1516	0.0425	3.5640	0.001	**	
saal1	ALX	-	ALO	0.0839	0.0607	1.3840	0.349		0.376
	GPS	-	ALO	-0.0941	0.0658	-1.4300	0.325		
	GPS	-	ALX	-0.1780	0.0582	-3.0580	0.006	**	
sla	ALX	-	ALO	-0.0579	0.0396	-1.4620	0.308		0.419
	GPS	-	ALO	-0.0644	0.0430	-1.4960	0.292		
	GPS	-	ALX	-0.0065	0.0379	-0.1710	0.984		
tlr2	ALX	-	ALO	-0.0193	0.0783	-0.2470	0.967		0.206
	GPS	-	ALO	0.2142	0.0837	2.5580	0.028	*	
	GPS	-	ALX	0.2335	0.0755	3.0940	0.006	**	
tnfr1	ALX	-	ALO	-0.0016	0.0693	-0.0230	1.000		0.684
	GPS	-	ALO	-0.1406	0.0765	-1.8370	0.157		
	GPS	-	ALX	-0.1390	0.0661	-2.1030	0.089	.	

Table S15 (continued). Differentially expressed genes between sham-exposed controls. LMMs included the origin of the fish as fixed effect, the weight of the fish as covariate and tank as random term.

	contrast			Estimate	Std. Error	z value	Pr(>|z|)		R2
adaptive									
stat4	ALX	-	ALO	-0.0661	0.0568	-1.1640	0.474		0.709
	GPS	-	ALO	-0.0563	0.0629	-0.8960	0.641		
	GPS	-	ALX	0.0097	0.0541	0.1800	0.982		
cd83	ALX	-	ALO	-0.0821	0.0397	-2.0670	0.096	.	0.481
	GPS	-	ALO	-0.1276	0.0434	-2.9410	0.009	**	
	GPS	-	ALX	-0.0455	0.0380	-1.1950	0.455		
igm	ALX	-	ALO	-0.4509	0.1667	-2.7060	0.019	*	0.169
	GPS	-	ALO	0.0681	0.1767	0.3850	0.921		
	GPS	-	ALX	0.5190	0.1609	3.2260	0.004	**	
stat6	ALX	-	ALO	-0.0476	0.0404	-1.1780	0.465		0.654
	GPS	-	ALO	-0.0856	0.0446	-1.9220	0.132		
	GPS	-	ALX	-0.0380	0.0386	-0.9850	0.585		
foxp3	ALX	-	ALO	-0.0399	0.0813	-0.4900	0.876		0.693
	GPS	-	ALO	0.0674	0.0900	0.7480	0.734		
	GPS	-	ALX	0.1072	0.0776	1.3830	0.349		
il16	ALX	-	ALO	-0.0632	0.0468	-1.3500	0.367		0.370
	GPS	-	ALO	0.0196	0.0509	0.3850	0.921		
	GPS	-	ALX	0.0828	0.0449	1.8430	0.155		
mhcII	ALX	-	ALO	-0.1014	0.0474	-2.1370	0.082	.	0.115
	GPS	-	ALO	-0.0154	0.0503	-0.3060	0.950		
	GPS	-	ALX	0.0860	0.0458	1.8780	0.145		
tcr-β	ALX	-	ALO	-0.0369	0.1004	-0.3680	0.928		0.192
	GPS	-	ALO	-0.2093	0.1077	-1.9430	0.126		
	GPS	-	ALX	-0.1724	0.0966	-1.7840	0.174		
complement									
c7	ALX	-	ALO	0.0300	0.0412	0.728	0.746		0.564
	GPS	-	ALO	-0.0291	0.0453	-0.643	0.795		
	GPS	-	ALX	-0.0591	0.0394	-1.502	0.289		
c9	ALX	-	ALO	-0.3773	0.1361	-2.773	0.015	*	0.134
	GPS	-	ALO	0.0323	0.1443	0.224	0.973		
	GPS	-	ALX	0.4097	0.1314	3.119	0.005	**	
cfb	ALX	-	ALO	-0.5242	0.2711	-1.933	0.129		0.172
	GPS	-	ALO	-0.4092	0.2906	-1.408	0.336		
	GPS	-	ALX	0.1150	0.2610	0.441	0.898		

SI.4.2. Gene expression profiles of S. solidus exposed stickleback (n = 101)

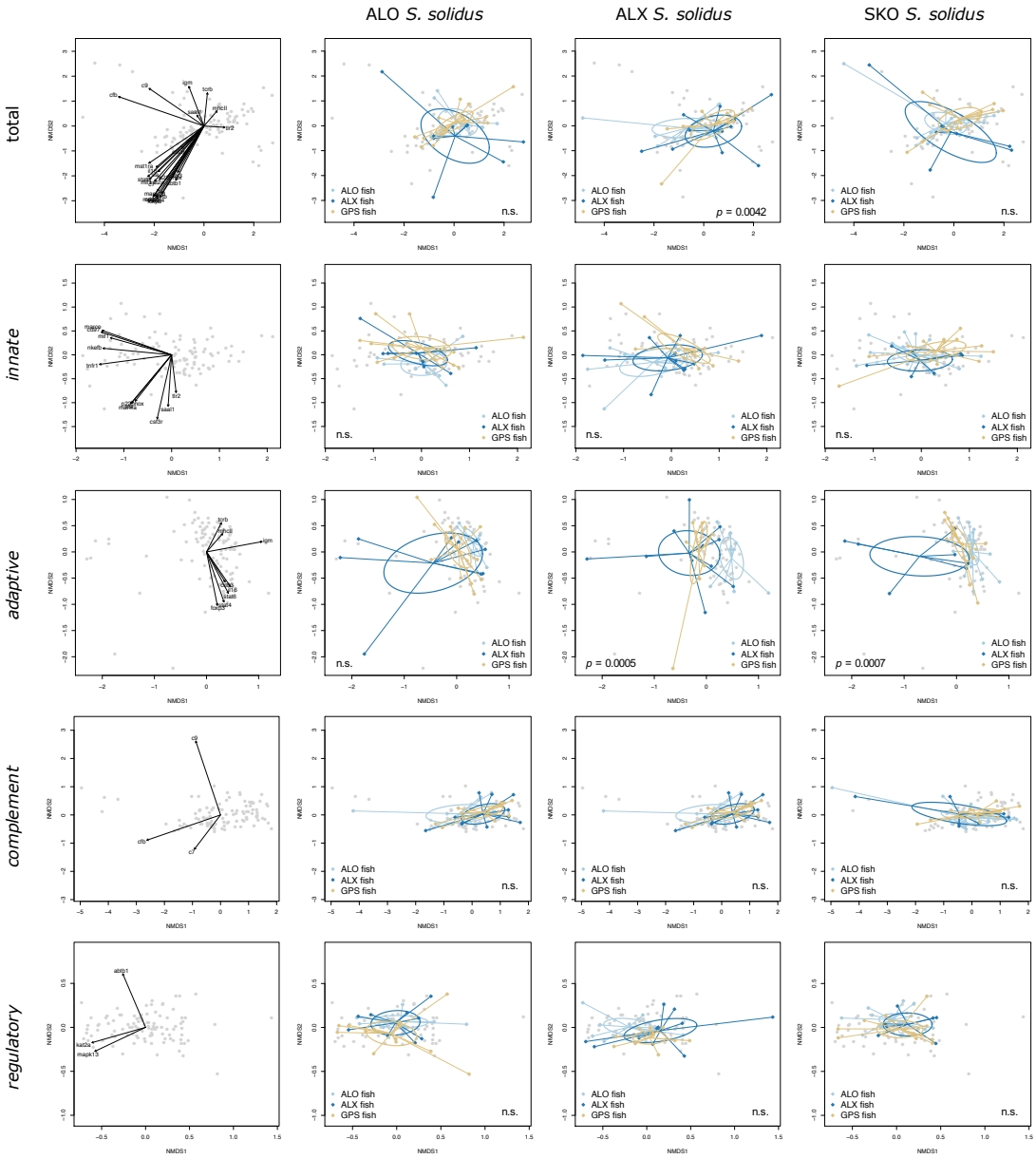


Figure S5. Gene expression profiles of *S. solidus* exposed stickleback. Sticklebacks originated from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Großer Plöner See (GPS; Germany); *S. solidus* came from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Lake Skogseidvatnet (SKO; Norway). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S16-S18.

Table S16. Differences between ALO exposed hosts (ALO, ALX, and GPS stickleback). The effect of host origin was not significant after FDR correction.

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	7.28	7.2800	2.2054	0.0670	0.0205	*
	Host origin	2	8.937	4.4685	1.3537	0.0823	0.2190	
	Residuals	28	92.426	3.3009		0.8507		
	Total	31	108.643			1		
innate	fish_weight	1	3.4334	3.4334	4.0349	0.1155	0.0294	*
	Host origin	2	2.4653	1.2327	1.4486	0.0829	0.4385	
	Residuals	28	23.8263	0.8509		0.8016		
	Total	31	29.725			1		
adaptive	fish_weight	1	1.938	1.9378	2.1494	0.0606	0.0993	.
	Host origin	2	4.817	2.4086	2.6717	0.1506	0.0120	*
	Residuals	28	25.243	0.9015		0.7889		
	Total	31	31.998			1		
complement	fish_weight	1	1.629	1.6286	1.1505	0.0385	0.1158	
	Host origin	2	1.055	0.5275	0.3726	0.0249	0.7936	
	Residuals	28	39.636	1.4156		0.9366		
	Total	31	42.319			1		
regulatory	fish_weight	1	0.3905	0.3905	2.0664	0.0621	0.1452	
	Host origin	2	0.6109	0.3054	1.6160	0.0971	0.3973	
	Residuals	28	5.2919	0.1890		0.8409		
	Total	31	6.2933			1		

Table S17. Differences between ALX exposed hosts (ALO, ALX, and GPS stickleback). Bold numbers indicate significance post FDR correction.

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	4.346	4.3461	1.1329	0.0340	0.9072	
	Host origin	2	16.117	8.0585	2.1007	0.1260	**0.0042**	**
	Residuals	28	107.411	3.8361		0.8400		
	Total	31	127.874			1		
innate	fish_weight	1	2.134	2.1336	1.9541	0.0577	0.7368	
	Host origin	2	4.263	2.1314	1.9520	0.1153	0.0102	*
	Residuals	28	30.573	1.0919		0.8270		
	Total	31	36.969			1		
adaptive	fish_weight	1	1.253	1.2534	1.4202	0.0392	0.7389	
	Host origin	2	6.032	3.0158	3.4170	0.1885	**0.0005**	***
	Residuals	28	24.713	0.8826		0.7723		
	Total	31	31.998			1		
complement	fish_weight	1	0.436	0.4356	0.2558	0.0082	0.9643	
	Host origin	2	5.145	2.5726	1.5108	0.0966	0.1592	
	Residuals	28	47.68	1.7029		0.8952		
	Total	31	53.261			1		
regulatory	fish_weight	1	0.5496	0.5496	2.8073	0.0791	0.4291	
	Host origin	2	0.9131	0.4566	2.3319	0.1315	0.0289	*
	Residuals	28	5.4821	0.1958		0.7894		
	Total	31	6.9449			1		

Table S18. Differences between SKO exposed hosts (ALO, ALX, and GPS stickleback). The bold number indicates significance post FDR.

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	9.014	9.0145	2.4115	0.0640	0.0113	*
	Host origin	2	8.582	4.2908	1.1478	0.0609	0.1455	
	Residuals	33	123.359	3.7381		0.8752		
	Total	36	140.955			1.0000		
innate	fish_weight	1	5.164	5.1636	6.3459	0.1544	0.0007	***
	Host origin	2	1.431	0.7155	0.8793	0.0428	0.3010	
	Residuals	33	26.852	0.8137		0.8028		
	Total	36	33.446			1.0000		
adaptive	fish_weight	1	2.0438	2.0438	3.3566	0.0732	0.0208	*
	Host origin	2	5.7858	2.8929	4.7511	0.2072	**0.0007**	***
	Residuals	33	20.0934	0.6089		0.7196		
	Total	36	27.923			1.0000		
complement	fish_weight	1	1.502	1.5022	0.6753	0.0198	0.2633	
	Host origin	2	1.131	0.5656	0.2543	0.0149	0.8305	
	Residuals	33	73.402	2.2243		0.9654		
	Total	36	76.036			1.0000		
regulatory	fish_weight	1	0.4202	0.4202	3.4071	0.0878	0.0864	.
	Host origin	2	0.294	0.1470	1.1919	0.0615	0.1907	
	Residuals	33	4.0696	0.1233		0.8507		
	Total	36	4.7838			1.0000		

Table S19. Differences between exposed ALO stickleback (ALO, ALX, and SKO *S. solidus*).

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	3.331	3.3309	0.9475	0.0274	0.4707	
	S. solidus	2	5.9	2.9498	0.8391	0.0485	0.1198	
	Residuals	32	112.494	3.5154		0.9242		
	Total	35	121.724			1		
innate	fish_weight	1	1.5996	1.5996	2.0020	0.0540	0.0612	.
	S. solidus	2	2.4722	1.2361	1.5471	0.0834	0.0775	.
	Residuals	32	25.5676	0.7990		0.8626		
	Total	35	29.6394			1		
adaptive	fish_weight	1	0.891	0.8910	2.2327	0.0591	0.2207	
	S. solidus	2	1.4086	0.7043	1.7649	0.0935	0.2487	
	Residuals	32	12.77	0.3991		0.8474		
	Total	35	15.0696			1		
complement	fish_weight	1	0.806	0.8060	0.3636	0.0110	0.9538	
	S. solidus	2	1.476	0.7382	0.3330	0.0202	0.0619	.
	Residuals	32	70.934	2.2167		0.9688		
	Total	35	73.216			1		
regulatory	fish_weight	1	0.0533	0.0533	0.4025	0.0110	0.2357	
	S. solidus	2	0.5706	0.2853	2.1563	0.1175	0.3171	
	Residuals	32	4.2335	0.1323		0.8716		
	Total	35	4.8574			1		

Table S20. Differences between exposed ALX stickleback (ALO, ALX, and SKO *S. solidus*)

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	9.538	9.5385	1.9458	0.0736	0.75
	S. solidus	2	7.249	3.6245	0.7394	0.0560	0.25
	Residuals	23	112.747	4.9021		0.8704	
	Total	26	129.535			1	
innate	fish_weight	1	4.8607	4.8607	5.7926	0.1911	0.875
	S. solidus	2	1.2729	0.6365	0.7585	0.0501	1
	Residuals	23	19.2995	0.8391		0.7588	
	Total	26	25.4331			1	
adaptive	fish_weight	1	3.026	3.0259	1.9475	0.0750	0.75
	S. solidus	2	1.573	0.7866	0.5063	0.0390	0.375
	Residuals	23	35.736	1.5537		0.8860	
	Total	26	40.335			1	
complement	fish_weight	1	0.38	0.3797	0.1610	0.0065	0.375
	S. solidus	2	4.252	2.1258	0.9011	0.0722	0.25
	Residuals	23	54.257	2.3590		0.9214	
	Total	26	58.889			1	
regulatory	fish_weight	1	1.5814	1.5814	8.6223	0.2647	0.875
	S. solidus	2	0.1754	0.0877	0.4782	0.0294	1
	Residuals	23	4.2184	0.1834		0.7060	
	Total	26	5.9753			1	

Table S21. Differences between exposed GPS stickleback (ALO, ALX, and SKO *S. solidus*)

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	5.533	5.5334	1.9471	0.0518	0.5
	S. solidus	2	4.749	2.3745	0.8356	0.0444	0.4844
	Residuals	34	96.622	2.8418		0.9038	
	Total	37	106.904			1	
innate	fish_weight	1	2.708	2.7076	2.5718	0.0672	0.5117
	S. solidus	2	1.82	0.9101	0.8644	0.0451	0.4766
	Residuals	34	35.796	1.0528		0.8877	
	Total	37	40.324			1	
adaptive	fish_weight	1	1.4861	1.4861	2.3920	0.0625	0.4844
	S. solidus	2	1.1784	0.5892	0.9484	0.0495	0.4844
	Residuals	34	21.1236	0.6213		0.8880	
	Total	37	23.7881			1	
complement	fish_weight	1	1.106	1.1057	1.0615	0.0290	0.6094
	S. solidus	2	1.571	0.7857	0.7543	0.0413	0.5664
	Residuals	34	35.416	1.0416		0.9297	
	Total	37	38.093			1	
regulatory	fish_weight	1	0.237	0.2370	1.3304	0.0364	0.5312
	S. solidus	2	0.2252	0.1126	0.6322	0.0346	0.5586
	Residuals	34	6.0561	0.1781		0.9291	
	Total	37	6.5182			1	

Table S22. Differentially expressed adaptive immune genes between ALX-exposed stickleback. LMMs included the origin of the fish as fixed effect, the weight of the fish as covariate and tank as random term. Bold letters indicate significance.

	contrast			Estimate	Std. Error	z value	Pr(>|z|)		R2
adaptive									
stat4	ALX	-	ALO	-0.2384	0.0782	-3.0500	0.007	**	0.729
	GPS	-	ALO	-0.2553	0.0781	-3.2680	0.003	**	
	GPS	-	ALX	-0.0169	0.0754	-0.2240	0.973		
cd83	ALX	-	ALO	-0.2117	0.0629	-3.3660	0.002	**	0.545
	GPS	-	ALO	-0.3393	0.0632	-5.3720	< 1e-04	***	
	GPS	-	ALX	-0.1276	0.0619	-2.0610	0.098	.	
igm	ALX	-	ALO	-0.6746	0.2247	-3.0020	0.008	**	0.239
	GPS	-	ALO	-0.3588	0.2270	-1.5800	0.254		
	GPS	-	ALX	0.3158	0.2226	1.4190	0.331		
stat6	ALX	-	ALO	-0.1869	0.0761	-2.4550	0.038	*	0.617
	GPS	-	ALO	-0.2993	0.0761	-3.9310	<0.001	***	
	GPS	-	ALX	-0.1124	0.0739	-1.5200	0.282		
foxp3	ALX	-	ALO	-0.1712	0.0856	-1.9990	0.112		0.84
	GPS	-	ALO	-0.1662	0.0855	-1.9430	0.127		
	GPS	-	ALX	0.0050	0.0821	0.0610	0.998		
il16	ALX	-	ALO	-0.1326	0.0747	-1.7750	0.178		0.416
	GPS	-	ALO	-0.1194	0.0748	-1.5960	0.247		
	GPS	-	ALX	0.0132	0.0730	0.1810	0.982		
mhcII	ALX	-	ALO	-0.0680	0.0573	-1.1870	0.461		0.493
	GPS	-	ALO	-0.0731	0.0573	-1.2760	0.408		
	GPS	-	ALX	-0.0051	0.0558	-0.0920	0.995		
tcr-β	ALX	-	ALO	-0.3580	0.1583	-2.2620	0.061	.	0.611
	GPS	-	ALO	-0.5859	0.1582	-3.7030	<0.001	***	
	GPS	-	ALX	-0.2279	0.1536	-1.4830	0.299		

Table S23. Differentially expressed genes between SKO-exposed stickleback. LMMs included the origin of the fish as fixed effect, the weight of the fish as covariate and tank as random term. Bold letters indicate significance.

	contrast			Estimate	Std. Error	z value	Pr(>|z|)		R2
adaptive									
stat4	ALX	-	ALO	-0.0743	0.0694	-1.0710	0.531		0.797
	GPS	-	ALO	-0.1401	0.0682	-2.0540	0.099	.	
	GPS	-	ALX	-0.0658	0.0748	-0.8800	0.653		
cd83	ALX	-	ALO	-0.1885	0.0531	-3.5500	0.002	**	0.618
	GPS	-	ALO	-0.1812	0.0513	-3.5350	0.001	**	
	GPS	-	ALX	0.0073	0.0573	0.1280	0.991		
igm	ALX	-	ALO	-1.0326	0.2173	-4.7530	< 1e-04	***	0.417
	GPS	-	ALO	-0.2372	0.2053	-1.1550	0.479		
	GPS	-	ALX	0.7955	0.2346	3.3910	0.002	**	
stat6	ALX	-	ALO	-0.1008	0.0604	-1.6680	0.217		0.72
	GPS	-	ALO	-0.1646	0.0591	-2.7870	0.015	*	
	GPS	-	ALX	-0.0638	0.0651	-0.9790	0.589		
foxp3	ALX	-	ALO	0.0508	0.1053	0.4830	0.879		0.72
	GPS	-	ALO	0.0747	0.1030	0.7250	0.748		
	GPS	-	ALX	0.0239	0.1135	0.2110	0.976		
il16	ALX	-	ALO	-0.0991	0.0602	-1.6460	0.226		
	GPS	-	ALO	0.0242	0.0581	0.4170	0.908		0.553
	GPS	-	ALX	0.1233	0.0650	1.8990	0.139		
mhcII	ALX	-	ALO	-0.1898	0.0626	-3.0320	0.007	**	
	GPS	-	ALO	-0.0257	0.0590	-0.4360	0.900		0.269
	GPS	-	ALX	0.1640	0.0676	2.4270	0.040	*	
tcr-β	ALX	-	ALO	0.0288	0.0892	0.3230	0.944		0.103
	GPS	-	ALO	-0.0501	0.0844	-0.5930	0.823		
	GPS	-	ALX	-0.0789	0.0963	-0.8190	0.691		

SI.4.3. Gene expression profiles of S. solidus infected stickleback (n = 80)

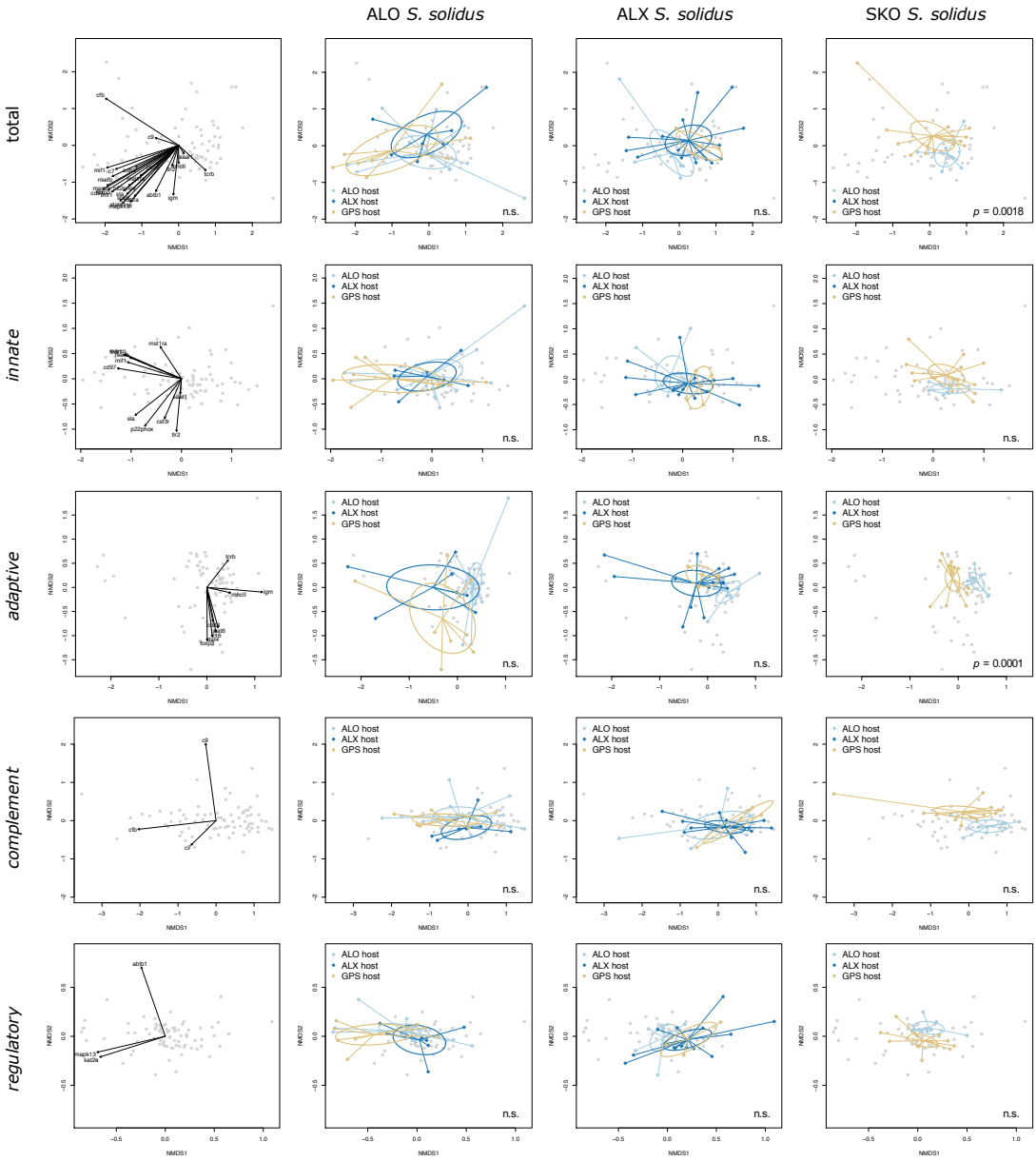


Figure S6. Gene expression profiles of *S. solidus* infected stickleback. Sticklebacks originated from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Großer Plöner See (GPS; Germany); *S. solidus* came from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Lake Skogseidvatnet (SKO; Norway). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S24-S26.

Table S24. Differences between ALO infected hosts (ALO, ALX, and GPS stickleback).

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	6.386	6.3857	1.8526	0.0550	0.9598
	Host origin	2	23.498	11.7488	3.4085	0.2025	0.3609
	Residuals	25	86.172	3.4469		0.7425	
	Total	28	116.056			1.0000	
innate	fish_weight	1	2.454	2.4538	2.2138	0.0635	0.9888
	Host origin	2	8.505	4.2526	3.8367	0.2200	0.6109
	Residuals	25	27.710	1.1084		0.7166	
	Total	28	38.669			1.0000	
adaptive	fish_weight	1	1.184	1.1837	0.9495	0.0292	0.9912
	Host origin	2	8.250	4.1252	3.3089	0.2032	0.1478
	Residuals	25	31.167	1.2467		0.7676	
	Total	28	40.601			1.0000	
complement	fish_weight	1	2.565	2.5655	2.6524	0.0804	0.6217
	Host origin	2	5.158	2.5789	2.6662	0.1617	0.4456
	Residuals	25	24.181	0.9672		0.7579	
	Total	28	31.904			1.0000	
regulatory	fish_weight	1	0.187	0.1867	1.1336	0.0306	0.8198
	Host origin	2	1.802	0.9010	5.4700	0.2951	0.1265
	Residuals	25	4.118	0.1647		0.6743	
	Total	28	6.107			1.0000	

Table S25. Differences between ALX infected hosts (ALO, ALX, and GPS stickleback).

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	5.078	5.0779	1.8928	0.0691	0.0439	*
	Host origin	2	4.018	2.0088	0.7488	0.0547	0.6715	
	Residuals	24	64.388	2.6828		0.8762		
	Total	27	73.484			1.0000		
innate	fish_weight	1	1.638	1.6380	2.2817	0.0823	0.0149	*
	Host origin	2	1.037	0.5185	0.7222	0.0521	0.5848	
	Residuals	24	17.229	0.7179		0.8656		
	Total	27	19.904			1.0000		
adaptive	fish_weight	1	1.494	1.4943	1.8558	0.0643	0.2052	
	Host origin	2	2.430	1.2152	1.5092	0.1045	0.1276	
	Residuals	24	19.324	0.8052		0.8312		
	Total	27	23.249			1.0000		
complement	fish_weight	1	1.557	1.5569	1.5115	0.0585	0.0990	.
	Host origin	2	0.331	0.1654	0.1606	0.0124	0.9778	
	Residuals	24	24.722	1.0301		0.9291		
	Total	27	26.610			1.0000		
regulatory	fish_weight	1	0.431	0.4313	2.9054	0.1015	0.0957	.
	Host origin	2	0.255	0.1275	0.8589	0.0600	0.3375	
	Residuals	24	3.562	0.1484		0.8385		
	Total	27	4.249			1.0000		

Table S26. Differences between SKO infected hosts (ALO and GPS).

Host effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	2.454	2.4536	1.3598	0.0558	0.0049	**
	Host origin	1	7.236	7.2363	4.0103	0.1646	0.1351	
	Residuals	19	34.284	1.8044		0.7796		
	Total	21	43.974			1		
innate	fish_weight	1	0.546	0.5456	1.1197	0.0534	0.0789	.
	Host origin	1	0.423	0.4228	0.8677	0.0413	0.7984	
	Residuals	19	9.258	0.4873		0.9053		
	Total	21	10.226			1		
adaptive	fish_weight	1	1.357	1.3571	4.9834	0.1628	0.0007	***
	Host origin	1	1.807	1.8071	6.6357	0.2167	**0.0001**	***
	Residuals	19	5.174	0.2723		0.6205		
	Total	21	8.338			1		
complement	fish_weight	1	0.430	0.4302	0.4306	0.0177	0.3339	
	Host origin	1	4.934	4.9342	4.9395	0.2027	0.2282	
	Residuals	19	18.980	0.9989		0.7796		
	Total	21	24.344			1		
regulatory	fish_weight	1	0.224	0.2240	3.3187	0.1418	0.0125	*
	Host origin	1	0.073	0.0732	1.0847	0.0464	0.4042	
	Residuals	19	1.282	0.0675		0.8119		
	Total	21	1.580			1		

Table S27. Differences within infected ALO stickleback (ALO, ALX, and SKO *S. solidus*).

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	6.002	6.0021	2.3392	0.0701	0.7481
	S. solidus	3	10.370	3.4567	1.3472	0.1211	0.4597
	Residuals	27	69.279	2.5659		0.8089	
	Total	31	85.651			1	
innate	fish_weight	1	1.482	1.4822	1.7465	0.0542	0.9548
	S. solidus	3	2.965	0.9882	1.1644	0.1084	0.9748
	Residuals	27	22.914	0.8487		0.8375	
	Total	31	27.361			1	
adaptive	fish_weight	1	0.870	0.8700	1.6518	0.0539	0.9899
	S. solidus	3	1.058	0.3528	0.6698	0.0655	0.9938
	Residuals	27	14.221	0.5267		0.8806	
	Total	31	16.150			1	
complement	fish_weight	1	3.598	3.5976	3.2771	0.0918	0.3526
	S. solidus	3	5.953	1.9842	1.8074	0.1519	0.2859
	Residuals	27	29.641	1.0978		0.7563	
	Total	31	39.191			1	
regulatory	fish_weight	1	0.054	0.0539	0.4342	0.0139	0.5675
	S. solidus	3	0.466	0.1554	1.2520	0.1204	0.6147
	Residuals	27	3.351	0.1241		0.8657	
	Total	31	3.871			1	

Table S28. Differences within infected ALX stickleback (ALO and ALX *S. solidus*).

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	5.200	5.1995	1.7172	0.0818	0.1793
	S. solidus	1	0.802	0.8018	0.2648	0.0126	0.8034
	Residuals	19	57.531	3.0279		0.9055	
	Total	21	63.532			1	
innate	fish_weight	1	1.956	1.9563	2.4438	0.1117	0.2391
	S. solidus	1	0.353	0.3532	0.4413	0.0202	0.4171
	Residuals	19	15.210	0.8005		0.8682	
	Total	21	17.519			1	
adaptive	fish_weight	1	1.367	1.3674	1.0375	0.0510	0.1332
	S. solidus	1	0.408	0.4078	0.3094	0.0152	0.7487
	Residuals	19	25.042	1.3180		0.9338	
	Total	21	26.817			1	
complement	fish_weight	1	1.085	1.0850	1.4085	0.0689	0.2339
	S. solidus	1	0.037	0.0367	0.0476	0.0023	0.7639
	Residuals	19	14.636	0.7703		0.9288	
	Total	21	15.758			1	
regulatory	fish_weight	1	0.853	0.8526	5.2762	0.2171	0.1198
	S. solidus	1	0.004	0.0041	0.0252	0.0010	0.9015
	Residuals	19	3.070	0.1616		0.7819	
	Total	21	3.927			1	

Table S29. Differences within infected GPS stickleback (ALO, ALX, and *S. solidus*).

Parasite effect		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	14.578	14.5779	5.9882	0.1708	0.9688
	S. solidus	2	17.216	8.6080	3.5359	0.2017	0.9727
	Residuals	22	53.558	2.4344		0.6275	
	Total	25	85.352			1	
innate	fish_weight	1	5.053	5.0533	7.4936	0.1907	0.7695
	S. solidus	2	6.605	3.3023	4.8970	0.2493	0.7956
	Residuals	22	14.836	0.6743		0.5600	
	Total	25	26.494			1	
adaptive	fish_weight	1	3.183	3.1830	4.7654	0.1402	0.6302
	S. solidus	2	4.829	2.4143	3.6145	0.2127	0.4518
	Residuals	22	14.695	0.6680		0.6472	
	Total	25	22.707			1	
complement	fish_weight	1	5.874	5.8743	5.8582	0.1821	0.9766
	S. solidus	2	4.322	2.1609	2.1550	0.1340	0.9701
	Residuals	22	22.060	1.0027		0.6839	
	Total	25	32.257			1	
regulatory	fish_weight	1	0.639	0.6393	5.7377	0.1337	0.8385
	S. solidus	2	1.691	0.8455	7.5892	0.3537	0.8385
	Residuals	22	2.451	0.1114		0.5126	
	Total	25	4.781			1	

Table S30. Differentially expressed genes between SKO infected stickleback. LMMs included the origin of the fish as fixed effect, the weight of the fish as covariate and tank as random term. Bold letters indicate significance.

	contrast			Estimate	Std. Error	z value	Pr(>|z|)		R2
adaptive									
stat4	GPS	-	ALO	-0.1941	0.1152	-1.6860	0.092	.	0.746
cd83	GPS	-	ALO	-0.3716	0.0730	-5.0890	<0.0001	***	0.873
igm	GPS	-	ALO	-0.5029	0.1209	-4.1600	<0.0001	***	0.811
stat6	GPS	-	ALO	-0.1812	0.0928	-1.9530	0.051	.	0.841
foxp3	GPS	-	ALO	0.0576	0.1058	0.5440	0.586		0.753
il16	GPS	-	ALO	-0.0709	0.1208	-0.5870	0.557		0.52
mhcII	GPS	-	ALO	-0.2617	0.0967	-2.7070	0.007	**	0.845
tcr-β	GPS	-	ALO	-0.4820	0.1089	-4.4260	<0.0001	***	0.683

SI.4.4. Effect of infection status on stickleback gene expression profiles

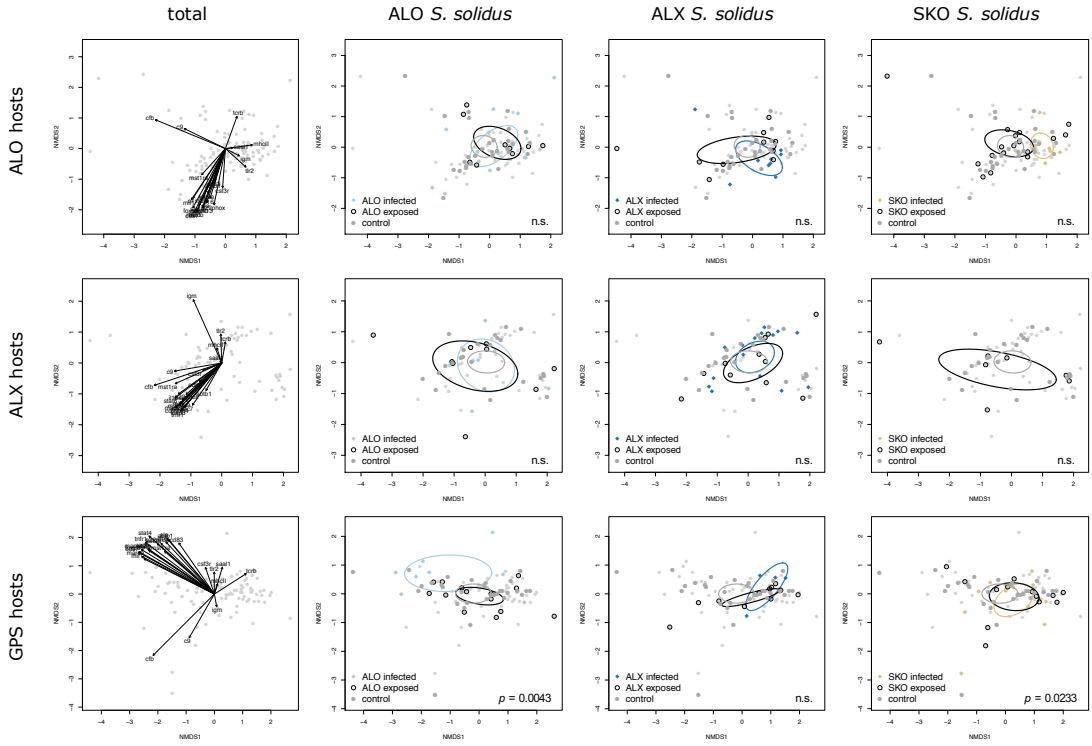


Figure S7. Immune and regulatory gene expression profiles of sham-exposed and *S. solidus* exposed and infected stickleback. Statistics follow Tables S31-S39.

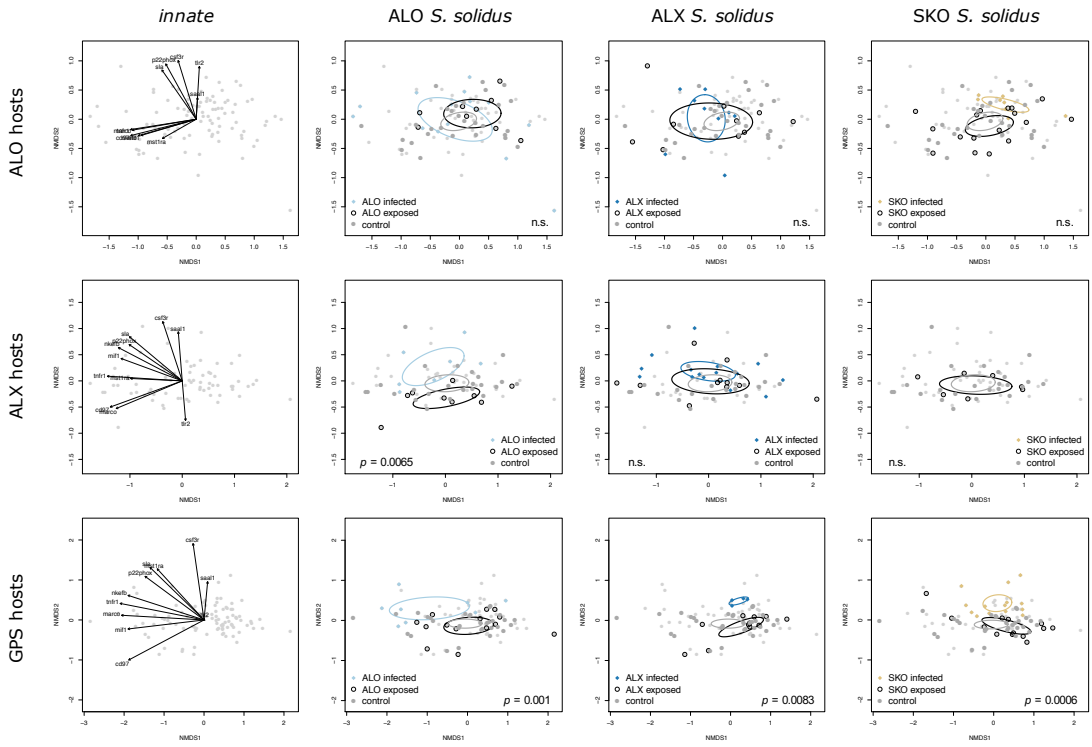


Figure S8. Gene expression profiles of eleven innate immune genes of sham-exposed and *S. solidus* exposed and infected stickleback. Statistics follow Tables S31-S39.

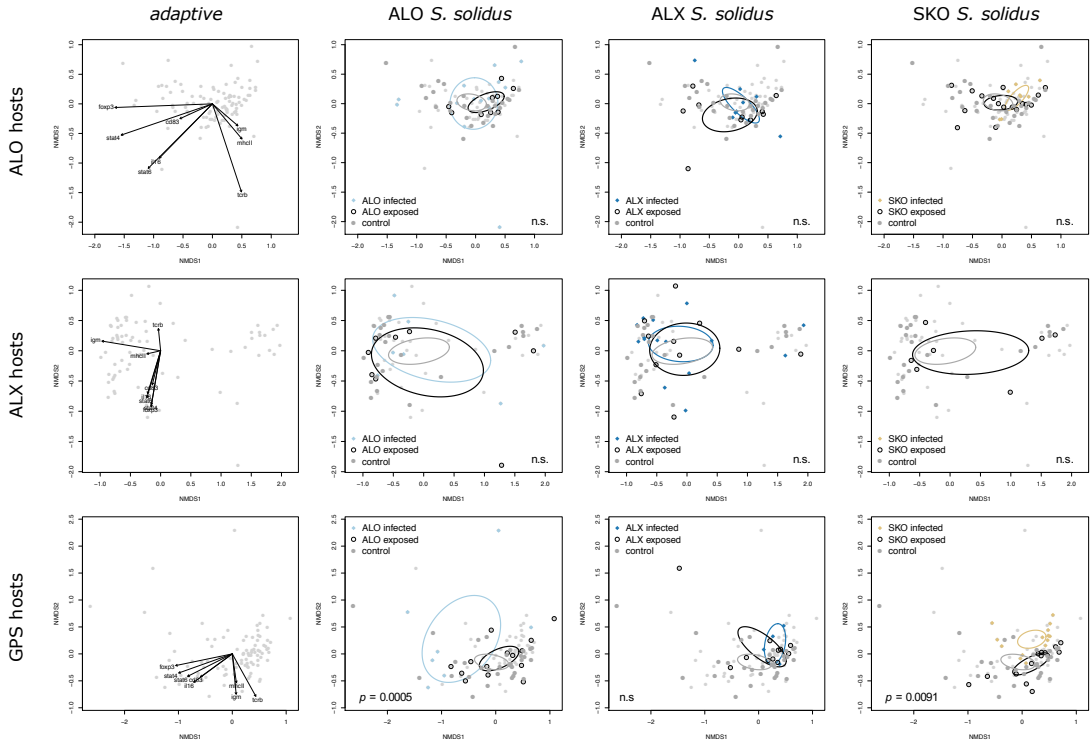


Figure S9. Gene expression profiles of eight adaptive immune genes of sham-exposed and *S. solidus* exposed and infected stickleback. Statistics follow Tables S31-S39.

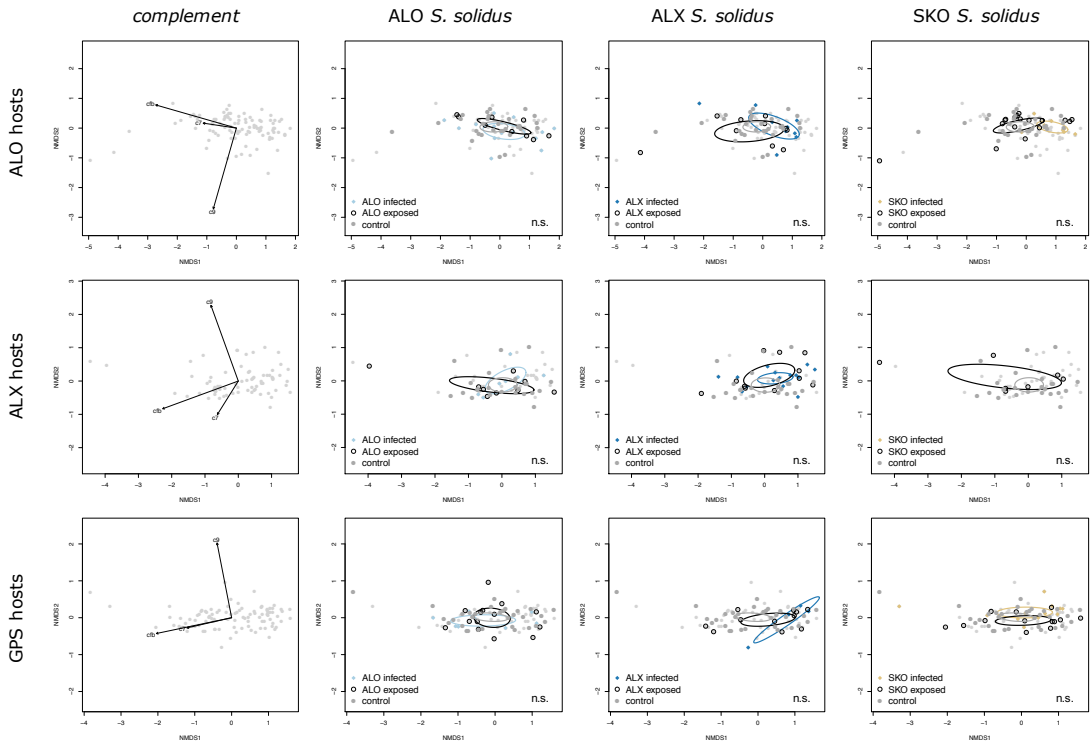


Figure S10. Gene expression profiles of three complement components of sham-exposed and *S. solidus* exposed and infected stickleback. Statistics follow Tables S31-S39.

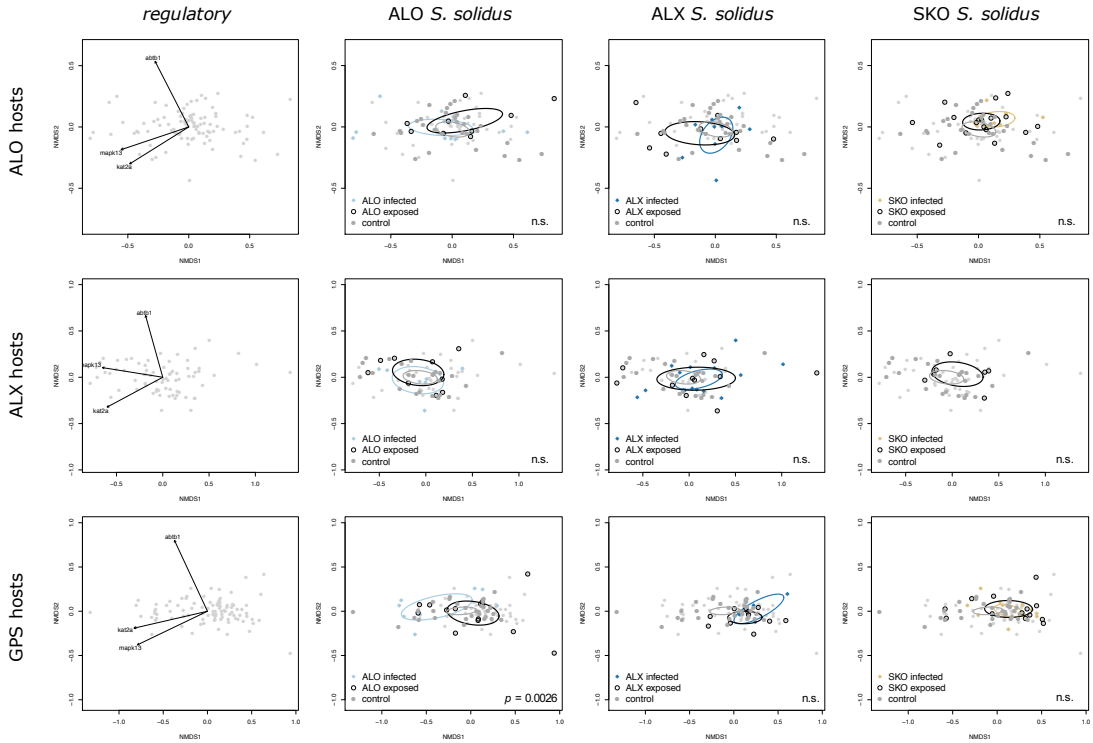


Figure S11. Gene expression profiles of three regulatory genes of sham-exposed and *S. solidus* exposed and infected stickleback. Statistics follow Statistics follow Tables S31-S39.

Table S31. Differences between gene expression profiles of control, exposed, and infected ALO stickleback. Fish were sham-exposed controls or exposed or infected with ALO *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	3.031	3.0312	1.0666	0.0210	0.5288
	group	2	4.950	2.4751	0.8709	0.0343	0.2645
	Residuals	48	136.411	2.8419		0.9447	
	Total	51	144.392			1	
innate	fish_weight	1	1.364	1.3641	1.5119	0.0294	0.6948
	group	2	1.691	0.8456	0.9372	0.0365	0.1353
	Residuals	48	43.306	0.9022		0.9341	
	Total	51	46.361			1	
adaptive	fish_weight	1	0.652	0.6516	1.1036	0.0219	0.5106
	group	2	0.800	0.4000	0.6776	0.0269	0.3213
	Residuals	48	28.338	0.5904		0.9513	
	Total	51	29.790			1	
complement	fish_weight	1	1.055	1.0549	0.8017	0.0159	0.2139
	group	2	2.330	1.1649	0.8852	0.0350	0.3458
	Residuals	48	63.165	1.3159		0.9491	
	Total	51	66.550			1	
regulatory	fish_weight	1	0.050	0.0500	0.3205	0.0064	0.8136
	group	2	0.298	0.1487	0.9534	0.0380	0.2550
	Residuals	48	7.488	0.1560		0.9557	
	Total	51	7.836			1	

Table S32. Differences between gene expression profiles of control, exposed, and infected ALO stickleback. Fish were sham-exposed controls or exposed or infected with ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	1.245	1.2451	0.4291	0.0092	0.3613
	group	2	6.693	3.3463	1.1532	0.0494	0.5808
	Residuals	44	127.679	2.9018		0.9415	
	Total	47	135.616			1	
innate	fish_weight	1	0.053	0.0526	0.0634	0.0014	0.9592
	group	2	1.545	0.7725	0.9320	0.0406	0.1795
	Residuals	44	36.471	0.8289		0.9580	
	Total	47	38.069			1	
adaptive	fish_weight	1	0.079	0.0785	0.1495	0.0032	0.7754
	S. solidus	2	1.193	0.5966	1.1359	0.0489	0.3767
	Residuals	44	23.110	0.5252		0.9478	
	Total	47	24.382			1	
complement	fish_weight	1	1.070	1.0704	0.6838	0.0145	0.2890
	S. solidus	2	4.131	2.0654	1.3193	0.0558	0.6280
	Residuals	44	68.881	1.5655		0.9298	
	Total	47	74.083			1	
regulatory	fish_weight	1	0.080	0.0801	0.6122	0.0135	0.2859
	S. solidus	2	0.114	0.0572	0.4373	0.0192	0.9546
	Residuals	44	5.753	0.1308		0.9673	
	Total	47	5.948			1	

Table S33. Differences between gene expression profiles of control, exposed, and infected ALO stickleback. Fish were sham-exposed controls or exposed or infected with SKO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	0.578	0.5779	0.2183	0.0040	0.8177	
	group	2	12.273	6.1365	2.3180	0.0845	0.0368	*
	Residuals	50	132.364	2.6473		0.9115		
	Total	53	145.215			1		
innate	fish_weight	1	0.289	0.2893	0.4015	0.0075	0.5965	
	group	2	2.182	1.0910	1.5143	0.0567	0.3911	
	Residuals	50	36.023	0.7205		0.9358		
	Total	53	38.494			1		
adaptive	fish_weight	1	0.124	0.1235	0.2757	0.0053	0.9571	
	group	2	0.815	0.4074	0.9098	0.0349	0.8004	
	Residuals	50	22.389	0.4478		0.9598		
	Total	53	23.327			1		
complement	fish_weight	1	0.236	0.2365	0.1594	0.0028	0.6362	
	group	2	9.299	4.6496	3.1341	0.1111	0.0256	*
	Residuals	50	74.176	1.4835		0.8861		
	Total	53	83.712			1		
regulatory	fish_weight	1	0.002	0.0022	0.0200	0.0004	0.9186	
	group	2	0.256	0.1280	1.1838	0.0452	0.5136	
	Residuals	50	5.405	0.1081		0.9544		
	Total	53	5.663			1		

Table S34. Differences between gene expression profiles of control, exposed, and infected ALX stickleback. Fish were sham-exposed controls or exposed or infected with ALO *S. solidus* (group). The bold number indicates significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	11.021	11.0211	2.9893	0.0744	0.2602	
	group	2	4.295	2.1475	0.5825	0.0290	0.5933	
	Residuals	36	132.727	3.6869		0.8965		
	Total	39	148.043			1		
innate	fish_weight	1	4.651	4.6514	5.6953	0.1285	0.0875	.
	group	2	2.143	1.0717	1.3122	0.0592	**0.0065**	**
	Residuals	36	29.402	0.8167		0.8123		
	Total	39	36.196			1		
adaptive	fish_weight	1	5.655	5.6555	3.6176	0.0890	0.3947	
	S. solidus	2	1.638	0.8191	0.5240	0.0258	0.5937	
	Residuals	36	56.279	1.5633		0.8853		
	Total	39	63.572			1		
complement	fish_weight	1	0.192	0.1918	0.1494	0.0040	0.3888	
	S. solidus	2	1.127	0.5633	0.4387	0.0237	0.6859	
	Residuals	36	46.221	1.2839		0.9723		
	Total	39	47.540			1		
regulatory	fish_weight	1	0.914	0.9137	6.3502	0.1487	0.0523	.
	S. solidus	2	0.053	0.0264	0.1836	0.0086	0.8540	
	Residuals	36	5.180	0.1439		0.8428		
	Total	39	6.147			1		

Table S35. Differences between gene expression profiles of control, exposed, and infected ALX stickleback. Fish were sham-exposed controls or exposed or infected with ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	10.066	10.0657	2.9645	0.0589	0.1415	
	group	2	4.735	2.3677	0.6973	0.0277	0.3738	
	Residuals	46	156.191	3.3954		0.9134		
	Total	49	170.992			1		
innate	fish_weight	1	3.777	3.7772	3.8419	0.0745	0.1931	
	group	2	1.710	0.8548	0.8695	0.0337	0.2303	
	Residuals	46	45.225	0.9831		0.8918		
	Total	49	50.712			1		
adaptive	fish_weight	1	4.335	4.3351	3.3082	0.0656	0.0540	.
	group	2	1.485	0.7425	0.5666	0.0225	0.4414	
	Residuals	46	60.279	1.3104		0.9120		
	Total	49	66.099			1		
complement	fish_weight	1	0.727	0.7272	0.7086	0.0147	0.7433	
	group	2	1.599	0.7995	0.7791	0.0323	0.3456	
	Residuals	46	47.208	1.0263		0.9530		
	Total	49	49.535			1		
regulatory	fish_weight	1	1.581	1.5809	7.8492	0.1451	0.1677	
	group	2	0.053	0.0264	0.1309	0.0048	0.9583	
	Residuals	46	9.265	0.2014		0.8501		
	Total	49	10.898			1		

Table S36. Differences between gene expression profiles of control and SKO *S. solidus* exposed ALX stickleback. ALX stickleback were not infected with SKO *S. solidus*.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	6.631	6.6314	1.7100	0.0550	0.3281	
	group	1	5.453	5.4528	1.4061	0.0452	0.3516	
	Residuals	28	108.583	3.8780		0.8999		
	Total	30	120.667			1		
innate	fish_weight	1	2.290	2.2901	2.5767	0.0837	0.4766	
	group	1	0.187	0.1870	0.2104	0.0068	0.9531	
	Residuals	28	24.886	0.8888		0.9095		
	Total	30	27.363			1		
adaptive	fish_weight	1	3.462	3.4618	2.4024	0.0763	0.0938	.
	S. solidus	1	1.538	1.5382	1.0675	0.0339	0.7344	
	Residuals	28	40.346	1.4409		0.8897		
	Total	30	45.346			1		
complement	fish_weight	1	0.729	0.7286	0.4798	0.0156	0.5859	
	S. solidus	1	3.614	3.6140	2.3801	0.0771	0.1797	
	Residuals	28	42.517	1.5185		0.9073		
	Total	30	46.860			1		
regulatory	fish_weight	1	0.446	0.4463	2.9607	0.0931	0.3359	
	S. solidus	1	0.129	0.1292	0.8572	0.0269	0.2109	
	Residuals	28	4.220	0.1507		0.8800		
	Total	30	4.796			1		

Table S37. Differences between gene expression profiles of control, exposed, and infected GPS stickleback. Fish were sham-exposed controls or exposed or infected with ALO *S. solidus* (group). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	11.946	11.9459	3.9016	0.0653	0.3403	
	group	2	20.889	10.4445	3.4112	0.1142	**0.0043**	**
	Residuals	49	150.029	3.0618		0.8204		
	Total	52	182.864			1		
innate	fish_weight	1	4.450	4.4501	3.9716	0.0620	0.1039	
	group	2	12.399	6.1993	5.5328	0.1728	**<0.001**	***
	Residuals	49	54.903	1.1205		0.7652		
	Total	52	71.751			1		
adaptive	fish_weight	1	2.975	2.9746	3.4904	0.0588	0.06189	.
	group	2	5.862	2.9310	3.4393	0.1159	**0.0005**	***
	Residuals	49	41.758	0.8522		0.8254		
	Total	52	50.595			1		
complement	fish_weight	1	3.934	3.9338	3.6625	0.0672	0.786	
	group	2	1.945	0.9725	0.9054	0.0332	0.6162	
	Residuals	49	52.630	1.0741		0.8995		
	Total	52	58.508			1		
regulatory	fish_weight	1	0.807	0.8068	4.6221	0.0745	0.1263	
	group	2	1.466	0.7332	4.2003	0.1354	**0.0026**	**
	Residuals	49	8.554	0.1746		0.7900		
	Total	52	10.827			1		

Table S38. Differences between gene expression profiles of control, exposed, and infected GPS stickleback. Fish were sham-exposed controls or exposed or infected with ALX *S. solidus* (group). The bold number indicates significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	5.507	5.5071	1.7895	0.0384	0.8428	
	group	2	8.586	4.2931	1.3950	0.0599	0.1168	
	Residuals	42	129.254	3.0775		0.9017		
	Total	45	143.347			1		
innate	fish_weight	1	1.455	1.4545	1.3561	0.0293	0.9809	
	group	2	3.178	1.5888	1.4813	0.0640	**0.0083**	**
	Residuals	42	45.048	1.0726		0.9068		
	Total	45	49.681			1		
adaptive	fish_weight	1	1.247	1.2467	1.6061	0.0349	0.3561	
	S. solidus	2	1.895	0.9475	1.2206	0.0530	0.0274	*
	Residuals	42	32.602	0.7762		0.9121		
	Total	45	35.743			1		
complement	fish_weight	1	2.725	2.7252	2.2092	0.0470	0.5687	
	S. solidus	2	3.399	1.6995	1.3777	0.0587	0.6120	
	Residuals	42	51.810	1.2336		0.8943		
	Total	45	57.934			1		
regulatory	fish_weight	1	0.127	0.1273	0.9078	0.0186	0.8146	
	S. solidus	2	0.834	0.4168	2.9731	0.1217	0.0376	*
	Residuals	42	5.888	0.1402		0.8597		
	Total	45	6.849			1.0000		

Table S39. Differences between gene expression profiles of control, exposed, and infected GPS stickleback. Fish were sham-exposed controls or exposed or infected with SKO *S. solidus* (group). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	14.391	14.3907	5.0082	0.0800	0.6693	
	group	2	10.406	5.2028	1.8106	0.0578	**0.0233**	*
	Residuals	54	155.165	2.8734		0.8622		
	Total	57	179.961			1		
innate	fish_weight	1	4.918	4.9176	4.9989	0.0769	0.6139	
	group	2	5.903	2.9516	3.004	0.0923	**0.0006**	***
	Residuals	54	53.122	0.9837		0.8308		
	Total	57	63.942			1		
adaptive	fish_weight	1	3.383	3.3828	5.4544	0.0844	0.3361	
	group	2	3.201	1.6004	2.5805	0.0799	**0.0091**	**
	Residuals	54	33.490	0.6202		0.8357		
	Total	57	40.074			1		
complement	fish_weight	1	5.784	5.7842	4.5459	0.0767	0.7208	
	group	2	0.921	0.4604	0.3618	0.0122	0.6871	
	Residuals	54	68.710	1.2724		0.9111		
	Total	57	75.415			1		
regulatory	fish_weight	1	0.684	0.6843	4.7639	0.0765	0.6673	
	group	2	0.509	0.2545	1.7719	0.0569	0.0750	.
	Residuals	54	7.757	0.1436		0.8667		
	Total	57	8.950			1		

SI.4.5. Gene expression differences between infected and control fish

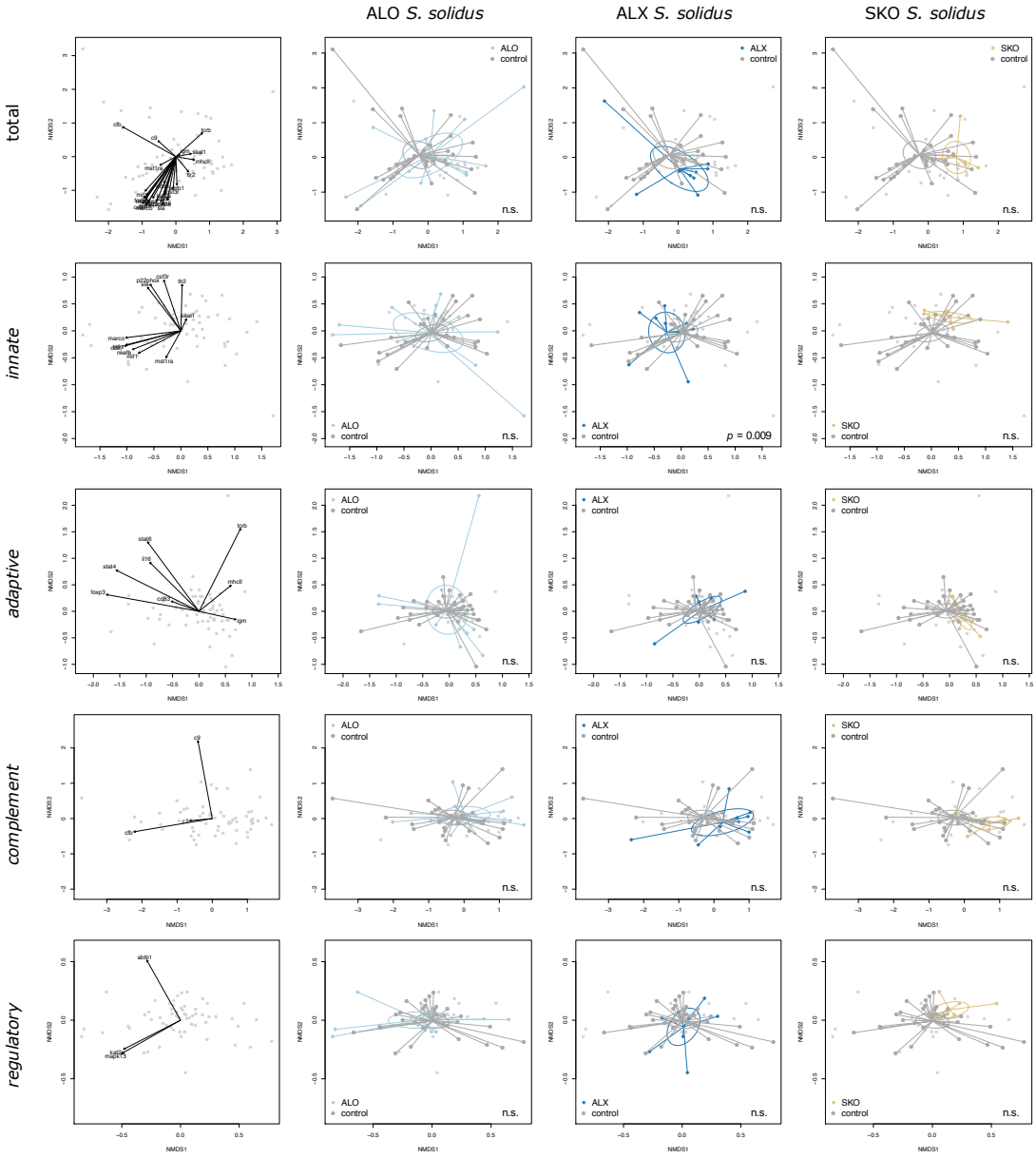


Figure S12. Gene expression profiles of *S. solidus* infected and control ALO stickleback. Sticklebacks originated from Walby Lake (ALO; Alaska); *S. solidus* came from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Lake Skogseidvatnet (SKO; Norway). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S40-S42.

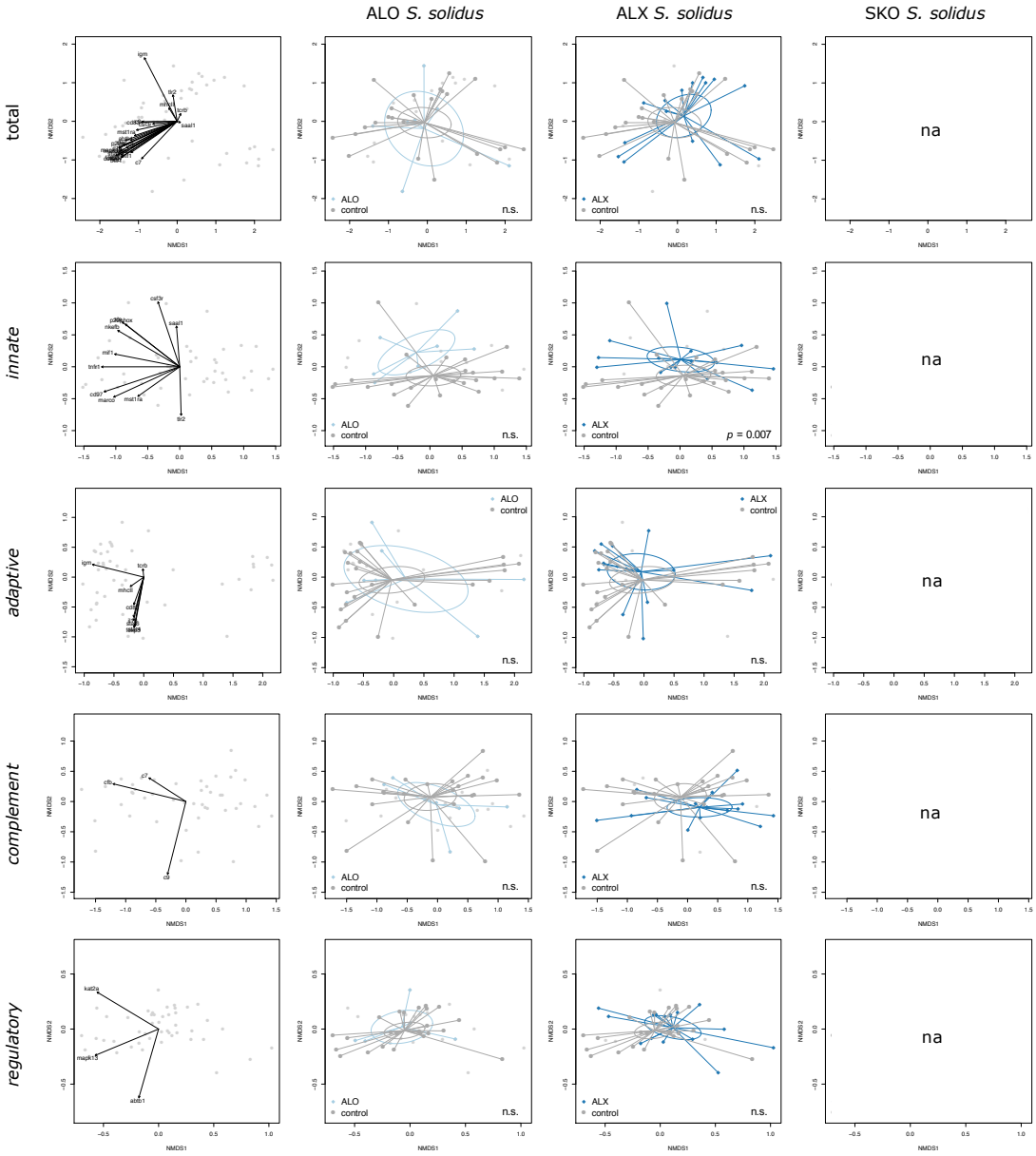


Figure S13. Gene expression profiles of *S. solidus* infected and control ALX stickleback. Sticklebacks originated from Wolf Lake (ALX; Alaska); *S. solidus* came from Walby Lake (ALO; Alaska) and Wolf Lake (ALX; Alaska); *S. solidus* from Lake Skogseidvatnet (SKO; Norway) did not infect ALX stickleback (as indicated by 'na'). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S43 and S44.

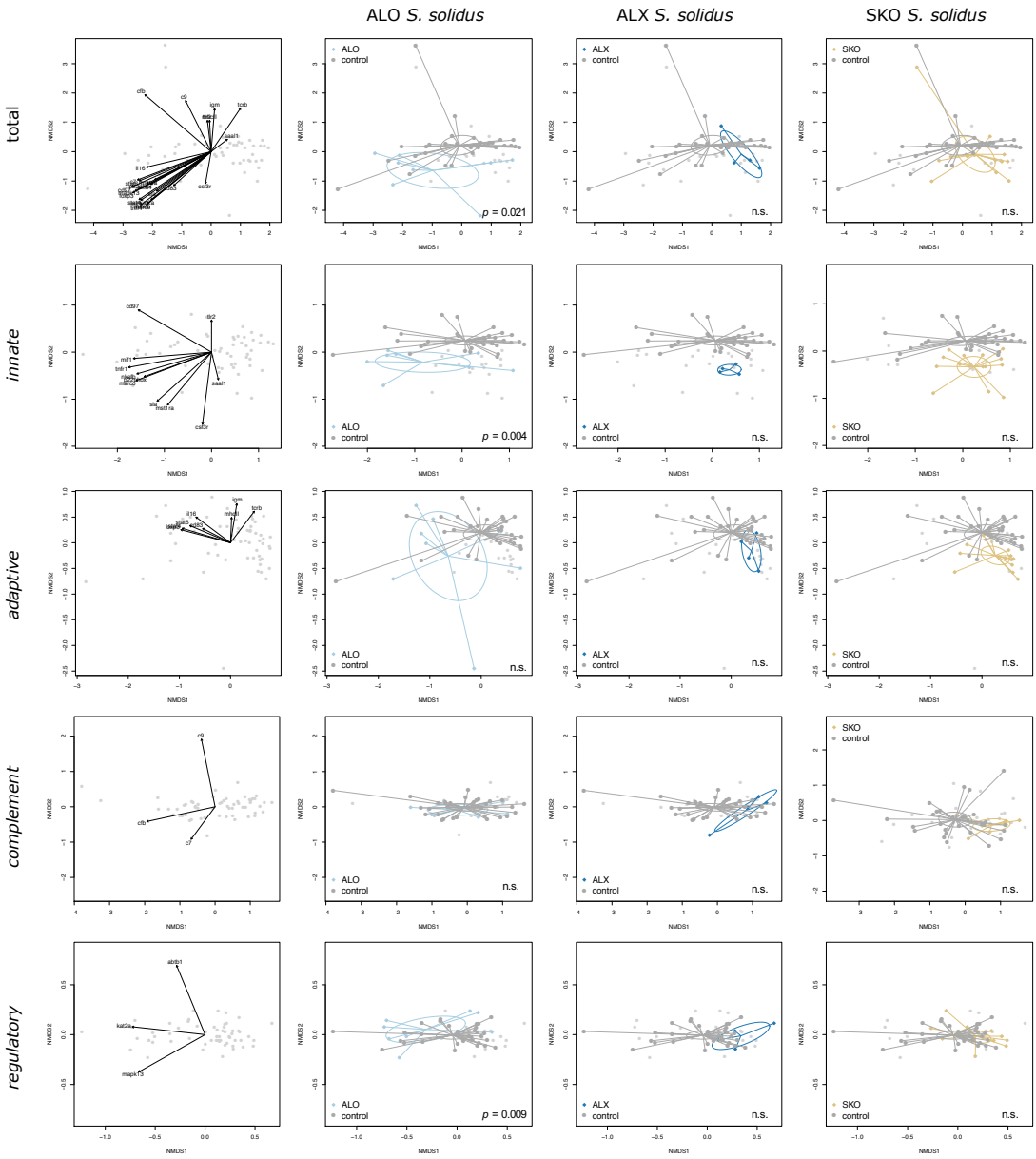


Figure S14. Gene expression profiles of *S. solidus* infected and control GPS stickleback. Sticklebacks originated from Lake Großer Plöner See (GPS; Germany); *S. solidus* came from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Lake Skogseidvatnet (SKO; Norway). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S45-S47.

Table S40. Differences between gene expression profiles of infected and control ALO stickleback. Fish were sham-exposed controls or infected with ALO *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	2.133	2.1327	0.6891	0.0163	0.9963
	group	1	1.862	1.8621	0.6017	0.0142	0.3698
	Residuals	41	126.886	3.0948		0.9695	
	Total	43	130.881			1	
innate	fish_weight	1	1.318	1.3177	1.3903	0.0323	0.9988
	group	1	0.657	0.6566	0.6928	0.0161	0.2437
	Residuals	41	38.858	0.9478		0.9517	
	Total	43	40.832			1	
adaptive	fish_weight	1	0.599	0.5993	0.9358	0.0221	0.7174
	S. solidus	1	0.226	0.2257	0.3524	0.0083	0.6274
	Residuals	41	26.259	0.6405		0.9695	
	Total	43	27.084			1	
complement	fish_weight	1	0.188	0.1882	0.1339	0.0032	0.8866
	S. solidus	1	0.885	0.8846	0.6297	0.0151	0.3622
	Residuals	41	57.598	1.4048		0.9817	
	Total	43	58.670			1	
regulatory	fish_weight	1	0.036	0.0362	0.2428	0.0058	0.9730
	S. solidus	1	0.119	0.1193	0.8010	0.0191	0.2096
	Residuals	41	6.108	0.1490		0.9752	
	Total	43	6.264			1	

Table S41. Differences between gene expression profiles of infected and control ALO stickleback. Fish were sham-exposed controls or infected with ALX *S. solidus* (group). The bold number indicates significance after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	1.160	1.1603	0.4352	0.0114	0.3980	
	group	1	4.508	4.5079	1.6906	0.0443	0.4541	
	Residuals	36	95.995	2.6665		0.9443		
	Total	38	101.663			1		
innate	fish_weight	1	0.147	0.1467	0.2013	0.0054	0.6771	
	group	1	1.005	1.0053	1.3802	0.0367	**0.0090**	**
	Residuals	36	26.221	0.7284		0.9579		
	Total	38	27.373			1		
adaptive	fish_weight	1	0.343	0.3433	0.6978	0.0186	0.0379	*
	group	1	0.405	0.4051	0.8235	0.0220	0.8805	
	Residuals	36	17.711	0.4920		0.9595		
	Total	38	18.460			1		
complement	fish_weight	1	0.574	0.5737	0.4177	0.0108	0.4690	
	group	1	3.093	3.0929	2.2517	0.0582	0.5590	
	Residuals	36	49.449	1.3736		0.9310		
	Total	38	53.115			1		
regulatory	fish_weight	1	0.117	0.1170	1.0272	0.0275	0.0768	.
	group	1	0.032	0.0324	0.2841	0.0076	0.9902	
	Residuals	36	4.102	0.1139		0.9649		
	Total	38	4.251			1		

Table S42. Differences between gene expression profiles of infected and control ALO stickleback. Fish were sham-exposed controls or infected with SKO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	0.995	0.9947	0.4151	0.0104	0.6204	
	group	1	10.697	10.6970	4.4643	0.1119	0.0203	*
	Residuals	35	83.864	2.3961		0.8777		
	Total	37	95.556			1		
innate	fish_weight	1	0.102	0.1017	0.1459	0.0039	1.0000	
	group	1	1.597	1.5974	2.2918	0.0612	0.5648	
	Residuals	35	24.395	0.6970		0.9349		
	Total	37	26.094			1		
adaptive	fish_weight	1	0.122	0.1222	0.2625	0.0071	0.9497	
	S. solidus	1	0.805	0.8048	1.7289	0.0467	0.5625	
	Residuals	35	16.292	0.4655		0.9462		
	Total	37	17.219			1		
complement	fish_weight	1	0.730	0.7297	0.6271	0.0147	0.4363	
	S. solidus	1	8.040	8.0400	6.9098	0.1624	0.0104	*
	Residuals	35	40.725	1.1636		0.8228		
	Total	37	49.494			1		
regulatory	fish_weight	1	0.063	0.0625	0.5705	0.0151	0.0232	*
	S. solidus	1	0.256	0.2557	2.3347	0.0616	0.5347	
	Residuals	35	3.834	0.1095		0.9234		
	Total	37	4.152			1		

Table S43. Differences between gene expression profiles of infected and control ALX stickleback. Fish were sham-exposed controls or infected with ALO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	7.152	7.1516	2.1166	0.0682	0.8229	
	group	1	3.078	3.0779	0.9109	0.0294	0.0729	.
	Residuals	28	94.609	3.3789		0.9024		
	Total	30	104.839			1		
innate	fish_weight	1	2.278	2.2777	2.5748	0.0803	0.5417	
	group	1	1.315	1.3152	1.4868	0.0464	0.0417	*
	Residuals	28	24.769	0.8846		0.8733		
	Total	30	28.362			1		
adaptive	fish_weight	1	4.411	4.4114	3.0605	0.0954	0.6562	
	group	1	1.464	1.4643	1.0159	0.0317	0.1042	
	Residuals	28	40.359	1.4414		0.8729		
	Total	30	46.234			1		
complement	fish_weight	1	0.256	0.2557	0.2756	0.0096	0.9583	
	group	1	0.293	0.2933	0.3161	0.0111	0.8333	
	Residuals	28	25.974	0.9277		0.9793		
	Total	30	26.523			1		
regulatory	fish_weight	1	0.379	0.3794	2.4279	0.0795	0.2500	
	group	1	0.018	0.0182	0.1165	0.0038	0.9271	
	Residuals	28	4.375	0.1563		0.9167		
	Total	30	4.773			1		

Table S44. Differences between gene expression profiles of infected and control ALX stickleback. Fish were sham-exposed controls or infected with ALX *S. solidus* (group). The bold number indicates significance after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	5.975	5.9753	1.7647	0.0454	0.2080	
	group	1	3.875	3.8746	1.1443	0.0294	0.0889	.
	Residuals	36	121.899	3.3861		0.9252		
	Total	38	131.749			1		
innate	fish_weight	1	2.080	2.0800	2.2077	0.0555	0.2492	
	group	1	1.476	1.4761	1.5667	0.0394	**0.0068**	**
	Residuals	36	33.917	0.9421		0.9051		
	Total	38	37.473			1		
adaptive	fish_weight	1	2.712	2.7116	1.9910	0.0512	0.2688	
	S. solidus	1	1.226	1.2262	0.9003	0.0232	0.1829	
	Residuals	36	49.030	1.3620		0.9257		
	Total	38	52.968			1		
complement	fish_weight	1	0.532	0.5317	0.5694	0.0151	0.1959	
	S. solidus	1	1.134	1.1344	1.2149	0.0322	0.3905	
	Residuals	36	33.614	0.9337		0.9528		
	Total	38	35.280			1		
regulatory	fish_weight	1	0.874	0.8737	5.0337	0.1220	0.2320	
	S. solidus	1	0.040	0.0404	0.2326	0.0056	0.9103	
	Residuals	36	6.248	0.1736		0.8724		
	Total	38	7.162			1		

Table S45. Differences between gene expression profiles of infected and control GPS stickleback. Fish were sham-exposed controls or infected with ALO *S. solidus* (group). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	8.066	8.0658	2.3646	0.0537	0.8715	
	group	1	19.474	19.4736	5.7089	0.1295	**0.0208**	*
	Residuals	36	122.799	3.4111		0.8168		
	Total	38	150.338			1		
innate	fish_weight	1	2.098	2.0977	1.8393	0.0385	0.8623	
	group	1	11.310	11.3102	9.9169	0.2077	**0.0035**	**
	Residuals	36	41.058	1.1405		0.7538		
	Total	38	54.466			1		
adaptive	fish_weight	1	2.428	2.4276	2.5712	0.0587	0.2512	
	group	1	4.949	4.9492	5.2419	0.1196	0.0428	*
	Residuals	36	33.989	0.9441		0.8217		
	Total	38	41.366			1		
complement	fish_weight	1	3.133	3.1331	2.6167	0.0647	0.9051	
	group	1	2.196	2.1964	1.8343	0.0454	0.4826	
	Residuals	36	43.105	1.1974		0.8900		
	Total	38	48.435			1		
regulatory	fish_weight	1	0.408	0.4076	2.5843	0.0565	0.2882	
	group	1	1.124	1.1235	7.1236	0.1559	**0.0093**	**
	Residuals	36	5.678	0.1577		0.7876		
	Total	38	7.209			1		

Table S46. Differences between gene expression profiles of infected and control GPS stickleback. Fish were sham-exposed controls or infected with ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	9.986	9.9861	3.0997	0.0854	0.8125	
	group	1	3.826	3.8265	1.1878	0.0327	0.1250	
	Residuals	32	103.093	3.2216		0.8819		
	Total	34	116.905			1		
innate	fish_weight	1	2.996	2.9959	2.7547	0.0763	0.9375	
	group	1	1.446	1.4465	1.3300	0.0369	0.0625	.
	Residuals	32	34.802	1.0876		0.8868		
	Total	34	39.244			1		
adaptive	fish_weight	1	1.689	1.6887	2.2126	0.0625	0.4375	
	S. solidus	1	0.893	0.8929	1.1700	0.0331	0.0625	.
	Residuals	32	24.423	0.7632		0.9044		
	Total	34	27.004			1		
complement	fish_weight	1	4.835	4.8350	3.8864	0.1054	0.5625	
	S. solidus	1	1.232	1.2321	0.9904	0.0269	0.1875	
	Residuals	32	39.810	1.2441		0.8678		
	Total	34	45.877			1		
regulatory	fish_weight	1	0.472	0.4716	3.0850	0.0832	0.4375	
	S. solidus	1	0.305	0.3052	1.9966	0.0538	0.1875	
	Residuals	32	4.892	0.1529		0.8630		
	Total	34	5.669			1		

Table S47. Differences between gene expression profiles of infected and control GPS stickleback. Fish were sham-exposed controls or infected with SKO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	12.128	12.1285	4.0019	0.0833	0.5112	
	group	1	6.235	6.2346	2.0572	0.0428	0.1946	
	Residuals	42	127.287	3.0306		0.8739		
	Total	44	145.650			1		
innate	fish_weight	1	3.883	3.8832	3.9387	0.0804	0.6023	
	group	1	3.033	3.0334	3.0767	0.0628	0.0281	*
	Residuals	42	41.408	0.9859		0.8569		
	Total	44	48.325			1		
adaptive	fish_weight	1	2.709	2.7085	4.1020	0.0822	0.1821	
	group	1	2.527	2.5266	3.8266	0.0766	0.1562	
	Residuals	42	27.732	0.6603		0.8412		
	Total	44	32.967			1		
complement	fish_weight	1	4.978	4.9776	3.8988	0.0841	0.5153	
	group	1	0.571	0.5707	0.4470	0.0097	0.8372	
	Residuals	42	53.621	1.2767		0.9062		
	Total	44	59.170			1		
regulatory	fish_weight	1	0.611	0.6112	4.4978	0.0949	0.6968	
	group	1	0.121	0.1211	0.8914	0.0188	0.0893	.
	Residuals	42	5.708	0.1359		0.8863		
	Total	44	6.440			1		

SI.4.6. Gene expression differences between infected and exposed fish

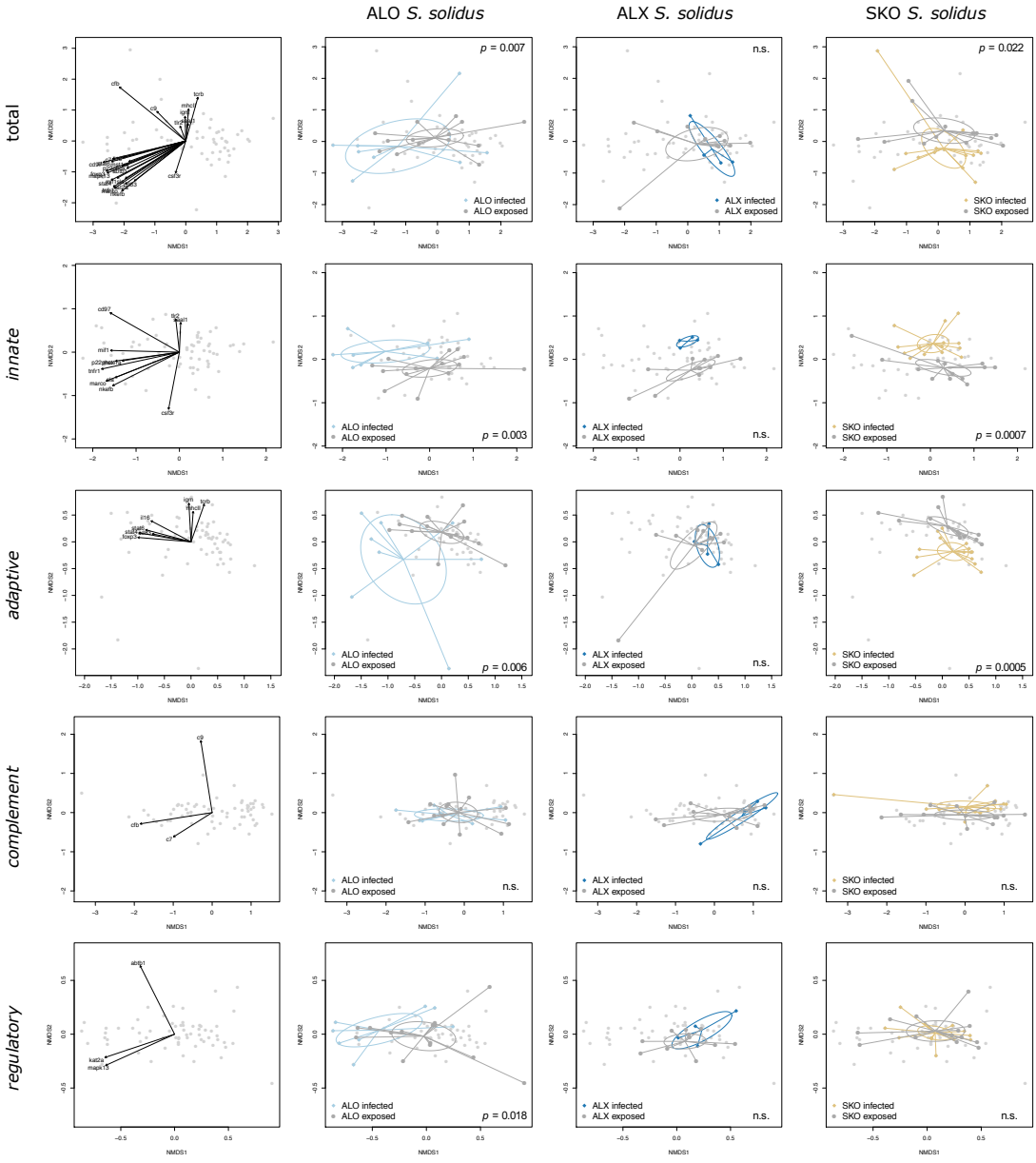


Figure S15. Gene expression profiles of *S. solidus* infected and exposed (but uninfected) GPS stickleback. Sticklebacks originated from Lake Großer Plöner See (GPS; Germany); *S. solidus* came from Walby Lake (ALO; Alaska), Wolf Lake (ALX; Alaska), and Lake Skogseidvatnet (SKO; Norway). Total RNA was extracted from head kidneys. We quantified expression levels of 25 targets (*total*) including eleven innate immune genes (*innate*: *marco*, *mst1ra*, *mif*, *tnfr1*, *saal1*, *tlr2*, *csf3r*, *p22^{phox}*, *nkef-b*, *sla1*, *cd97*), eight adaptive immune genes (*adaptive: stat4*, *stat6*, *igm*, *cd83*, *foxp3*, *il-16*, *mhcII*, *tcr-β*), three complement component genes (*complement: cfb*, *c7*, *c9*), and three regulatory genes (*regulatory*: *abtb1*, *kat2a*, *mapk13*). NMDS plots are based on log10 transformed calibrated normalized relative quantities (CNRQ). Statistics follow Tables S53-S55.

Table S48. Differences between gene expression profiles of infected and exposed ALO stickleback. Fish were exposed to or infected with ALO *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	3.744	3.7438	1.1424	0.0518	0.9960
	group	1	3.030	3.0303	0.9246	0.0419	0.4391
	Residuals	20	65.545	3.2772		0.9063	
	Total	22	72.319			1	
innate	fish_weight	1	2.193	2.1929	2.1110	0.0890	0.9925
	group	1	1.674	1.6735	1.6110	0.0679	0.1539
	Residuals	20	20.776	1.0388		0.8431	
	Total	22	24.643			1	
adaptive	fish_weight	1	1.300	1.3003	2.0822	0.0886	0.9664
	S. solidus	1	0.888	0.8882	1.4222	0.0605	0.2198
	Residuals	20	12.490	0.6245		0.8509	
	Total	22	14.679			1	
complement	fish_weight	1	0.056	0.0559	0.0380	0.0019	0.9991
	S. solidus	1	0.140	0.1400	0.0951	0.0047	0.8333
	Residuals	20	29.466	1.4733		0.9934	
	Total	22	29.662			1	
regulatory	fish_weight	1	0.201	0.2007	1.1034	0.0477	0.6881
	S. solidus	1	0.366	0.3664	2.0140	0.0871	0.3365
	Residuals	20	3.638	0.1819		0.8652	
	Total	22	4.205			1	

Table S49. Differences between gene expression profiles of infected and exposed ALO stickleback. Fish were exposed to or infected with ALX *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	2.093	2.0934	0.5888	0.0321	0.0463	*
	group	1	6.328	6.3278	1.7797	0.0969	0.0521	.
	Residuals	16	56.888	3.5555		0.8711		
	Total	18	65.309			1		
innate	fish_weight	1	1.149	1.1490	1.2899	0.0721	0.0362	*
	group	1	0.546	0.5460	0.6129	0.0342	0.5697	
	Residuals	16	14.252	0.8908		0.8937		
	Total	18	15.947			1		
adaptive	fish_weight	1	0.192	0.1917	0.3768	0.0208	0.5388	
	group	1	0.884	0.8838	1.7373	0.0959	0.5101	
	Residuals	16	8.140	0.5087		0.8833		
	Total	18	9.215			1		
complement	fish_weight	1	0.733	0.7334	0.3555	0.0190	0.1030	
	group	1	4.801	4.8010	2.3275	0.1246	0.0182	*
	Residuals	16	33.004	2.0627		0.8564		
	Total	18	38.538			1		
regulatory	fish_weight	1	0.021	0.0208	0.1568	0.0092	0.8108	
	group	1	0.117	0.1174	0.8862	0.0520	0.8941	
	Residuals	16	2.120	0.1325		0.9388		
	Total	18	2.258			1		

Table S50. Differences between gene expression profiles of infected and exposed ALO stickleback. Fish were exposed to or infected with SKO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	3.812	3.8116	1.3526	0.0521	0.0548	.
	group	1	7.416	7.4157	2.6316	0.1013	0.0730	.
	Residuals	22	61.994	2.8179		0.8467		
	Total	24	73.221			1		
innate	fish_weight	1	0.790	0.7900	1.2090	0.0481	0.2931	
	group	1	1.270	1.2696	1.9430	0.0773	0.3760	
	Residuals	22	14.375	0.6534		0.8747		
	Total	24	16.434			1		
adaptive	fish_weight	1	0.316	0.3155	0.9342	0.0385	0.2899	
	S. solidus	1	0.447	0.4466	1.3224	0.0545	0.6950	
	Residuals	22	7.429	0.3377		0.9070		
	Total	24	8.191			1		
complement	fish_weight	1	2.674	2.6739	1.5154	0.0568	0.0498	*
	S. solidus	1	5.598	5.5981	3.1726	0.1189	0.0744	.
	Residuals	22	38.819	1.7645		0.8243		
	Total	24	47.091			1		
regulatory	fish_weight	1	0.045	0.0445	0.5501	0.0231	0.3499	
	S. solidus	1	0.102	0.1021	1.2604	0.0529	0.5936	
	Residuals	22	1.781	0.0810		0.9240		
	Total	24	1.928			1		

Table S51. Differences between gene expression profiles of infected and exposed ALX stickleback. Fish were exposed to or infected with ALO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	7.045	7.0449	1.6032	0.1049	0.3403	
	group	1	3.022	3.0223	0.6878	0.0450	0.5000	
	Residuals	13	57.125	4.3942		0.8502		
	Total	15	67.192			1		
innate	fish_weight	1	3.490	3.4896	5.8074	0.2659	0.0139	*
	group	1	1.823	1.8234	3.0344	0.1389	0.0278	*
	Residuals	13	7.812	0.6009		0.5952		
	Total	15	13.125			1		
adaptive	fish_weight	1	2.472	2.4717	1.3020	0.0895	0.5729	
	group	1	0.472	0.4720	0.2486	0.0171	0.7326	
	Residuals	13	24.679	1.8984		0.8934		
	Total	15	27.622			1		
complement	fish_weight	1	0.415	0.4146	0.2272	0.0167	0.1424	
	group	1	0.726	0.7258	0.3976	0.0292	0.6944	
	Residuals	13	23.728	1.8252		0.9541		
	Total	15	24.868			1		
regulatory	fish_weight	1	0.764	0.7644	7.2611	0.3523	0.4097	
	group	1	0.037	0.0368	0.3498	0.0170	0.6806	
	Residuals	13	1.369	0.1053		0.6307		
	Total	15	2.170			1		

Table S52. Differences between gene expression profiles of infected and exposed ALX stickleback. Fish were exposed to or infected with ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	6.182	6.1819	1.7463	0.0689	0.4977
	group	1	2.104	2.1040	0.5944	0.0235	0.4953
	Residuals	23	81.422	3.5401		0.9076	
	Total	25	89.708			1	
innate	fish_weight	1	2.756	2.7562	2.6581	0.1006	0.3264
	group	1	0.783	0.7828	0.7549	0.0286	0.2768
	Residuals	23	23.849	1.0369		0.8708	
	Total	25	27.388			1	
adaptive	fish_weight	1	1.811	1.8114	1.4903	0.0592	0.4515
	S. solidus	1	0.845	0.8453	0.6954	0.0276	0.4633
	Residuals	23	27.956	1.2155		0.9132	
	Total	25	30.613			1	
complement	fish_weight	1	0.552	0.5518	0.5115	0.0214	0.7128
	S. solidus	1	0.458	0.4578	0.4243	0.0177	0.4196
	Residuals	23	24.813	1.0788		0.9609	
	Total	25	25.823			1	
regulatory	fish_weight	1	1.179	1.1791	5.0028	0.1782	0.1847
	S. solidus	1	0.018	0.0182	0.0773	0.0028	0.9244
	Residuals	23	5.421	0.2357		0.8191	
	Total	25	6.618			1	

Table S53. Differences between gene expression profiles of infected and exposed GPS stickleback. Fish were exposed to or infected with ALO *S. solidus* (group). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	10.117	10.1166	3.5163	0.1242	0.2928	
	group	1	16.680	16.6799	5.7975	0.2048	**0.0068**	**
	Residuals	19	54.665	2.8771		0.6711		
	Total	21	81.461			1		
innate	fish_weight	1	3.910	3.9102	3.6167	0.1149	0.2160	
	group	1	9.567	9.5672	8.8490	0.2812	**0.0026**	**
	Residuals	19	20.542	1.0812		0.6038		
	Total	21	34.019			1		
adaptive	fish_weight	1	3.042	3.0419	3.3704	0.1224	0.1384	
	group	1	4.656	4.6563	5.1590	0.1874	**0.0062**	**
	Residuals	19	17.149	0.9026		0.6902		
	Total	21	24.847			1		
complement	fish_weight	1	2.464	2.4644	3.2348	0.1356	0.5945	
	group	1	1.239	1.2391	1.6265	0.0682	0.2300	
	Residuals	19	14.475	0.7618		0.7963		
	Total	21	18.178			1		
regulatory	fish_weight	1	0.709	0.7089	3.6038	0.1200	0.3331	
	group	1	1.462	1.4624	7.4342	0.2475	**0.0177**	*
	Residuals	19	3.738	0.1967		0.6325		
	Total	21	5.909			1		

Table S54. Differences between gene expression profiles of infected and exposed GPS stickleback. Fish were exposed to or infected with ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	1.826	1.8257	0.6563	0.0497	0.5833
	group	1	1.566	1.5658	0.5629	0.0426	0.1667
	Residuals	12	33.381	2.7818		0.9078	
	Total	14	36.773			1	
innate	fish_weight	1	0.667	0.6666	0.7761	0.0565	0.6944
	group	1	0.832	0.8319	0.9685	0.0705	0.1250
	Residuals	12	10.307	0.8589		0.8731	
	Total	14	11.805			1	
adaptive	fish_weight	1	0.348	0.3478	0.4759	0.0371	0.6389
	S. solidus	1	0.269	0.2692	0.3683	0.0287	0.7153
	Residuals	12	8.770	0.7309		0.9343	
	Total	14	9.387			1	
complement	fish_weight	1	0.811	0.8108	0.7222	0.0555	0.2500
	S. solidus	1	0.319	0.3190	0.2842	0.0219	0.2083
	Residuals	12	13.472	1.1227		0.9226	
	Total	14	14.602			1	
regulatory	fish_weight	1	0.009	0.0092	0.1091	0.0079	0.9097
	S. solidus	1	0.146	0.1458	1.7272	0.1248	0.2431
	Residuals	12	1.013	0.0844		0.8673	
	Total	14	1.168			1	

Table S55. Differences between gene expression profiles of infected and exposed GPS stickleback. Fish were exposed to or infected with SKO *S. solidus* (group). Bold numbers indicate significance post FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	7.258	7.2580	2.8284	0.0963	0.5633	
	group	1	6.524	6.5235	2.5422	0.0866	**0.0217**	*
	Residuals	24	61.587	2.5661		0.8172		
	Total	26	75.369			1		
innate	fish_weight	1	2.115	2.1154	2.6651	0.0839	0.3165	
	group	1	4.052	4.0523	5.1053	0.1607	**0.0007**	***
	Residuals	24	19.050	0.7937		0.7554		
	Total	26	25.217			1		
adaptive	fish_weight	1	2.053	2.0529	5.0903	0.1523	0.0895	.
	group	1	1.744	1.7442	4.3249	0.1294	**0.0005**	***
	Residuals	24	9.679	0.4033		0.7182		
	Total	26	13.476			1		
complement	fish_weight	1	2.952	2.9518	2.3082	0.0860	0.7866	
	group	1	0.694	0.6939	0.5426	0.0202	0.5132	
	Residuals	24	30.692	1.2788		0.8938		
	Total	26	34.337			1		
regulatory	fish_weight	1	0.163	0.1629	1.2720	0.0498	0.7509	
	group	1	0.035	0.0354	0.2761	0.0108	0.4619	
	Residuals	24	3.073	0.1280		0.9394		
	Total	26	3.271			1		

SI.4.7. Gene expression differences between control and exposed fish

Table S56. Differences between gene expression profiles of control and exposed ALO stickleback. Fish were sham-exposed or exposed to ALO S. solidus (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	8.943	8.9426	3.6914	0.0935	0.0625	.
	group	1	1.884	1.8845	0.7779	0.0197	0.4540	
	Residuals	35	84.789	2.4225		0.8868		
	Total	37	95.616			1		
innate	fish_weight	1	0.523	0.5231	0.7056	0.0193	0.3698	
	group	1	0.663	0.6631	0.8946	0.0244	0.4193	
	Residuals	35	25.944	0.7412		0.9563		
	Total	37	27.130			1		
adaptive	fish_weight	1	0.184	0.1838	0.3773	0.0104	0.5651	
	S. solidus	1	0.422	0.4218	0.8661	0.0239	0.3889	
	Residuals	35	17.046	0.4870		0.9657		
	Total	37	17.652			1		
complement	fish_weight	1	8.225	8.2252	7.5143	0.1741	0.0582	.
	S. solidus	1	0.716	0.7163	0.6544	0.0152	0.5096	
	Residuals	35	38.311	1.0946		0.8108		
	Total	37	47.253			1		
regulatory	fish_weight	1	0.013	0.0127	0.0879	0.0025	0.7743	
	S. solidus	1	0.084	0.0837	0.5813	0.0163	0.4931	
	Residuals	35	5.042	0.1440		0.9812		
	Total	37	5.138			1		

Table S57. Differences between gene expression profiles of control and exposed ALO stickleback. Fish were sham-exposed or exposed to ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	3.332	3.3317	1.0784	0.0287	0.2441	
	group	1	1.486	1.4859	0.4809	0.0128	0.6335	
	Residuals	36	111.224	3.0896		0.9585		
	Total	38	116.041			1		
innate	fish_weight	1	0.119	0.1193	0.1351	0.0037	0.5872	
	group	1	0.550	0.5497	0.6221	0.0169	0.0970	.
	Residuals	36	31.808	0.8836		0.9794		
	Total	38	32.477			1		
adaptive	fish_weight	1	0.126	0.1256	0.2257	0.0060	0.3809	
	group	1	0.646	0.6461	1.1608	0.0311	0.0612	.
	Residuals	36	20.037	0.5566		0.9629		
	Total	38	20.808			1		
complement	fish_weight	1	3.047	3.0474	1.9603	0.0515	0.2428	
	group	1	0.183	0.1827	0.1175	0.0031	0.8105	
	Residuals	36	55.963	1.5545		0.9454		
	Total	38	59.193			1		
regulatory	fish_weight	1	0.049	0.0490	0.3410	0.0092	0.4785	
	group	1	0.109	0.1086	0.7565	0.0204	0.0684	.
	Residuals	36	5.169	0.1436		0.9704		
	Total	38	5.326			1		

Table S58. Differences between gene expression profiles of control and exposed ALO stickleback. Fish were sham-exposed or exposed to SKO *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
total	fish_weight	1	2.162	2.1619	0.7275	0.0166	0.2720
	group	1	0.617	0.6168	0.2075	0.0047	0.7854
	Residuals	43	127.788	2.9718		0.9787	
	Total	45	130.567			1	
innate	fish_weight	1	0.376	0.3760	0.4884	0.0111	0.1246
	group	1	0.477	0.4767	0.6192	0.0140	0.1292
	Residuals	43	33.104	0.7699		0.9749	
	Total	45	33.956			1	
adaptive	fish_weight	1	0.198	0.1984	0.4090	0.0094	0.5956
	S. solidus	1	0.040	0.0398	0.0821	0.0019	0.9221
	Residuals	43	20.858	0.4851		0.9887	
	Total	45	21.096			1	
complement	fish_weight	1	1.587	1.5867	0.9693	0.0220	0.2926
	S. solidus	1	0.074	0.0741	0.0452	0.0010	0.9156
	Residuals	43	70.388	1.6369		0.9770	
	Total	45	72.049			1	
regulatory	fish_weight	1	0.003	0.0029	0.0241	0.0006	0.9817
	S. solidus	1	0.051	0.0512	0.4307	0.0099	0.3043
	Residuals	43	5.110	0.1188		0.9895	
	Total	45	5.164			1	

Table S59. Differences between gene expression profiles of control and exposed ALX stickleback. Fish were sham-exposed or exposed to ALO *S. solidus* (group). Differences were not significant after FDR correction

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	10.108	10.1080	2.5714	0.0778	0.1992	
	group	1	1.898	1.8979	0.4828	0.0146	0.6016	
	Residuals	30	117.928	3.9309		0.9076		
	Total	32	129.934			1		
innate	fish_weight	1	3.724	3.7242	4.3105	0.1236	0.0430	*
	group	1	0.486	0.4863	0.5628	0.0161	0.3203	
	Residuals	30	25.919	0.8640		0.8603		
	Total	32	30.130			1		
adaptive	fish_weight	1	5.536	5.5363	3.5848	0.1056	0.3281	
	group	1	0.583	0.5833	0.3777	0.0111	0.4375	
	Residuals	30	46.331	1.5444		0.8833		
	Total	32	52.451			1		
complement	fish_weight	1	0.351	0.3511	0.2504	0.0081	0.4219	
	group	1	0.814	0.8144	0.5808	0.0188	0.5625	
	Residuals	30	42.064	1.4021		0.9730		
	Total	32	43.229			1		
regulatory	fish_weight	1	0.758	0.7577	5.0093	0.1423	0.0117	*
	group	1	0.030	0.0295	0.1953	0.0056	0.7344	
	Residuals	30	4.538	0.1513		0.8522		
	Total	32	5.325			1		

Table S60. Differences between gene expression profiles of control and exposed ALX stickleback. Fish were sham-exposed or exposed to ALX *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	9.528	9.5279	2.6255	0.0750	0.0938	.
	group	1	1.357	1.3570	0.3739	0.0107	0.5260	
	Residuals	32	116.125	3.6289		0.9143		
	Total	34	127.010			1		
innate	fish_weight	1	3.028	3.0280	2.9829	0.0846	0.1918	
	group	1	0.265	0.2654	0.2614	0.0074	0.6319	
	Residuals	32	32.483	1.0151		0.9080		
	Total	34	35.777			1		
adaptive	fish_weight	1	5.574	5.5744	4.2453	0.1163	0.0373	*
	S. solidus	1	0.327	0.3267	0.2488	0.0068	0.6849	
	Residuals	32	42.018	1.3131		0.8769		
	Total	34	47.919			1		
complement	fish_weight	1	0.224	0.2239	0.1996	0.0061	0.8498	
	S. solidus	1	0.731	0.7306	0.6512	0.0198	0.4149	
	Residuals	32	35.902	1.1219		0.9741		
	Total	34	36.856			1		
regulatory	fish_weight	1	0.986	0.9856	4.6553	0.1264	0.1875	
	S. solidus	1	0.035	0.0348	0.1644	0.0045	0.4913	
	Residuals	32	6.775	0.2117		0.8691		
	Total	34	7.795			1		

Table S61. Differences between gene expression profiles of control and exposed ALX stickleback. Fish were sham-exposed or exposed to SKO *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	6.662	6.6624	1.6774	0.0540	0.3281	
	group	1	5.466	5.4661	1.3762	0.0443	0.3672	
	Residuals	28	111.212	3.9719		0.9017		
	Total	30	123.341			1		
innate	fish_weight	1	2.290	2.2901	2.5767	0.0837	0.4766	
	group	1	0.187	0.1870	0.2104	0.0068	0.9531	
	Residuals	28	24.886	0.8888		0.9095		
	Total	30	27.363			1		
adaptive	fish_weight	1	3.462	3.4618	2.4024	0.0763	0.0938	.
	group	1	1.538	1.5382	1.0675	0.0339	0.7344	
	Residuals	28	40.346	1.4409		0.8897		
	Total	30	45.346			1		
complement	fish_weight	1	0.729	0.7286	0.4798	0.0156	0.5859	
	group	1	3.614	3.6140	2.3801	0.0771	0.1797	
	Residuals	28	42.517	1.5185		0.9073		
	Total	30	46.860			1		
regulatory	fish_weight	1	0.446	0.4463	2.9607	0.0931	0.3359	
	group	1	0.129	0.1292	0.8572	0.0269	0.2109	
	Residuals	28	4.220	0.1507		0.8800		
	Total	30	4.796			1		

Table S62. Differences between gene expression profiles of control and exposed GPS stickleback. Fish were sham-exposed or exposed to ALO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	10.984	10.9838	3.5108	0.0768	0.1576	
	group	1	0.674	0.6737	0.2153	0.0047	0.7915	
	Residuals	42	131.401	3.1286		0.9185		
	Total	44	143.059			1		
innate	fish_weight	1	5.012	5.0119	4.4411	0.0950	0.0398	*
	group	1	0.369	0.3686	0.3266	0.0070	0.4246	
	Residuals	42	47.398	1.1285		0.8981		
	Total	44	52.779			1		
adaptive	fish_weight	1	2.293	2.2934	3.0985	0.0683	0.0714	.
	S. solidus	1	0.211	0.2110	0.2851	0.0063	0.4110	
	Residuals	42	31.088	0.7402		0.9255		
	Total	44	33.592			1		
complement	fish_weight	1	2.942	2.9419	2.6135	0.0585	0.5571	
	S. solidus	1	0.065	0.0653	0.0580	0.0013	0.8963	
	Residuals	42	47.277	1.1256		0.9402		
	Total	44	50.284			1		
regulatory	fish_weight	1	0.740	0.7399	4.1399	0.0888	0.0864	.
	S. solidus	1	0.085	0.0847	0.4739	0.0102	0.3566	
	Residuals	42	7.506	0.1787		0.9010		
	Total	44	8.331			1		

Table S63. Differences between gene expression profiles of control and exposed GPS stickleback. Fish were sham-exposed or exposed to ALX *S. solidus* (group).

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	3.473	3.4725	1.0415	0.0251	0.6929	
	group	1	5.064	5.0635	1.5187	0.0365	0.3650	
	Residuals	39	130.026	3.3340		0.9384		
	Total	41	138.562			1		
innate	fish_weight	1	1.144	1.1440	1.0153	0.0244	0.8867	
	group	1	1.728	1.7280	1.5336	0.0369	0.1793	
	Residuals	39	43.943	1.1267		0.9387		
	Total	41	46.815			1		
adaptive	fish_weight	1	1.142	1.1416	1.4097	0.0338	0.1189	
	group	1	1.027	1.0275	1.2688	0.0304	0.0734	.
	Residuals	39	31.583	0.8098		0.9357		
	Total	41	33.752			1		
complement	fish_weight	1	1.146	1.1463	0.8974	0.0217	0.6337	
	group	1	1.971	1.9709	1.5430	0.0372	0.7036	
	Residuals	39	49.816	1.2773		0.9411		
	Total	41	52.933			1		
regulatory	fish_weight	1	0.082	0.0816	0.5636	0.0131	0.9462	
	group	1	0.496	0.4958	3.4241	0.0797	0.0532	.
	Residuals	39	5.647	0.1448		0.9072		
	Total	41	6.224			1		

Table S64. Differences between gene expression profiles of control and exposed GPS stickleback. Fish were sham-exposed or exposed to SKO *S. solidus* (group). Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	10.947	10.9471	3.3859	0.0749	0.8677	
	group	1	2.653	2.6531	0.8206	0.0182	0.1188	
	Residuals	41	132.558	3.2331		0.9070		
	Total	43	146.158			1		
innate	fish_weight	1	4.962	4.9624	4.5008	0.0963	0.8774	
	group	1	1.367	1.3672	1.2400	0.0265	0.0506	.
	Residuals	41	45.205	1.1026		0.8772		
	Total	43	51.535			1		
adaptive	fish_weight	1	1.970	1.9697	2.7663	0.0621	0.8853	
	S. solidus	1	0.576	0.5763	0.8094	0.0182	0.1616	
	Residuals	41	29.194	0.7121		0.9198		
	Total	43	31.740			1		
complement	fish_weight	1	3.513	3.5131	2.7265	0.0620	0.7778	
	S. solidus	1	0.291	0.2914	0.2262	0.0052	0.5052	
	Residuals	41	52.828	1.2885		0.9328		
	Total	43	56.633			1		
regulatory	fish_weight	1	0.502	0.5019	3.0768	0.0656	0.8784	
	S. solidus	1	0.460	0.4602	2.8212	0.0602	0.0495	*
	Residuals	41	6.688	0.1631		0.8742		
	Total	43	7.650			1		

SI.4.7. Sympatric versus allopatric combinations (Alaskan hosts and parasites)

Table S65. Differences between gene expression profiles of exposed sticklebacks. Alaskan sticklebacks were exposed in sympatric or allopatric combinations. Differences were not significant after FDR correction.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	8.681	8.6813	2.0897	0.0532	0.3447	
	combination	1	4.965	4.9647	1.1951	0.0304	0.1639	
	Residuals	36	149.556	4.1543		0.9164		
	Total	38	163.202			1		
innate	fish_weight	1	4.101	4.1014	4.4745	0.1080	0.5977	
	combination	1	0.862	0.8615	0.9399	0.0227	0.0493	*
	Residuals	36	32.998	0.9166		0.8693		
	Total	38	37.961			1		
adaptive	fish_weight	1	2.856	2.8557	2.4891	0.0635	0.4414	
	combination	1	0.816	0.8156	0.7109	0.0181	0.3717	
	Residuals	36	41.302	1.1473		0.9184		
	Total	38	44.973			1		
complement	fish_weight	1	0.945	0.9454	0.4904	0.0129	0.1126	
	combination	1	2.759	2.7589	1.4311	0.0377	0.2326	
	Residuals	36	69.400	1.9278		0.9493		
	Total	38	73.105			1		
regulatory	fish_weight	1	0.958	0.9576	4.7666	0.1085	0.4828	
	combination	1	0.635	0.6351	3.1613	0.0720	0.0172	*
	Residuals	36	7.232	0.2009		0.8195		
	Total	38	8.825			1		

Table S66. Differences between gene expression profiles of infected sticklebacks. Alaskan sticklebacks were infected in sympatric or allopatric combinations.

		Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
total	fish_weight	1	13.285	13.2854	4.2542	0.0895	0.1109	
	combination	1	0.949	0.9489	0.3038	0.0064	0.8562	
	Residuals	43	134.285	3.1229		0.9042		
	Total	45	148.519			1		
innate	fish_weight	1	4.639	4.6394	5.2143	0.1070	0.3272	
	combination	1	0.470	0.4703	0.5286	0.0108	0.4752	
	Residuals	43	38.259	0.8897		0.8822		
	Total	45	43.368			1		
adaptive	fish_weight	1	2.624	2.6238	2.4700	0.0542	0.2827	
	combination	1	0.093	0.0933	0.0879	0.0019	0.9689	
	Residuals	43	45.678	1.0623		0.9439		
	Total	45	48.395			1		
complement	fish_weight	1	5.116	5.1161	4.8983	0.1015	0.0599	.
	combination	1	0.372	0.3716	0.3558	0.0074	0.6151	
	Residuals	43	44.912	1.0445		0.8911		
	Total	45	50.400			1		
regulatory	fish_weight	1	0.966	0.9655	5.9470	0.1212	0.1171	
	combination	1	0.018	0.0180	0.1108	0.0023	0.8541	
	Residuals	43	6.981	0.1624		0.8765		
	Total	45	7.965			1		

REFERENCES

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecol. *26*, 32–46.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting Linear Mixed-Effects Models using lme4. ArXiv14065823 Stat.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Methodol. *57*, 289–300.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. Biom. J. *50*, 346–363.

Johnson, P.C.D. (2014). Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods Ecol. Evol. *5*, 944–946.

Lefcheck, J.S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. *7*, 573–579.

Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. *4*, 133–142.

Oksanen, J.F., Blanchet, G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G., Solymos, P., Stevens, M.H.H., and Wagner, H. (2015). vegan: community ecology package. 2.0-0. R package version 2.3-0.

EIDESSTATTLICHE ERKLÄRUNG

Hiermit bestätige ich, Agnes Piecyk, dass die folgende Dissertation

Evolutionary and ecological perspectives on epidemiological traits in helminth infections of sticklebacks

von mir, unter Beratung meiner Betreuer, selbstständig verfasst wurde, nach Inhalt und Form meine eigene Arbeit ist und keine weiteren Quellen und Hilfsmittel als die angegebenen verwendet wurden.
Die vorliegende Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden und wurde weder im Rahmen eines Prüfungsverfahrens an anderer Stelle vorgelegt noch veröffentlicht. Veröffentlichte oder zur Veröffentlichung eingereichte Manuskripte wurden kenntlich gemacht. Mir wurde kein akadamischer Grad entzogen.

Kiel, Februar 2019

Agnes Piecyk