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In vitro leukocyte response of three-spined sticklebacks (*Gasterosteus aculeatus*) to helminth parasite antigens

Frederik Franke[a], Anna K. Rahn[b], Janine Dittmar[a], Noémie Erin[c], Jennifer K. Rieger[d], David Haase[d], Irene E. Samonte-Padilla[c], Joseph Lange[a], Per J. Jakobsen[e], Miguel Hermida[f], Carlos Fernández[f], Joachim Kurtz[a], Theo C.M. Bakker[b], Thorsten B.H. Reusch[d], Martin Kalbe[c], Jörn P. Scharsack[a,*]

[a] *Department of Animal Evolutionary Ecology, Institute for Evolution and Biodiversity, University of Münster, Hüfferstrasse 1, 48149 Münster, Germany*
[b] *Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany*
[c] *Department of Evolutionary Ecology, Max-Planck Institute of Evolutionary Biology, August-Thienemann Str 2, 24306 Plön, Germany*
[d] *Department of Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz Centre for Ocean Research, Düsternbrooker Weg 20, 24105 Kiel, Germany*
[e] *Institute for Biology, University of Bergen, Thor Møhlensgate 55, 5020 Bergen, Norway*
[f] *Departamento de Xenetica, Facultade de Veterinaria, Universidade de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*

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ABSTRACT

Helminth parasites of teleost fish have evolved strategies to evade and manipulate the immune responses of their hosts. Responsiveness of fish host immunity to helminth antigens may therefore vary depending on the degree of host-parasite counter-adaptation. Generalist parasites, infective for a number of host species, might be unable to adapt optimally to the immune system of a certain host species, while specialist parasites might display high levels of adaptation to a particular host species. The degree of adaptations may further differ between sympatric and allopatric host-parasite combinations. Here, we test these hypotheses by *in vitro* exposure of head kidney leukocytes from three-spined sticklebacks (*Gasterosteus aculeatus*) to antigens from parasites with a broad fish host range (*Diplostomum pseudospathaceum*, *Triaenophorus nodulosus*), a specific fish parasite of cyprinids (*Ligula intestinalis*) and parasites highly specific only to a single fish species as second intermediate host (*Schistocephalus pungitii*, which does not infect *G. aculeatus*, and *Schistocephalus solidus*, infecting *G. aculeatus*). *In vitro* responses of stickleback leukocytes to *S. solidus* antigens from six European populations, with *S. solidus* prevalence from <1% to 66% were tested in a fully crossed experimental design. Leukocyte cultures were analysed by means of flow cytometry and a chemiluminescence assay to quantify respiratory burst activity. We detected decreasing magnitudes of *in vitro* responses to antigens from generalist to specialist parasites and among specialists, from parasites that do not infect *G. aculeatus* to a *G. aculeatus*-infecting species. Generalist parasites seem to maintain their ability to infect different host species at the costs of relatively higher immunogenicity compared to specialist parasites. In a comparison of sympatric and allopatric combinations of stickleback leukocytes and antigens from *S. solidus*, magnitudes of *in vitro* responses were dependent on the prevalence of the parasite in the population of origin, rather than on sympatry. Antigens from Norwegian (prevalence 30–50%) and Spanish (40–66%) *S. solidus* induced generally higher *in vitro* responses compared to *S. solidus* from two German (<1%) populations. Likewise, leukocytes from stickleback populations with a high *S. solidus* prevalence showed higher *in vitro* responses to *S. solidus* antigens compared to populations with low *S. solidus* prevalence. This suggests a rather low degree of local adaptation in *S. solidus* populations, which might be due to high gene flow among populations because of their extremely mobile final hosts, fish-eating birds.

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[*] Corresponding author. Tel.: +49 251 83 32550.
E-mail address: joern.scharsack@uni-muenster.de (J.P. Scharsack).

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1. Introduction

Helminths are frequent parasites of natural fish populations, but interactions of helminths with the piscine immune system are under-investigated. This might be attributed to the fact that helminth infections in aquaculture are often relatively easy to control (*e.g.* by control of invertebrate intermediate hosts) and rarely have prominent commercial impact [1]. However, in their natural habitat, parasites often drastically reduce host fitness and thus pose strong selection pressures on their hosts, which therefore have evolved powerful counter-measures to control infection [2]. The success of helminth parasites largely depends on their ability to evade and/or manipulate the generally efficient immune system of their fish hosts [3,4]. The evolutionary arms race of host-parasite counter adaptations (often described as Red Queen Dynamics) promote parasite virulence and infection success on the one hand [5–7], but host immunocompetence and prevention of infection on the other [8–11]. In cyprinids and salmonids, activation of granulocytes is considered to be an important part of the immune defence against parasitic helminths [12–16]. In sticklebacks, traits of cellular innate immunity, such as respiratory burst activity, were elevated in a population that was adapted to higher parasite infection pressure [9]. Adaptation of stickleback immunocompetence to local parasites is presumably supported by selection for certain MHC class II genotypes [11]. The basis of functional cellular immunity in such differential adaptive situations is not well investigated to date in teleost fish.

1.1. *Parasites with a broad and a narrow host range – Diplostomum and Schistocephalus*

Helminths are experts in evasion and manipulation of their hosts' immune functions and the respective strategies may depend on the host range. Generalist parasites might not be able to adapt their antigenicity (antigenic surface) for the immune system of a certain host species and might instead use other immune evasion strategies. An example is the trematode *Diplostomum pseudospathaceum*, which infects the immunological inert eye lens of various freshwater fish species. After penetrating the skin (or gills) of their fish host, the tissue migrating larval stage (diplostomulum) of *D. pseudospathaceum* finds its way along the blood vessels to the eye lenses. Antigens from such a generalist parasite might trigger stronger immune responses compared to antigens from specialist parasites, such as the cestode *Schistocephalus solidus*. The adult stage of the tapeworm *S. solidus* reproduces in the gut of warm-blooded vertebrates, most often fish-eating birds. Eggs are released in the faeces of the final host. A first, free-swimming larval stage (coracidium) hatches in water, and develops to the second larval stage (procercoid) after ingestion by a cyclopoid copepod. The third larval stage (plerocercoid) develops in the body cavity of the obligatory and specific second intermediate host: the three-spined stickleback (*Gasterosteus aculeatus*). Besides the immune system, the aggressive environment of the stickleback's stomach may prevent infection [17,18], but once in the body cavity of the three-spined stickleback host, clearance of *S. solidus* plerocercoids is rare [19]. Experimental transfer of *S. solidus* plerocercoids to fish species other than three-spined sticklebacks lead to rapid death of the larvae [20,21], underlining the specific adaptation of *S. solidus* to three-spined sticklebacks, but suggesting that the immune system of fish is in principle able to clear *S. solidus* infections. In three-spined sticklebacks, established plerocercoids of *S. solidus* take all the resources that the parasite needs from the host and grow to up to 20–30% (w/w) of their host's body weight [19], thereby reducing the fitness of the hosts and resulting in decreased or even absent reproduction [22–26].

1.2. *Host-parasite local mutual adaption*

A specialist like *S. solidus* might even have optimised its surface antigens (immune evasion) for a frequently infected local host population. If this is the case, immunity of sympatric hosts might have a weaker response to the parasites' antigens compared to immunity of allopatric hosts. We thus hypothesize that antigenicity (strength of *in vitro* leukocyte response) decreases from generalist to specialist parasites and among specialists from parasites that do not infect *G. aculeatus* to *G. aculeatus*-infecting parasites, and among *G. aculeatus*-infecting parasites from allopatric to sympatric host-parasite combinations.

Previous studies of local adaptation of teleost fish hosts and their parasites have mainly focussed at infectivity and host mortality, but have rarely included immunological patterns of adaptation [5–7,27–30]. Some of these studies observed local advantages of the (co-evolved) host population. In those studies, hosts were genetically best adapted to the local parasite population and showed inferior performance in preventing infections with non-local parasites of the same species [27,28]. Such situations would disadvantage immigrant hosts, but favour migrating parasites, thus promoting gene flow in the parasites. Other studies failed to detect local adaption in host-parasite systems [29] but a larger third group of studies describes a co-evolutionary local advantage of the parasite population, which became more infective for local compared to non-local hosts of the same species [5–7]. Such a constellation would promote immigrant hosts and disadvantage foreign parasites, which may promote gene flow among hosts. Therefore, parasites may play an important role in the dynamic process of diversification and speciation of their teleost fish hosts and *vice versa* [9].

1.3. *The present study*

In this study, responses of three-spined stickleback head kidney leukocytes (HKL) to antigens of helminth fish parasites were investigated with an *in vitro* system, enabling large-scale comparisons between parasite species, as well as comparisons across different host populations. Since activation of granulocytes is important in the immune defence of fish against parasitic helminths [12–16] and in sticklebacks cellular innate immunity was elevated in a population with higher parasite infection pressure [9], we quantified the respiratory burst (RB) activity of HKL. We hypothesized that *in vitro* exposure of HKL to parasite antigens might influence leukocyte viability and the frequencies of cellular subsets and therefore analysed numbers of viable HKL and the granulocytes to lymphocytes ratio (G/L ratio) after *in vitro* stimulation.

We investigated HKL *in vitro* responses to antigens from generalist parasites, such as the eye fluke *D. pseudospathaceum* that infects, among other fish species, also three-spined sticklebacks (*G. aculeatus*) [31], and the cestode *Triaenophorus nodulosus*, with several fish species including *G. aculeatus* as second intermediate hosts [32]. Furthermore, antigens from *Ligula intestinalis* were used, a parasite specific to cyprinids as second intermediate hosts, which does not infect *G. aculeatus*. Finally, tapeworm antigens from two highly specialised *Schistocephalus* species, *Schistocephalus pungitii*, specific for nine-spined sticklebacks (*Pungitius pungitius*) and *S. solidus*, specific for *G. aculeatus*, were used (Table 1). From the latter, seven hosts and corresponding parasite populations from across Europe were tested to investigate potential local adaptation in the *G. aculeatus*–*S. solidus* system.

After *in vitro* stimulation, stickleback HKL were analysed by means of flow cytometry to determine the cell viability and the granulocyte to lymphocyte ratio. In addition, the respiratory burst