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Testing for local adaptation in the *Gasterosteus–Gyrodactylus* host–parasite system

Nellie Konijnendijk, Joost A.M. Raeymaekers*, Sandra Vandeuren, Lize Jacquemin and Filip A.M. Volckaert

Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Leuven, Belgium

ABSTRACT

Background: Parasites are often assumed to be locally adapted to their hosts, while a growing body of literature shows this is not a fixed rule. We used the threespine stickleback (*Gasterosteus aculeatus*) and its host-specific parasitic flatworm *Gyrodactylus gasterostei* of the Belgian lowland–upland system to test for local adaptation and assess whether findings are consistent over different life stages.

Question: Is the *Gasterosteus–Gyrodactylus* host–parasite model system an example of local adaptation?

Hypothesis: Parasites have higher infection success on sympatric than on allopatric host populations.

Methods: F1 laboratory-bred stickleback originating from a lowland and upland population were infected with parasites of lowland and upland origin. We monitored parasite numbers per individual for 6 weeks and for two life stages and calculated the effect size of local adaptation.

Results: Infection success of parasites was not higher on sympatric than on allopatric host populations. Instead, total worm load differed among sub-adult host populations, but not among adult host populations. This suggests immune competence differs among host populations at a specific life stage, rather than local adaptation of the parasite.

Keywords: *Gasterosteus aculeatus*, *Gyrodactylus gasterostei*, host–parasite interactions, local adaptation, immune competence, stickleback.

INTRODUCTION

Local adaptation is a key process in adaptive evolution and is crucial in generating phenotypic diversity. It is the process by which different environments exert local selective pressure and this maximizes individual fitness within the local environment. As a result, individuals of the local population have higher fitness in their local environment than individuals from a non-local population. Recent theoretical and empirical studies (Kawecki and

Correspondence: N. Konijnendijk, Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium. e-mail: nellie.konijnendijk@bio.kuleuven.be
**Present address*: Zoological Institute, University of Basel, Vesalgasse 1, CH-4051 Basel, Switzerland.

Ebert, 2004; Morgan *et al.*, 2005; Nosil *et al.*, 2005; Hoekstra *et al.*, 2006; Garant *et al.*, 2007; Gandon and Nuismer, 2009) have contributed a great deal to our understanding of local adaptation, although it is still hard to predict when local adaptation will happen in nature (Hereford, 2009). Several meta-analyses of either reciprocal transplant or common garden experiments show that although examples of local adaptation are numerous, maladapted phenotypes are not the exception (Kaltz and Shykoff, 1998; Van Zandt and Mopper, 1998; Greischar and Koskella, 2007; Hoeksema and Forde, 2008; Leimu and Fischer, 2008; Fraser *et al.*, 2011). Conditions that favour local adaptation include: low gene flow, costs or constraints to plasticity, more spatial than temporal variation in selection, and limited fluctuations in habitat quality (Kawecki and Ebert, 2004).

Host–parasite systems are good models for testing the generality and prerequisites for local adaptation. The interactions between a host and its parasite are generally strong (Kawecki and Ebert, 2004; Greischar and Koskella, 2007), and unlike adaptation to the physical environment adaptive peaks shift continuously in response to the co-evolving species. A meta-analysis across many host–parasite systems revealed that relative gene flow is the strongest predictor for local adaptation (Hoeksema and Forde, 2008). Parasites with a high rate of gene flow compared with their host are on average better adapted to their local host than are parasites with a low rate of gene flow, most likely because gene flow introduces new alleles on which local selection can act (Gandon *et al.*, 1996; Gandon, 2002). In contrast, virulence (Greischar and Koskella, 2007), generation time, relative organismal complexity, and taxonomic similarity (Hoeksema and Forde, 2008) are not reliable predictors of local adaptation These findings allow us to make better predictions regarding whether local adaptation will happen in natural conditions.

Several outcomes are possible for tests of local adaptation in host–parasite systems (Fig. 1). For instance, parasite strains might vary in infection success (scenario in Fig. 1a), causing differential infection rates regardless of host origin (Altizer, 2001). Alternatively, differences in infection rates among host populations (scenario in Fig. 1c) might be an effect of differential immune competence or behavioural strategies in the host populations independent of the parasite under study. Both scenarios lead to differential infection rates, and both can exist without local adaptation being responsible for the observed pattern. Only the scenario in Fig. 1e would be a conclusive outcome for local adaptation of the parasite to the host.

To distinguish among these various scenarios, Thrall *et al.* (2002) and Kawecki and Ebert (2004) suggest reciprocal testing. Hoeksema and Forde (2008) found that adding non-reciprocal tests strongly influences the outcome of tests for local adaptation. A number of additional design elements are important to determine the extent of, and the genetic mechanisms underlying, local adaptation. For instance, when testing genetic mechanisms, it is important to rule out phenotypic plasticity and prior exposure to the parasite using F1 or if possible F2 individuals of the host species that are naïve with respect to the parasite under study (Eizaguirre *et al.*, 2012). As a result of the complexity of these necessary designs, studies that definitively test for local adaptation in host–parasite systems are rare. In fish, for example, to our knowledge only three studies have used reciprocal testing of host–parasite interactions. Two suggested local adaptation (Ballabeni and Ward, 1993; Voutilainen *et al.*, 2009), while the third showed no quantitative difference in infection success among hosts or parasites (Sasal *et al.*, 2000). The first two of these studies used laboratory-reared individuals, demonstrating that there is a genetic basis for local adaptation in these study systems.

The threespine stickleback (*Gasterosteus aculeatus*) represents an excellent host species to test for local adaptation in host–parasite systems. The species has already been used to

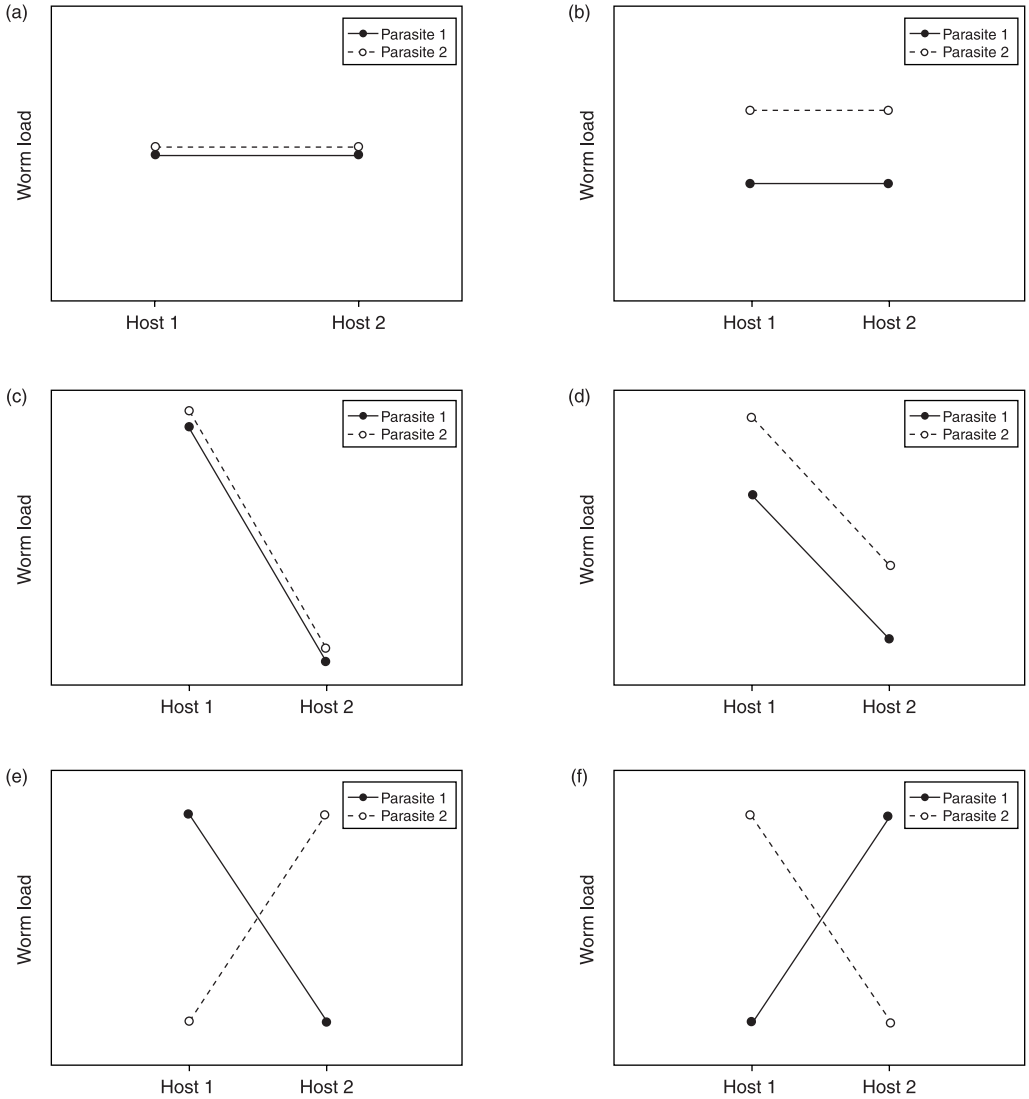

Fig. 1. Possible (net) outcomes of reciprocal infection experiments. (a) No adaptation by either parasite or host to the other *or* no adaptation by both host and parasite, with no net effect. (b) Intrinsic difference between parasites *or* adaptation by parasite 2 to host 2 leading to improved performance on both hosts (no trade-off). (c) Intrinsic difference between hosts *or* adaptation by host 1 to parasite 1 leading to improved resistance against both parasites (no trade-off). (d) Combination of (b) and (c) where the effects are additive. (e) Local adaptation of the parasite to the host *or* maladaptation of the host. (f) Local maladaptation of the parasite to the host *or* adaptation of the host.

better understand host–parasite dynamics and divergence in immune resistance in natural populations (Wegner *et al.*, 2003; Kalbe and Kurtz, 2006; MacColl, 2009; Eizaguirre *et al.*, 2011). Experiments with this model system can be easily carried out in the laboratory and repeated several times to check for repeatability across life stages. Moreover, the availability of laboratory crosses

allows the use of F1 individuals that have not been in contact with any parasites before to rule out effects of phenotypic plasticity and prior experience. Finally, the parasitic fauna of *G. aculeatus* is well characterized (Kalbe *et al.*, 2002).

Gyrodactylus gasterostei is a Monogonean flatworm that is a common and often dominant parasite in stickleback populations (Harris, 1985b, 1998; Raeymaekers *et al.*, 2008). Gene flow in *G. gasterostei* is likely to be much lower than in its host for two reasons: (1) it has a direct life cycle (Harris, 1985a) and no free-living stage (Cable *et al.*, 2002), which limits its spread, and (2) it is viviparous, with asexual reproduction as the predominant mode (Harris, 1998). *Gyrodactylus gasterostei* generation time can be as short as one day (Harris, 1985a), much shorter than in its host species. The abundance and occurrence of this parasite vary widely among stickleback populations and host populations frequently exposed to *Gyrodactylus* parasites have lower infection rates after experimental infection than populations that only encounter this parasite infrequently (de Roij *et al.*, 2011). This suggests that local adaptation of the host to this parasite is possible. In contrast, Raeymaekers *et al.* (2011) found that German host populations were less susceptible to infection with parasites from Belgian origin than the sympatric Belgian host population, suggesting that parasites in direct contact with the local host population might be better adapted to their local host. However, both studies used parasites from a single origin for experimental infection, and therefore could not address whether differential infection patterns were caused by differences in host immune resistance irrespective of the parasite rather than local adaptation. In this study, we test whether differences in infection patterns can be explained by local adaptation and whether the patterns are stable across life stages.

We use a full factorial design of two populations of threespine stickleback and their respective co-occurring ecto-parasite *Gyrodactylus gasterostei* to differentiate among the various scenarios summarized in Fig. 1. F1 laboratory-bred sub-adult fish were infected with parasites isolated from wild source populations. Parasite numbers per individual host were monitored for 6 weeks. Infection patterns in sympatric and allopatric host–parasite combinations were then compared and used as a proxy for effect size of local adaptation. To verify consistency of patterns across life stages, the experiment was repeated with adult animals from a subset of the same families.

MATERIALS AND METHODS

Origin of hosts and parasites

We used laboratory-bred threespine stickleback (*Gasterosteus aculeatus* L.; Gasterosteidae) as infection hosts. Their parents originated from Maldegem (51°10′29″N, 3°28′10.45″E) and Neerijse (50°48′58.84″N, 4°38′2.62″E) in the lowland region and the upland region of Belgium respectively. These locations are approximately 110 km apart. Locations resemble each other in water conductivity, width, depth, and vegetation, but differ in species composition as the lowland area also harbours ninespine stickleback (*Pungitius pungitius*), whereas in the upland habitat this species is absent. While the upland population is limited to a single river drainage, the lowland population has connections to many water bodies as well as an indirect connection to the sea, which results in gene flow with anadromous individuals (Raeymaekers *et al.*, 2005). All individuals were members of an F1 generation derived from artificial crosses of the two respective populations and reared under standardized conditions. Fish were kept under a light regime of 14 h day/10 h night, a water temperature

of 17 °C, and were raised on infusoria (*Colipidium* sp.) from Day 1 to Day 4, *Artemia salina* until 2 months after hatching, and thawed chironomid larvae from then onwards. For each of the two stickleback populations, a total of eight independent F1 families were used in the experiments, performed in September 2010 (Experiment 1), February 2011 (Experiment 2), and September 2011 (Experiment 3). Two weeks before each experiment, source fish infected with the flatworm parasite *Gyrodactylus gasterostei* were captured from the two host origin locations with hand nets and kept on their original host for 2 weeks at 17°C, causing rapid population growth of the parasites. Parasites from a random subset of these fish were preserved on glass slides for species confirmation.

Infection experiments

The three experiments were performed 3 months (Experiment 1), 8 months (Experiment 2), and 15 months (Experiment 3) after hatching. Experiments 1 and 2 were performed on hosts that had yet to reach the reproductive state (i.e. sub-adult fish). The first experiment tested the infection rate of the upland parasite on both hosts and the second experiment tested the infection rate of the lowland parasite on both hosts. These experiments were conducted separately because of housing capacity and available manpower constraints and therefore are not strictly reciprocal. They were both done under similar conditions and with fish that had not yet reached the adult stage. We nevertheless analysed all experiments separately to circumvent inappropriate pooling. In the third experiment, fish that had passed the reproductive stage (i.e. adult fish) were infected. For this experiment, infection rates of both parasite strains were monitored in parallel so the design was strictly reciprocal. The sample size was 100 individuals ($n = 50$ per host population split over 7 families each), 62 individuals ($n = 31$ per host population split over 4 families each), and 80 individuals ($n = 20$ per host population split over 6 families each) for the first, second, and third experiment, respectively. Overall, we used individuals of 8 families per population (most families thus being replicated over experiments). All individual fish were used only once.

One week before infection, individual fish were transferred to 2-litre containers filled with de-chlorinated water at 11°C to ensure optimal growth conditions of the parasite for the experiment (Cable *et al.*, 2002; de Roij *et al.*, 2011; Raeymaekers *et al.*, 2011). Water was changed weekly. Feeding consisted of adding thawed mosquito larvae to the tanks three times a week. This diet kept the fish on a constant weight while ensuring there was no overfeeding that might conceal effects of the infection (Barber *et al.*, 2008). Weight and standard length were recorded both before and after the experiment.

Fish were infected with an individual parasite, harvested from infected fish collected in the field. We collected a parasite from the infected host with a needle, held the needle close to the new experimental host that had been tranquillized with MS-222 ($0.1\ g \cdot L^{-1}$) to allow parasite transfer to the fins of its new host, before returning the new host to the 2-litre container. Infection success was verified one week later. Parasite loss due to suboptimal experimental conditions versus host resistance is difficult to distinguish. The experimental infection was thus repeated if the parasite was absent one week after infection. Fish that did not retain any parasites after this second infection were scored as resistant to infection.

Parasite numbers on each fish were recorded once per week for six consecutive weeks. After this period, fish were euthanized with an overdose of MS-222 ($2\ g \cdot L^{-1}$), fin clipped, weighed, measured, and photographed.

Data analysis

Resistance among families was measured as the percentage of individuals with the capacity to dispose of the parasite twice one week after infection. Re-infection and resistance frequencies were compared among groups with a Pearson χ^2-test. A general linear model (GLM) was designed in the software package STATISTICA v.11.0 (StatSoft, 2012) for each experiment separately, with population as a fixed factor and family (nested within population) as a random factor for Experiments 1 and 2. The GLM for Experiment 3 also tested for parasite origin and the host population × parasite origin interaction effect. The dependent variable 'total worm load' was calculated as the total number of parasites at 5 weeks, which was the average peak of infection. Total worm load was log transformed to fulfil the criteria of normality. All GLMs were performed with and without resistant individuals to segregate effects of infection intensity and resistance. Finally, we calculated the effect size of local adaptation (E), using the equation formulated by Rosenberg *et al.* (2000) to compare our results with previous findings (Hoeksema and Forde, 2008) and to give a quantitative measure of local adaptation. E is calculated as the log response ratio of sympatric to allopatric performance:

$$E = \ln (X_S \cdot X_A^{-1}),$$

where X_S is the mean performance of the parasite in sympatric pairings and X_A is the mean performance of the parasite in allopatric pairings. A positive value for E represents local adaptation of the parasite to its host, while a negative value represents maladaptation.

RESULTS

Across all experiments, 13.8% of the upland hosts and 7.6% of the lowland hosts were resistant against the infection. Resistance did not differ among life stages ($\chi^2 = 0.547$, d.f. = 1, $P = 0.46$), parasite origin ($\chi^2 = 0.120$, d.f. = 1, $P = 0.73$), or experiments ($\chi^2 = 1.167$, d.f. = 3, $P = 0.76$). However, in sub-adults, resistance was higher in the upland host population than in the lowland host population. This was true for both the upland parasite ($\chi^2 = 4.107$, d.f. = 1, $P = 0.04$) and the lowland parasite ($\chi^2 = 6.495$, d.f. = 1, $P = 0.01$). In general, adult individuals were more resistant than sub-adult individuals, but this was not significant. This might be due to the low rate of resistant adult upland hosts infected with lowland parasites (see Table 1).

The infection patterns observed were very similar to those previously reported in *Gyrodactylus* infection experiments (Bakke *et al.*, 2002; Boeger *et al.*, 2005; de Roij *et al.*, 2011; Raeymaekers

Table 1. Frequency of individuals that were resistant after infection with either the upland or lowland parasite

	Parasite, upland			Parasite, lowland		
	n	Resistant individuals	S.E.	*n*	Resistant individuals	S.E.
Host, upland sub-adult	51	0.18	0.053	27	0.26	0.084
Host, lowland sub-adult	45	0.04	0.031	32	0.03	0.031
Host, upland adult	19	0.32	0.107	20	0.10	0.067
Host, lowland adult	20	0.11	0.070	17	0.12	0.078

et al., 2011), with in general an initial growth phase, a peak of infection (in this case around 5 weeks after the start of the experiment), and a rapid decline in the week after this peak.

In Experiment 1 (sub-adult fish, upland parasite) and Experiment 2 (sub-adult fish, lowland parasite), there was a similar significant host effect (Table 2; Fig. 2). In both cases, fish from the lowland population had a significantly higher total worm load than fish from the upland population. This effect was consistent among families. In contrast to Experiment 2, host populations did not differ significantly from one another in Experiment 3 (Table 2; Fig. 2). Furthermore, there was a significant family effect, while there was no parasite or host population × parasite origin interaction effect (Table 2). Analyses were completed with and without resistant individuals included. Analysis without resistant individuals only altered one result, a significant family effect observed in sub-adults infected with upland parasites (group A in Table 2 and Fig. 2). All results given include resistant fish in the analysis.

The effect size of local adaptation was $E = -0.002$ and $E = -0.149$ for the sub-adult and adult life stage respectively, suggesting that parasites are not adapted to their local host populations in sub-adult fish and might be slightly maladapted to their host in the adult stage. The effect size of the adult stage is not very big, however, and we conclude from Fig. 2c that this would only apply to the upland parasites.

Table 2. GLM table of log-transformed total worm load on (A) sub-adult hosts infected with parasites of upland origin, (B) sub-adult hosts infected with parasites of lowland origin, (C) adult hosts infected with parasites of either upland or lowland origin

Effect	SS	$d.f._{effect}$	MS_{effect}	$d.f._{error}$	MS_{error}	F	P
(A) *Sub-adult hosts infected with parasites of upland origin*							
Intercept	108.38	1	108.32	12.52	0.48	225.63	**< 0.0000**
Population	5.78	1	5.78	12.52	0.48	12.03	**0.0044**
Family (Population)	5.86	12	0.49	82.00	0.27	1.79	0.0632
Error	22.35	82	0.27				
(B) *Sub-adult hosts infected with parasites of lowland origin*							
Intercept	65.07	1	65.07	6.62	0.09	746.86	**< 0.0000**
Population	5.46	1	5.46	6.63	0.09	62.71	**0.0001**
Family (Population)	0.50	6	0.08	51.00	0.33	0.25	0.9562
Error	16.99	51	0.33				
(C) *Adult hosts infected with parasites of either upland or lowland origin*							
Intercept	177.01	1	177.01	8.03	0.98	179.95	**< 0.0000**
Population	1.14	1	1.14	8.03	0.98	1.16	0.3127
Family (Population)	7.91	8	0.99	64.00	0.28	3.56	**0.0018**
Parasite	0.14	1	0.14	64.00	0.28	0.50	0.4837
Parasite × Population	0.11	1	0.11	64.00	0.28	0.38	0.5378
Error	17.75	64	0.28				

Note: Family (nested within population) was included as a random effect. Significant *P*-values are in **bold**. SS = sum of squares, $d.f._{effect}$ = degrees of freedom for each individual predictor variable added, MS_{effect} = mean square for the model, $d.f._{error}$ = corrected degrees of freedom – $d.f._{effect}$, MS_{error} = mean square for the error.

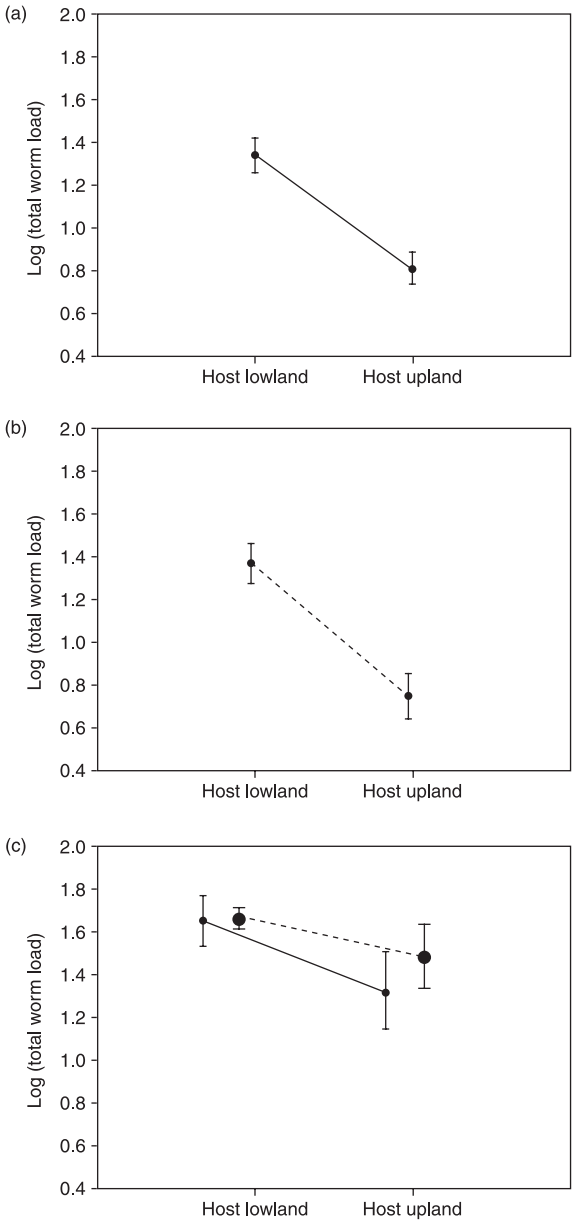

Fig. 2. Log-transformed total worm load in the three experiments. (a) Sub-adult fish infected with parasites of upland origin (solid line). (b) Sub-adult fish infected with parasites of lowland origin (dashed line). (c) Adult fish infected with either parasites of upland (solid line) or lowland origin (dashed line). Error bars depict standard errors.

DISCUSSION

When sub-adult threespine stickleback were challenged with the parasite *Gyrodactylus gasterostei* over a 6-week period, total worm load strongly differed between the lowland and upland populations. This effect was observed regardless of parasite origin. Experiments with the upland (Experiment 1) and lowland parasite (Experiment 2) generated similar results and resemble the scenario in Fig. 1c. We suspect this is due to differential immune resistance between the sub-adult host populations. A third experiment testing both parasite origins simultaneously on adult fish rather resembled the scenario in Fig. 1a, but confirmed that the infection success of the parasite does not depend on whether it occurred in sympatry or in allopatry with the host. Therefore, our data do not support a general pattern of local adaptation of the parasite (scenario in Fig. 1e). As parasite origin does not have an effect on the worm load, we assume there are very limited differences in parasite infectivity across locations. Instead, worm load seems to be affected by life stage, as the results of Experiment 3 indicate that the more resistant upland population loses its immunological advantage at the reproductive stage. The effect size of local adaptation was slightly negative, indicating no adaptation of parasites in Experiments 1 and 2 and slight maladaptation in Experiment 3. Both effect sizes were well within the range reported previously (Hoeksema and Forde, 2008).

Local adaptation of the parasite might be absent for several reasons. Absolute gene flow among *Gyrodactylus gasterostei* populations might be low, limiting the genetic variation on which local selection can act (Gandon *et al.*, 1996; Gandon, 2002). High gene flow might also hinder local adaptation by the inflow of maladapted genes (Gandon *et al.*, 1996; Gandon, 2002; Gandon and Michalakis, 2002; Garant *et al.*, 2007). Characterizing the parasite population's genetic structure could address both the role of low versus high parasite gene flow as well as host–parasite relative gene flow, supposedly the strongest predictor of local adaptation (Hoeksema and Forde, 2008).

We did not observe local adaptation of the host (scenario depicted in Fig. 1f) either. Fitness costs of infection may not be sufficiently strong to evoke host adaptation in our system. *Gyrodactylus* infections resulted in major health costs for Atlantic salmon *Salmo salar* and Arctic charr *Salvelinus alpinus*, leading to loss of fish stock (Bakke *et al.*, 1992; Bakke and Harris, 1998; Winger *et al.*, 2008). *Gyrodactylus* worms also trigger immunological responses (Lester, 1972). However, fitness costs in *Gasterosteus aculeatus* are likely less severe than in Atlantic salmon or Arctic charr. *Gasterosteus aculeatus* infection is rarely fatal in experimental studies (de Roij *et al.*, 2011; Raeymaekers *et al.*, 2011; present study). Eizaguirre *et al.* (2012) did show that over 10 months and under natural conditions stickleback weight can be affected by *Gyrodactylus* sp. when individuals do not have parasite-adapted MHC genes. These genes vary among populations and affect resistance against *Gyrodactylus* (Eizaguirre *et al.*, 2009, 2011). We did not find an effect of parasite infection on fish weight (results not shown), but this could be due to the short time span between measurements. The lack of severe fitness costs might, however, prevent local adaptation by the host. Virulence has not been one of the best predictors for local adaptation, but might be important in creating selective pressure nevertheless (Greischar and Koskella, 2007).

A number of non-mutually exclusive processes might explain the lower infection rates in the upland population than in the lowland population. Encounter rates between hosts and *Gyrodactylus gasterostei* parasites may be higher in the upland population if there is increased parasite prevalence or host density, increasing *Gyrodactylus*-mediated selection

and immunogenetic adaptation. Alternatively, gene flow in the lowland host population might constrain adaptive divergence (Hendry *et al.*, 2002). In our system, the lowland population is connected to many other water bodies and migrating fish populations, which results in high gene flow (Raeymaekers *et al.*, 2012). This could explain why the lowland host population suffers from a higher worm load than the upland population. However, Roth *et al.* (2012a) showed host immune adaptation of *Syngnathus typhie* (pipefish) to its associated bacteria from the genus *vibrio*, even with high gene flow among hosts. To understand the impact of gene flow in the host, we need to increase the number of populations with high and low gene flow.

Another possibility is that immune resistance in the lowland population trades off with other fitness-related traits due to pleiotropic effects or energy constraints. In this case, other ecological variables that differ between these populations might cause selection on other fitness-related traits (Lochmiller and Deerenberg, 2000), such as growth (Soler *et al.*, 2003), predation-related traits (Cotter *et al.*, 2004), and reproduction (Macnab *et al.*, 2009). Such trade-offs could limit energy invested in immune resistance (Zuk and Stoehr, 2002; Birrer *et al.*, 2012). Differences in immune resistance may also result from genetic hitchhiking, where selection for genes related to other traits are genetically linked to immune genes and inhibit an optimal immune defence (van Oosterhout, 2009). It is possible but unlikely that one population was better adapted to our experimental setting. Both populations had been in the laboratory for an equal period of time and the fish in the experiment were raised in the laboratory from fertilization onwards. Possible differential parental effects that have been shown to play a role in immune resistance (e.g. Sadd and Schmid-Hempel, 2007; Hasselquist and Nilsson, 2009; Roth *et al.*, 2012b; Zhang *et al.*, 2013) cannot be excluded as we used F1 individuals.

Repeated experiments in different life stages lead to the conclusion that immune resistance is not stable across life stages. Previous studies indicated that freshwater fish experience decreased immune function after the reproductive stage in experimental settings (Poulin, 1993). This could be explained by the fact that (1) at this stage investment in immunity is of less importance to the individual, (2) there is a trade-off between immune resistance and reproduction (Macnab *et al.*, 2009), or (3) that experimental conditions are not representative of natural conditions where individuals would have a lower life expectancy. Another explanation for our findings could be that instead of measuring a change between life stages, we are measuring a change due to, for example, feeding regime. This variable is hard to keep constant for fish of different sizes and might change in importance between life stages. Food intake may mask the effects on immune resistance (Barber *et al.*, 2008) and thus we should be careful when interpreting this life stage variation. Sample size in Experiment 3 might also have been too small to discern subtle differences among host populations. Ramirez *et al.* (2012) show with an agent-based model that susceptibility loci present in only 10–30% of the population cannot be detected with sample sizes of 20–30 fish per factor combination. However, we did find differences in sub-adult fish with similar sample sizes (i.e. Experiment 2), and did a power analysis to circumvent Type II errors in Experiment 3 by bootstrapping the dataset of the first two experiments (results not shown). We therefore find it unlikely our sample size was too small to detect host differentiation. Resistance was stable across life stages, suggesting that resistance and infectivity are not regulated by the same genes or are not affected in the same way across life stages.

CONCLUSION

Although there are striking examples of local adaptation to parasites (Ballabeni and Ward, 1993), we highlight the importance of carefully interpreting suggestive evidence of one-way tests of local adaptation and show that in the *Gasterosteus–Gyrodactylus* example differential host immune resistance may instead explain differential accumulation of parasites. To explain why local adaptation appears lacking, we must understand the strength of selection and the population genetic structure of both parasite and host. To understand why immune resistance is different among populations, we have to (1) rule out adaptation to the laboratory with extra replication of populations incorporated in a full factorial design, (2) test for possible trade-offs or genetic linkage with other important fitness-related traits, and (3) rule out the effects of drift and inbreeding. Finally, to understand why immune resistance changes with life stage, we need to test infection rates in more natural settings such as reciprocal transplant experiments. These present technical challenges, but Nuismer and Gandon (2008) suggested improved experimental designs for these kind of experiments so that adaptation to the environment ($G \times E$), adaptation to the genotype frequency distribution of the interacting species ($G \times G$), and joint adaptation to the fitness consequences of interactions and the genotype frequency distribution of the interacting species ($G \times G \times E$), can be separated and compared with the results of common garden experiments.

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