

Divergent resistance to a monogenean flatworm among three-spined stickleback populations

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Summary

1. Given their ubiquity, we might expect parasites to play an important role in the adaptive divergence of host populations. Specifically, adaptation to local parasite communities is predicted to influence the evolution of a number of host traits such as parasite resistance.
2. To investigate the possibility that divergent parasite-mediated selection drives population-level variation in parasite resistance, we artificially infected lab-reared three-spined sticklebacks with the monogenean flatworm *Gyrodactylus gasterostei*. The fish were derived from five populations from North Uist, Scotland, that were chosen because they differed in natural infection levels of *Gyrodactylus arcuatus*.
3. We found substantial differences in resistance to *G. gasterostei* among populations. Resistance was defined largely by the ability to limit the size of the worm population rather than by the timing of the host response.
4. Experimental resistance was not significantly correlated with natural infection levels of *G. arcuatus*. However, in general, populations with greater exposure to *G. arcuatus* were shown to be more resistant to *G. gasterostei*. Fish from the only naturally unexposed population showed the highest susceptibility, which may be the result of less selection to maintain resistance.
5. Taken together, these results suggest that the divergent selection mediated by *Gyrodactylus* may play a role in driving population-level variation in resistance to this parasite.

Key-words: *Gyrodactylus gasterostei*, natural infection, parasite-mediated selection, parasite resistance, parasite tolerance

Introduction

Spatial variation in traits related to fitness is thought to be a consequence of adaptation to local ecological conditions (Schluter 2000). Divergence in ecologically relevant traits may be driven by a number of selective factors (Rundle & Nosil 2005). The role of competition (Schluter 1994; Pfennig *et al.* 2007) and predation (Reznick & Endler 1982; Nosil & Crespi 2006) have been well studied in this context, but the influence of parasites on this process has been comparatively neglected (but see e.g. Buckling & Rainey 2002; Laine 2009). Divergent parasite-mediated selection may affect the evolution of many host life history traits (Møller 1997; Fredensborg & Poulin 2006), but defence traits are likely to be under the strongest selection, as they determine parasite resistance. All else being equal, directional selection should drive alleles coding for resistance to fixation and erode

genetic variation (Mousseau & Roff 1987; Houle 1992). However, extensive additive genetic variation in parasite resistance within natural populations is commonplace (Henter & Via 1995; Ebert, Zschokke-Rohringer & Carius 1998; Uller, Olsson & Madsen 2003; Jackson & Tinsley 2005). This diversity may be maintained via several mechanisms, including: (i) negative frequency-dependent selection (Carius, Little & Ebert 2001; Koskella & Lively 2009), (ii) costs of resistance (Sheldon & Verhulst 1996; Rigby, Hechinger & Stevens 2002), (iii) fluctuating selection associated with environmental heterogeneity (Blanford *et al.* 2003; Lazzaro & Little 2009), (iv) heterozygote advantage (MacDougall-Shackleton *et al.* 2005) and (v) sexual selection (Hamilton & Zuk 1982; Penn & Potts 1999).

An important aspect of host–parasite interactions that governs the evolution and maintenance of parasite resistance among populations is spatial variation in parasite distributions. The magnitude of parasite-mediated selection may be determined not only by the prevalence of certain parasite

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species, but also by their abundance. There are two possible outcomes of divergent parasite-mediated selection in terms of parasite resistance. On the one hand, populations at greater risk of infection should be under stronger selection to evolve resistance, which should lead to a positive correlation between natural parasite distributions and investment in parasite resistance. On the other hand, resistant populations may keep parasite abundance and/or prevalence at a low level, which should generate a negative correlation between parasite distribution and parasite resistance. The few studies that have been conducted in this context provide support for the former scenario. For example, Bryan-Walker, Leung & Poulin (2007) compared resistance to a trematode parasite, *Mariotrema novaezealandensis*, in two populations of amphipods, *Paracalliope novizealandiae*, and found that the population not exposed to the parasite had significantly lower resistance in an infection experiment. Likewise, albeit at the level of parasite diversity, Corby-Harris & Promislow (2008) showed that natural bacterial species richness was positively associated with resistance to a bacterial species, *Lactococcus lactis* across 20 natural populations of *Drosophila melanogaster*. Cable & van Oosterhout (2007a) documented the opposite pattern for two Trinidadian guppy (*Poecilia reticulata*) populations infected with a laboratory strain of the monogenean flatworm *Gyrodactylus turnbulli*: the population naturally exposed to higher *Gyrodactylus* burdens was significantly more susceptible. However, with the exception of these and a few other studies (e.g. Little & Ebert 2000; Kalbe & Kurtz 2006; Hasu, Benesh & Valtonen 2009), our understanding of patterns of divergence in parasite resistance among natural populations and their relationship to infection levels in the wild remains limited, especially for vertebrate-macroparasite interactions.

Artificial infection experiments involving outbred individuals from a number of natural populations allow us to test the prediction that divergent parasite-mediated selection drives divergent parasite resistance. Here, using lab-reared individuals from five populations of three-spined sticklebacks, *Gasterosteus aculeatus* (Fig. 1), we investigate divergence in resistance to *Gyrodactylus gasterostei*, a monogenean flatworm. Sticklebacks are a well-established model for the study of ecologically-based divergent selection (McKinnon & Rundle 2002). In the last 10 000 years marine sticklebacks have repeatedly invaded freshwater environments and as a result have undergone rapid evolution in a number of traits. Morphological divergence stemming from these invasion



Fig. 1. The study organism, the three-spined stickleback, *Gasterosteus aculeatus*.

events has been particularly well characterized (Colosimo *et al.* 2005), but considerable variation in life history (Baker *et al.* 2008) and behavioural (Boughman 2001) traits among populations has also been documented. There is a growing interest in understanding how host–parasite interactions fit into this context (MacColl 2009a). Sticklebacks have a diverse and well-documented parasite fauna (Wootton 1976; Barber 2007) and therefore constitute a model species for investigating divergent parasite-mediated selection.

Gyrodactylids form a dominant component of many stickleback parasite communities (e.g. Kalbe, Wegner & Reusch 2002; MacColl 2009b). *Gyrodactylus* spp. are viviparous and lack a specific transmission stage. As worms reproduce directly on the host and have short generation times (2–4 days; Bakke, Cable & Harris 2007), population growth can be exponential, often leading to host mortality. Furthermore, *Gyrodactylus* is known to affect mate-choice and courtship behaviours (Houde & Torio 1992; Lopez 1998). Considering their detrimental effects on host fitness, gyrodactylids are likely to exert strong selection on hosts. Most of our knowledge of gyrodactylid–host interactions comes from the *G. salaris*–salmon (*Salmo salar*) and the *G. turnbulli*–guppy systems. In both cases, there is evidence for geographic variation in parasite resistance. For example, van Oosterhout, Harris & Cable (2003) and Cable & van Oosterhout (2007b) showed that resistance to *G. turnbulli* varied among two populations of Trinidadian guppies. Likewise, different genetic stocks of Atlantic salmon differ in their ability to resist *G. salaris* (Bakke, Jansen & Hansen 1990; Dalgaard, Nielsen & Buchmann 2003). However, pathogenicity may vary widely among gyrodactylid species (Bakke, Cable & Harris 2007) and the extent to which different species drive divergence in parasite resistance remains unknown. Although we have some knowledge of the biology (Glaser 1974; Harris 1982) and population dynamics (Raeymaekers *et al.* 2008) of *G. gasterostei*, this study sought to shed light on pathogenicity and infection dynamics of *G. gasterostei*, and spatial variation in the host response to this parasite species.

The objectives of the study were fourfold: first, to look for differences in natural *Gyrodactylus* abundance among the five stickleback populations; second, to examine variation in resistance to *G. gasterostei* among these populations by carrying out an artificial infection experiment; third, to determine whether this variation is related to natural infection level; and fourth, to look for an association between *G. gasterostei* infection and stickleback growth. This last question was of interest because it has recently been suggested that *Gyrodactylus* may affect host life history evolution in terms of growth, at least in guppies (Cable & van Oosterhout 2007a). Therefore, we were motivated to examine the relationship between parasite resistance and growth. We stress that *Gyrodactylus arcuatus*, not *G. gasterostei*, is the native *Gyrodactylus* species in our stickleback populations. A different *Gyrodactylus* species was chosen for the infection experiment because it removed the possibility of close coevolution between host and parasite populations. However, as there may be sufficient overlap in the host response to both parasite species, it still allowed us to

make general inferences about the evolution of resistance to *Gyrodactylus*. Overall, we found substantial differences in *G. gasterostei* resistance among populations which were partially correlated with natural *G. arcuatus* abundance.

Materials and methods

STUDY POPULATIONS AND PARASITES

All work was conducted under licence from the UK Home Office and with the approval of the University of Nottingham Ethical Review Committee. Fish were collected from five geographically isolated lochs on North Uist, Outer Hebrides, Scotland during May 2008. These lochs were chosen specifically to represent a range of natural *Gyrodactylus* infection levels (Table 1). Abundance and prevalence of *G. arcuatus*, the native *Gyrodactylus* species of three-spined sticklebacks on North Uist, were estimated by sampling approximately 10–20 fish per loch in May 2008. For each population, F₁ offspring used in infection experiments were obtained by making eight unrelated full-sib crosses from wild-caught fish. To make a cross, eggs were stripped from a gravid female and placed into a petri dish containing a small volume of 1‰ salt solution. Males were killed, by overdose of anaesthetic (400 mg L⁻¹ MS222), and were dissected to remove testes. Fine forceps were used to tease apart testes and release sperm, which was gently mixed with the eggs (Barber & Arnott 2000). Two to three hours later, fertilization was confirmed by low-power microscopy, and testes were removed from the fertilized clutches. Fertilized eggs were transferred to a falcon tube containing 50 mL of 1‰ salt solution. Eggs were then transported on ice to aquaria at the University of Nottingham, where they were placed in a plastic cup with a mesh screen on the bottom suspended in a well-aerated tank containing dechlorinated water (e.g. Marchinko & Schluter 2007). Water was treated with Methylene blue to reduce the possibility of fungal infection. After 10 days, egg cups were transferred to individual half-tank partitions of 100 L tanks and the eggs were allowed to hatch. Following hatching, full-sib families were thinned to groups of 15. Clutches from each population were distributed haphazardly between tanks across the temperature-controlled room (13.5 ± 1 °C). Fry were fed with infusoria (*Colpidium* spp.) for the first five days, then daily with brine shrimp (*Artemia salina*) naupliae until 64 days post-hatching. Thereafter, fish received chironomid larvae ('bloodworm'; defrosted from frozen) daily. Fish were maintained at a daylight regime mimicking the natural photoperiod on North Uist.

Gyrodactylus gasterostei is a common fin parasite (Glaser 1974; Harris 1985) of three-spined sticklebacks. Sticklebacks infected with *G. gasterostei* were caught from a stream in Clifton, Nottingham (52°55'N; 1°10'W) at the end of February, two weeks prior to the start of the experimental infection. Gyrodactylids were identified as *G. gasterostei* by confirming the absence of excretory bladders, a defining anatomical feature of this *Gyrodactylus* species, under a dis-

secting microscope. Donor fish were housed in groups of 16–20 to encourage growth of parasite populations.

EXPERIMENTAL DESIGN

In total, 150 ten-month-old sticklebacks were exposed to *G. gasterostei*: 30 fish per population balanced for logistic purposes across two blocks. For each population, fish from eight full-sib families were included, with the exception of Hosta, for which only seven families were available. Families were also balanced across both blocks, such that at least one fish per family was included in both blocks. Fish were housed individually in a 3-L tank containing one litre of dechlorinated tap water. This enabled the infection profile of each fish to be monitored accurately. Water temperature was maintained at 12 °C (± 0.5 °C), a well-established temperature for carrying out gyrodactylid infections (Bakke, Harris & Cable 2002). Populations were distributed equally across the room to balance any microclimatic effects on parasite population growth.

INFECTION PROTOCOL

Donor fish, selected randomly from the infected fish population, were killed with an overdose of MS222 (400 mg L⁻¹) and placed in a Petri dish containing a small amount of dechlorinated water. *Gyrodactylus* worms were removed using insect pins. Prior to infection, the standard length of each recipient fish was measured to the nearest 0.1 mm. Infection of naive, lab-bred fish was achieved by holding the caudal fin of a lightly anaesthetized (MS222, 100 mg L⁻¹) experimental fish near two previously isolated *Gyrodactylus* until the worms moved onto the fin. Generally, this process was extremely rapid: most worms transferred within 5–10 s. On the day following infection, each fish was scanned carefully using a binocular microscope to determine establishment success. If a fish had lost both parasites, it was immediately re-infected with two new worms from another randomly selected donor fish. Fish from different populations were infected in a sequential order such that all populations were exposed as uniformly as possible to worms from each donor fish, minimizing any variation in infection response profiles due to worm origin.

Starting on day 4, the number of parasites on each fish was counted every four days until the end of the experimental period (day 62), by which time all but 10 fish had lost the infection. Monitoring parasite levels involved careful scanning of the caudal, anal, dorsal and pectoral fins as well as the dorsal spines, pelvic spines and girdle, caudal peduncle, flanks and head. Both stereomicroscopic and sub-stage illumination were used to accurately determine the number of *Gyrodactylus*. Prior to scanning, fish were lightly anaesthetized. On day 62, fish were killed by overdose of MS222, measured as before (standard length to the nearest 0.1 mm) and sexed by dissection. Water was changed every 4 days and fish were fed to satiation once per day with bloodworm, defrosted from frozen. Throughout the

Table 1. The five stickleback populations from North Uist, Scotland used in the study. Data on *Gyrodactylus arcuatus* prevalence and abundance were obtained by sampling approximately 20 sticklebacks from each loch in May 2008. Abundance on individual fish was quantified by counting the number of worms on the caudal, anal and dorsal fins

Population	<i>G. arcuatus</i> abundance	<i>G. arcuatus</i> prevalence	<i>N</i>	Geographic location
Chadha Ruaidh	0	0 (0.0, 15.9)	21	57°35'N; 7°11'W
Hosta	2.45 ± 0.51	90.0 (68.0, 98.2)	20	57°37'N; 7°29'W
Lochmaddy	12.63 ± 2.42	100.0 (86.1, 100.0)	24	57°36'N; 7°10'W
Reivil	6.27 ± 3.25	90.9 (59.6, 99.5)	11	57°36'N; 7°30'W
Tormasad	0.10 ± 0.07	10.0 (1.8, 32.0)	20	57°33'N; 7°19'W

Abundance values are given with standard error of the mean, whereas prevalence values are given with 95% confidence intervals (in brackets).

infection experiment, if fish were in a state of poor health, here defined as the cessation of feeding and reduction in movement, they were euthanized by overdose of MS222 and destruction of the brain, as required by conditions of the experimental licence. For the purposes of the experiment, this was defined as mortality. By the end of the 62-day period, 13 fish had been euthanized, mostly because of infection with the secondary, opportunistic fungus of the genus *Saprolegnia*, which normally results in death of the fish once the fungus becomes visible (Pickering & Willoughby 1982).

STATISTICAL ANALYSIS

All statistical analyses were conducted in GENSTAT (release 12; VSN International Ltd, Hemel Hempstead, UK). For all models, parameter estimates are given for significant terms only. Natural abundance of *G. arcuatus* was modelled using a generalized linear model (GLM) with a negative binomial error distribution and logit link function. 'Population' was the only fixed effect in this model.

The *Gyrodactylus* infection process is highly dynamic and is characterized by a standard sequence of events: the parasite population grows, reaches a peak, then starts to decline and is eventually cleared by the host (Fig. 2). Where possible, five response variables were extracted from the infection response profiles of individual fish. 'Peak' was the parasite abundance at the peak of the infection. 'AUC' was the sum of worm abundances counted throughout the experiment, a measure equivalent to total worm burden over the infection time course. 'Average r to peak', the average daily growth rate of the parasite population, was calculated as: $r = [\ln(N_{\text{peak}}) - \ln(N_0)]/t$, where N_{peak} = 'peak', N_0 is the number of parasites on the host at the start of the infection (two worms), and t is the day at which the peak was reached (i.e. 'time until peak'). 'Time lost post-peak' was the time until clearance following the peak. For those fish that had not lost the infection by day 62, this day was noted as the day of clearance.

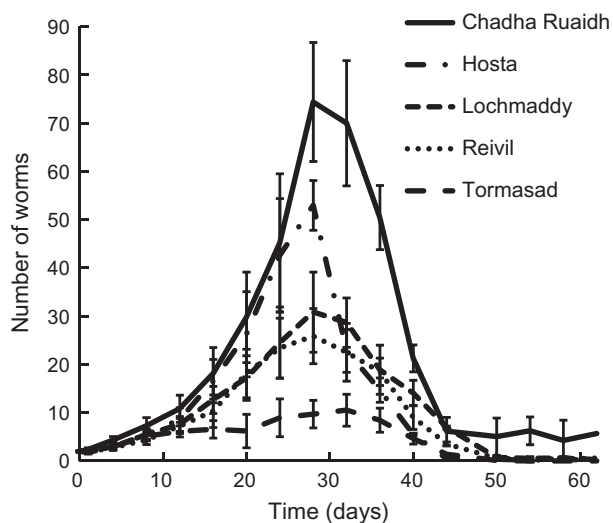


Fig. 2. Mean infection response profile of all five stickleback populations across the 62-day experimental period. Parasite abundance of *Gyrodactylus gasterostei* on individual fish was counted every 4 days, following artificial infection with two *G. gasterostei* on Day 0. Data from fish that died were included until the time of death. Error bars represent standard errors of the mean number of worms per fish at each time point.

To reduce the number of response variables to two explanatory variables that captured the most variation in infection response profiles, a principal components analysis (PCA) was conducted on the five response variables. The resulting scores of principal component 1 (PC1) and principal component 2 (PC2) were used to make inferences about variation in resistance to *G. gasterostei*. Although this approach obscures interesting and subtle differences in temporal aspects of each infection response profile, single measures of resistance provide a more rigorous basis for statistical analysis. Due to the strong effect of AUC and peak on PC1 scores (see Results), models with these two response variable generated qualitatively similar results to those using PC1. Nevertheless, we chose to use PC1 because it gave the best objective descriptor of the dynamics of the infection. It was not possible to calculate PC scores for those fish (13) missing values of any of the response variables due to mortality.

GLMs with a binomial distribution and a logit link function were used to model parasite establishment and host mortality data. The binary response variable, 'established?' took a value of 1 if the two parasites remained on the fish the first day following infection and 0 if they did not. Likewise, the response variable 'died?' took a value of 1 if fish died during the experimental period and 0 if fish survived. Full models included block, sex, and population as fixed effects and initial length as a covariate. For the mortality model, an additional effect, 'daily r ', describing average daily growth rate of the parasite population up until the point of death or until the peak of the infection, was included to examine whether mortality was associated with infection levels. Similarly, a daily $r \times$ population term was fitted to assess whether this relationship was consistent across populations. Significance of fixed effects was assessed by comparing the change in deviance upon dropping the effect to a chi-square distribution with the appropriate degrees of freedom. Additionally, the relationship between mortality and *G. gasterostei* resistance at the population level was examined by Pearson correlation.

Variation in infection response profiles was analysed using a general linear model, with PC1 or PC2 score as the response variable. To achieve normality, PC1 and PC2 scores were log and square-root transformed respectively. The model comprised the fixed effects block, sex, population, the covariate initial length and the initial length \times population interaction. Family nested within population was initially included as a random effect; however, the term was non-significant for both PC1 and PC2 models (likelihood ratio test based on comparing the deviance of the reduced model without the random interaction term and the deviance of the full model (Galwey 2006) – PC1: $\chi^2_1 = 0.78$, $P = 0.19$; PC2: $\chi^2_1 = 0.37$, $P = 0.27$). Therefore, a general linear model rather than a linear mixed model (LMM) was used. Stepwise regression was used to construct a minimal adequate model by sequentially dropping non-significant explanatory terms (*sensu* Crawley 2007). If main effects were marginal to interaction terms, significance of main effects was tested in the presence of interactions. *Post-hoc* contrasts on population PC1 score means were used to examine differences among populations in more detail. The correlation between mean population (log-transformed) PC1 scores and natural abundance of *G. arcuatus* was tested using a Pearson correlation.

To examine if there was variation in fish growth rate, and a relationship between parasite resistance and fish growth, a LMM was used. Specific growth rate (SGR), the average daily percentage increase in fish length, was calculated using the equation: $SGR = 100 * [\ln(L_{62}) - \ln(L_0)] / 62$, where L_0 and L_{62} denote the length measured before, and at the end of, the experimental infection, respectively. SGR was square-root transformed to achieve normality. The full model consisted of family nested within population as a random

effect and block, sex, initial length, population, PC1 score (untransformed) and population \times PC1 as fixed effects. Again, non-significant explanatory terms were dropped from the full model to generate a minimum adequate model. Significance of fixed effects was determined by Wald F -tests.

Results

NATURAL *GYRODACTYLUS ARCUATUS* INFECTION LEVELS

Natural *G. arcuatus* abundance varied significantly among populations ($\chi^2_4 = 54.98$, $P < 0.001$; Table 1). *G. arcuatus* appeared to be absent from one population, Chadha Ruaidh.

PARASITE ESTABLISHMENT AND HOST MORTALITY IN THE INFECTION EXPERIMENT

Eight fish lost their infection within 24 h; however, all eight fish were successfully re-infected the following day. *G. gasterosteii* establishment did not vary significantly among populations ($\chi^2_4 = 5.00$, $P = 0.288$). It also did not differ significantly between males and females ($\chi^2_1 = 0.01$, $P = 0.931$), blocks ($\chi^2_1 = 0.00$, $P = 0.991$) or as a result of variation in fish length ($\chi^2_1 = 0.00$, $P = 0.956$). Although there was some small-scale variation in mortality among populations (number of deaths per population: Chadha Ruaidh, 5, Hosta, 2, Lochmaddy, 1, Reivil, 3, Tormasad, 2), the effect of population was not significant ($\chi^2_4 = 3.99$, $P = 0.407$). Likewise, mortality was not dependent on the average daily growth rate of the parasite population (daily r : $\chi^2_1 = 0.11$, $P = 0.745$) or its interaction with population ($\chi^2_4 = 3.82$, $P = 0.431$). Sex ($\chi^2_1 = 0.32$, $P = 0.574$), length ($\chi^2_1 = 1.77$, $P = 0.184$) and block ($\chi^2_1 = 2.58$, $P = 0.108$) also failed to explain significant variation in host mortality. The relationship between mortality and population mean PC1 score was positive but not significant ($r = 0.69$, $P = 0.200$).

RESISTANCE TO *GYRODACTYLUS GASTEROSTEI*

Principal component 1 (PC1) explained 46.92% of the variation in infection response profiles and was determined mainly by the peak and AUC (Table 2). These two variables were strongly positively correlated ($r = 0.94$; Table 3). PC2

Table 2. Loadings from a principal components analysis (PCA) of five response variables, extracted from infection response profiles of individual fish. PC1 and PC2 accounted for 46.92% and 28.87% of variation in infection response profiles respectively

Response variable	PC1 loading	PC2 loading
AUC	0.638	0.004
Peak	0.630	0.027
Average r to peak	0.229	0.704
Time until peak	0.348	-0.647
Time lost post-peak	0.152	0.292

accounted for 28.87% of variation and was influenced largely by average r to peak and time until peak (Table 2), which were negatively correlated ($r = -0.41$; Table 3).

There was no significant difference in PC1 scores between the two experimental blocks, and therefore results from both blocks were pooled. Mean infection response profiles were markedly different for each population (Fig. 2) and PC1 scores varied significantly among populations (Table 4). Chadha Ruaidh, the only population not naturally exposed to *G. arcuatus*, had a significantly higher PC1 score than Reivil, Tormasad and Lochmaddy (contrast: $F_{1,122} = 20.04$, $P < 0.001$). Furthermore, Tormasad had a significantly lower PC1 score than any other population (contrast: $F_{1,122} = 27.55$, $P < 0.001$). There was no significant difference in PC1 score between Chadha Ruaidh and Hosta (contrast: $F_{1,122} = 0.36$, $P = 0.552$). Initial length was negatively correlated with PC1 score across populations, but there was no significant difference in PC1 score between males and females (Table 4). Mean population PC1 score was weakly negatively correlated with natural *G. arcuatus* abundance rank score, although this relationship was not significant ($r = -0.23$, $P = 0.701$; Fig. 3). PC2 scores were significantly affected by block, being marginally higher in Block 2 than Block 1, but not by sex, population, initial length and initial length \times population (Table 4).

ASSOCIATIONS BETWEEN *GYRODACTYLUS GASTEROSTEI* RESISTANCE AND FISH GROWTH

Specific growth rate varied significantly among families (likelihood ratio test of family \times population random effect: $\chi^2_1 = 11.84$, $P < 0.001$). There was no significant effect of block on SGR ($F_{1,96} = 0.78$, $P = 0.379$); hence results from both blocks were pooled. PC1 score did not explain significant variation in SGR ($F_{1,103} = 0.01$, $P = 0.911$) and this relationship did not vary among populations, as indicated by the non-significant PC1 \times population interaction ($F_{4,103} = 0.54$, $P = 0.708$). However, SGR was significantly affected by population ($F_{4,35} = 31.78$, $P < 0.001$), length ($F_{4,35} = 31.78$, $P < 0.001$) and sex ($F_{1,127} = 93.55$, $P < 0.001$). Length was negatively correlated with SGR (parameter estimate \pm SE = -0.152 ± 0.001), such that larger fish grew proportionately slower. Females grew faster than males (parameter estimates \pm SE: females = 0.349 ± 0.007 , males = 0.243 ± 0.008).

Table 3. A matrix of correlation coefficients between the five response variables included in the principal components analysis

	AUC	Peak	Average r to peak	Time until peak	Time lost post-peak
AUC					
Peak	0.94				
Average r to peak	0.31	0.37			
Time until peak	0.47	0.43	-0.41		
Time lost post-peak	0.21	0.09	0.13	-0.05	

Response variable	Fixed effect	F	d.f.	P	Estimate ± SE
Log (PC1)	Initial length	6.08	1, 122	0.015	-0.011 ± 0.005
	Population	9.57	4, 122	<0.001	
	Chadha Ruaidh				0.995 ± 0.179
	Hosta				0.960 ± 0.194
	Lochmaddy				0.816 ± 0.169
	Reivil				0.876 ± 0.192
	Tormasad				0.686 ± 0.174
	Sex	0.91	1, 121	0.341	-
	Block	0.06	1, 120	0.803	-
	Initial length × Population	0.91	4, 116	0.462	-
Sqrt (PC2)	Initial length	2.29	1, 125	0.133	-
	Population	0.35	4, 121	0.843	-
	Sex	0.01	1, 120	0.932	-
	Block	5.64	1, 126	0.019	
	Block 1				1.621 ± 0.044
	Block 2				1.767 ± 0.043
	Initial length × Population	1.26	4, 116	0.292	-

Table 4. Results of general linear models of PC1 and PC2 scores, used to characterize variation in infection response profiles of individual sticklebacks

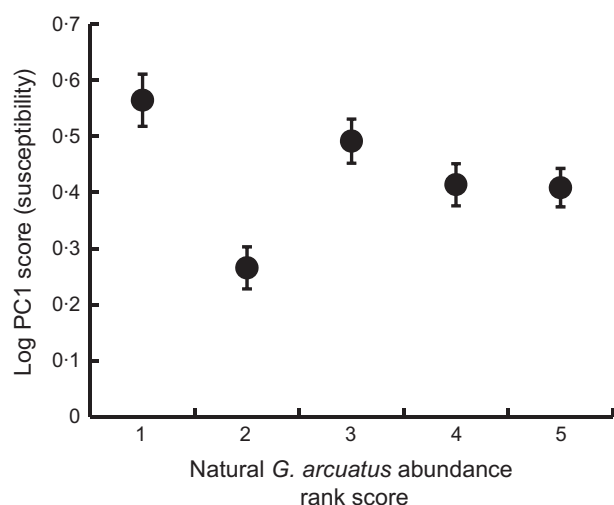


Fig. 3. Correlation between population means of log-transformed PC1 scores and natural *Gyrodactylus arcuatus* abundance rank score. PC1 scores served as a proxy for resistance to *Gyrodactylus gasterostei*: a high PC1 value indicates susceptibility, whereas a low PC1 value indicates resistance. Rank score increase corresponds to an increase in natural *G. arcuatus* abundance. The correlation was negative and not significant ($r = -0.23$, $P = 0.701$).

Discussion

GEOGRAPHIC VARIATION IN GYRODACTYLUS RESISTANCE

We found substantial variation in resistance to *Gyrodactylus gasterostei* among the five populations of three-spined sticklebacks studied. Population-level variation in resistance has been reported in other *Gyrodactylus*-host systems (Madhavi & Anderson 1985; van Oosterhout, Harris & Cable 2003; Bakke *et al.* 2004), but this is one of the few demonstrations of variation in resistance to *Gyrodactylus* among stickleback populations. A multivariate technique, used to analyse

variation in infection response profiles of individual fish, revealed that resistance to *G. gasterostei* was defined largely by the ability to limit the size of the worm population, rather than by the timing of the host response to infection. Moreover, as there were no significant population-level differences in establishment of *G. gasterostei*, these results reflect variation among populations in their response to the infection, rather than variation in the suitability of fish from different populations as hosts for *G. gasterostei*. Hereafter, resistance will be defined as being inversely proportional to PC1 score, such that a low PC1 score denotes resistance and a high PC1 score denotes susceptibility.

The results of the artificial infection experiment, utilizing a common garden, suggest that *G. gasterostei* resistance has a genetic basis, echoing studies in guppies, *Poecilia reticulata* (Madhavi & Anderson 1985; Cable & van Oosterhout 2007a), salmon, *Salmo salar* (Bakke, Soleng & Harris 1999; Gilbey *et al.* 2006) and topminnows of the genus *Poeciliopsis* (Leberg & Vrijenhoek 1994; Hedrick, Kim & Parker 2001). In addition to population-level differences in resistance, fish families varied in their response to infection. This may reflect true genetic variation, although our choice of a F1 generation full-sib experimental design means we cannot exclude the contribution of maternal effects or dominance variance (Lynch & Walsh 1998). This variation also could have been the result of host genotype × parasite genotype interactions (e.g. Carius, Little & Ebert 2001) generated by genetic variation in virulence in the *G. gasterostei* population. In any case, our study provides the first line of evidence for the potential of *Gyrodactylus*-mediated selection in stickleback populations, both within and among populations, because variation in infection response profiles was influenced strongly by host genetics (Anderson & May 1982; Little 2002).

The mechanistic basis of the variation in *G. gasterostei* resistance is currently unknown. Several arms of the innate immune system have been implicated with the host response to *Gyrodactylus* (Buchmann & Lindenstrøm 2002), especially the alternative complement pathway (Buchmann

1998; Harris, Soleng & Bakke 1998). It would be worthwhile examining baseline complement levels of all five populations and to assess their relationship with experimental levels of resistance. This has been demonstrated for three strains of Atlantic salmon that vary greatly in resistance to *G. salaris*: higher levels of complement factor C3 in mucous corresponded to greater *G. salaris* resistance (Bakke *et al.* 2000). Alternatively, *Gyrodactylus* resistance may be related to the density of mucous cells (Buchmann & Uldal 1997). Interestingly, PC2 scores, which were determined mainly by the time until the peak of the infection was reached, did not vary significantly among populations, implying the existence of an immune cascade leading to clearance of the infection that is common to all populations. Therefore, quantitative, rather than qualitative differences in immune parameters may be responsible for variation in resistance among populations. Although the adaptive immune system is unlikely to have a large effect on the *Gyrodactylus* infections of naive, lab-bred fish, such as those conducted here, recent studies have found associations between major histocompatibility complex (MHC) genetic diversity and natural *Gyrodactylus* abundance (Eizaguirre *et al.* 2009; Fraser & Neff 2010), suggesting that the adaptive immune response may play a role in determining parasite resistance in the wild.

THE RELATIONSHIP BETWEEN *GYRODACTYLUS* RESISTANCE AND NATURAL *GYRODACTYLUS* INFECTION

Resistance to *G. gasterostei* was not significantly correlated with natural *G. arcuatus* abundance. However, a weak pattern emerged. Generally, fish from populations exposed to higher levels of *G. arcuatus* in the wild were more resistant to *G. gasterostei* (had a lower PC1 score) in the artificial infection experiment. Tormasad was the exception to this pattern, showing high resistance despite low natural burdens. Chadha Ruaidh, the only naturally uninfected population, demonstrated particularly low resistance. This supports findings from other studies that have compared evolutionary naive and exposed host populations. For example, Hasu, Benesh & Valtonen (2009) examined variation in experimental resistance to an acanthocephalan parasite (*Acanthocephalus lucii*) among naturally exposed and unexposed isopod (*Asellus aquaticus*) populations and showed that unexposed populations were markedly more susceptible. Similarly, Kalbe & Kurtz (2006) reported that a population of three-spined sticklebacks naturally exposed to the eye fluke *Diplostomum pseudospathaceum* was significantly more resistant than a naturally unexposed population. In the absence of the parasite, there may be less selection to maintain resistance (Webster, Gower & Blair 2004; Lohse, Gutierrez & Kaltz 2006; Hasu, Benesh & Valtonen 2009), when resistance is costly (Sheldon & Verhulst 1996; Rigby, Hechinger & Stevens 2002). Here, we studied a number of populations that spanned a gradient of natural infection levels, rather than considering only naturally exposed and unexposed populations. Our data suggest that differences in *Gyrodactylus*-

mediated selection, as inferred from natural infection levels, may play a role in driving variation in *G. gasterostei* resistance among populations.

There are several caveats to our approach of correlating natural infection levels with experimental levels of parasite resistance, which may go some way to explaining the noisiness of the pattern. First, using current infection levels to infer historical levels of parasite-mediated selection is problematic, given that there may be temporal variation in parasite distributions within a host population. Furthermore, the small sample size used in this study (20 fish per population) may not capture the true extent of spatial heterogeneity natural infection levels. However, data we have collected on spatiotemporal variation in *G. arcuatus* distribution indicate that differences among populations are stable in the short term (de Roij & MacColl, unpublished data), lending support to the idea that the single, snapshot measure can be informative, at least for the North Uist system. Secondly, we chose to use a non-native *Gyrodactylus* species to investigate divergent resistance to this parasite. This decision was based on the need to remove the possibility of close coevolution between the host and parasite species, and the possibility that there may be sufficient overlap in the host response to both *Gyrodactylus* species. This allowed us to draw general conclusions about the evolution of resistance to *Gyrodactylus*. However, it is necessary to repeat the infection experiment with *G. arcuatus* to confirm that the observed differences in *Gyrodactylus* resistance are relevant to infection scenarios in the wild. Thirdly, the interpretation that a positive relationship between natural infection levels and experimental resistance is evidence for divergent *Gyrodactylus*-mediated selection assumes that natural infection levels are determined mainly by the environment (exposure rate), not host genetics. If the opposite were true, we might expect to have seen a negative relationship between natural infection levels and parasite resistance. In reality, both exposure and host genetics influence parasite distribution in host populations (Scott 1991; Grosholz 1994; Little & Ebert 2000; Karvonen *et al.* 2004). Lastly, our study included just five host populations. Inclusion of more populations could alter the sign and strength of the relationship. Nevertheless, we observed an interesting pattern that warrants further investigation. We encourage others to conduct similar studies that examine the relationship between a gradient of natural parasite infection levels and resistance to those parasite species, particularly for vertebrate-macroparasite interactions where such data are scarce.

MORTALITY IN THE INFECTION EXPERIMENT AND TOLERANCE TO *GYRODACTYLUS* INFECTION

Mortality in the infection experiment was low. This supports other *G. gasterostei* infection experiments (Harris 1982) that also found low levels of mortality associated with infection. Mortality was independent of the growth rate of the parasite population, which is sometimes used as a proxy for *Gyrodactylus* pathogenicity (Bakke, Cable & Harris 2007). Moreover, many fish sustained large infections without any

apparent pathology. By comparison, small *G. turnbulli* infections can be lethal for guppies (Scott & Anderson 1984; Cable & van Oosterhout 2007a). There are two possible explanations for the low mortality observed in our study. On the one hand, low pathogenicity may be a property of the strain of *G. gasterostei* used in the infection experiment, or more generally, of *G. gasterostei* as a species. On the other hand, host tolerance to infection, defined as the ability to limit the detrimental fitness effects of the parasite (Restif & Koella 2004; Raberg, Sim & Read 2007; Raberg, Graham & Read 2009), may play an important role in the *G. gasterostei*–stickleback interaction. Minimizing *G. gasterostei*-associated mortality may form a separate or complementary defence mechanism to resistance to *G. gasterostei*. Furthermore, there may be variation in this strategy among populations, such that more tolerant populations are able to sustain higher parasite levels and minimize the fitness effects of *Gyrodactylus* infection relative to less tolerant populations. Interestingly, mortality did not vary significantly among populations, suggesting that there may not be genetic variation in tolerance to *G. gasterostei*. However, this idea needs to be tested explicitly in separate infection experiments.

GYRODACTYLUS RESISTANCE AND STICKLEBACK SIZE/GROWTH

We found no significant effect of *G. gasterostei* resistance on stickleback growth and this was consistent across all five populations. Unfortunately, due to the experimental design it was not possible to distinguish the effects of the host response to infection (resistance) from the effects of the infection itself. Negative effects of *Gyrodactylus* infection on host growth have been reported previously (Barker, Cone & Burt 2002), but there are few studies that have investigated growth effects of *Gyrodactylus* infection formally. In contrast to the lack of an effect of parasite resistance on growth rate during the infection experiment, fish length, measured before the start of the infection experiment, explained significant variation in *G. gasterostei* resistance. This suggests there may be an interaction between fish growth and parasite resistance. Larger fish were more resistant to *G. gasterostei*, contradicting results from a study in guppies that found that larger guppies supported larger number of *Gyrodactylus* and were more likely to die as a result of the infection (Cable & van Oosterhout 2007a). Larger fish are assumed to have a greater surface area that provides more niche space for parasites (Poulin 2000). A possible explanation for the pattern observed here is that there is a positive genetic correlation between parasite resistance and host growth (Coltman *et al.* 2001). We are currently exploring the possibility of a genetic correlation between *Gyrodactylus* resistance and host growth rate; specifically, whether there is a trade-off between these two traits (de Roij & MacColl, unpublished data). If such a relationship exists, selection mediated by *Gyrodactylus* may have consequences for stickleback life history evolution in terms of growth. The significant variation in growth rate among host populations certainly suggests that there is ample inter-

population variation for this life history trait. In any case, it has yet to be determined whether resistance to *Gyrodactylus* is evolutionarily or physiologically costly. Costliness of parasite resistance, like spatial variation in parasite distributions, forms a potential mechanism constraining the evolution of parasite resistance within and among host populations (Rigby, Hechinger & Stevens 2002).

Conclusions

In summary, we have shown that there are substantial differences in *G. gasterostei* resistance among stickleback populations on North Uist. Resistance was best defined by the ability to limit the size of the infection rather than by preventing establishment of the parasite or the timing of the host response. The population-level variation observed here most likely has a genetic basis, although the mechanism(s) conferring resistance have yet to be explored. There was a weak positive correlation between resistance to *G. gasterostei* and natural abundance of *G. arcuatus*, suggesting that population-level differences in resistance to *Gyrodactylus* may be driven partly by divergent selection mediated by this parasite, as inferred from natural infection levels of *G. arcuatus*. More generally, our study illustrates the potential of the stickleback-*Gyrodactylus* interaction as a tractable model for investigating divergent parasite-mediated selection and the evolution of parasite resistance.

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