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Abiotic environmental variation drives virulence evolution in a fish host–parasite geographic mosaic

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Abstract

1. Parasite virulence varies greatly. Theory predicts that this arises from parasites optimising a trade-off between the mortality they inflict on current hosts, and their transmission to future hosts. The effect of the environment on this co-evolution is rarely considered.
2. Geographic mosaics are fertile systems for studying co-evolution, but again, the diversity of outcomes is often assumed to result from co-evolutionary dynamism, rather than being moulded by the environment.
3. Here, we quantify variation in virulence among lakes in a geographic mosaic of co-evolution between a trematode ectoparasite (*Gyrodactylus arcuatus*) and its three-spined stickleback (*Gasterosteus aculeatus*) host.
4. Virulence varies greatly in this system, and parasites are generally locally adapted to their hosts.
5. Parasites are also locally adapted to the water in their own lake, and virulence is strongly related to lake pH, the dominant axis of abiotic environmental variation in this system.
6. These results suggest that the evolution of virulence can be substantially affected by the abiotic environment, which has important implications for understanding co-evolution. There are also implications for the evolutionary management of disease, e.g. ectoparasites in aquaculture, the impacts of which might be expected to reduce given ongoing acidification of aquatic ecosystems.

KEYWORDS
co-evolution, disease, *Gasterosteus aculeatus*, *Gyrodactylus*, local adaptation, three-spined stickleback, trematode

1 | INTRODUCTION

The geographic mosaic of co-evolution has provided an attractive, if controversial, metaphor for the study of spatial variation in the evolution of biotic interactions (Gomulkiewicz et al., 2007; Nuismer, 2006; Thompson, 2005). Numerous empirical studies interpreted in this way provide compelling examples of the possible diversity of evolutionary outcomes, especially when antagonistic co-evolution is inferred (Benkman, Holimon, & Smith, 2001; Berenbaum & Zangerl, 2006; Brodie, Ridenhour, & Brodie, 2002; Kraaijeveld, Ferrari, & Godfray, 2003). An implicit assumption of some of the best known examples has been that co-evolutionary dynamism by itself, or related biotic interactions, are enough to account for the spatial diversity of outcomes (Benkman et al., 2001; Berenbaum & Zangerl, 2006; Brodie et al., 2002). In contrast, there has been surprisingly little investigation of the possibility that these outcomes are also, or instead, the result of variation in the wider (abiotic) environment in which they take place (Lively, Roode, Duffy, Graham, & Koskella, 2014), although such relationships could have important implications for our understanding of the consequences of global environmental change (Budria & Candolin,

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2014; MacLeod & Poulin, 2012). Here, we examine spatial variation in the outcome (virulence) of the interaction between the three-spined stickleback (*Gasterosteus aculeatus*) and its monogenean trematode ectoparasite, *Gyrodactylus arcuatus*, in a geographic mosaic of isolated lakes which exhibit strong abiotic variation in the aquatic environment.

The evolutionary outcome of host–parasite interactions has been intensively studied both theoretically (Frank, 1996) and empirically (De Roode, Yates & Altizer 2008; Ebert, 1994; Herre, 1995). In standard theory (Anderson & May, 1979; May & Anderson, 1979), virulence is supposed to evolve to a level that optimises the trade-off between the increased risk of mortality inflicted on the current host, and the probability of transmission to new hosts, both of which are assumed to be positively correlated with the growth rate of the infection. In this sense, the outcome of the host–parasite interaction is assumed to be driven by factors internal to the interaction (Zhan, Mundt, Hoffer, & Mcdonald, 2002). However, it has long been recognised that important effects on the outcome may result from external variation. In the classic example of virulence evolution in myxomatosis, it has been speculated that substantial differences in virulence between the UK and France may be the result of different vectors (Kerr & Best, 1988). The extent to which environmental variation drives virulence evolution is an open question (Lively et al., 2014). Studying the variation in virulence among strains of parasite species may reveal the cause of such variation and it may contribute to a better understanding of how to control parasitic infections (Bull, 1994; De Roode, Yates et al., 2008; Lopez Pascua, Gandon, & Buckling, 2012), and how they are likely to respond to environmental change (Budria & Candolin, 2014; MacLeod & Poulin, 2012).

We examined variation in the virulence of *G. arcuatus* (using an index of the growth rate of infections), among lakes on the Scottish island of North Uist, where there is substantial spatial variation in both the abundance of the parasite (De Roij & MacColl, 2012) and the aquatic abiotic environment, largely associated with variation in pH, which defines the dominant axis of environmental variation on North Uist (MacColl, El Nagar, & de Roij, 2013; Magalhaes, D'Agostino, Hohenlohe, & MacColl, 2016; Waterston, Holden, Campbell, & Maitland, 1979). Our aim was to assess the extent of local adaptation between parasites and hosts, and to quantify the degree to which variation in virulence was associated with abiotic environmental variation. The genus *Gyrodactylus* is commonly seen on the fins, gills and skin of many fish species. Because *Gyrodactylus* are ectoparasites, in direct contact with their environment at all times, we hypothesised that the abiotic aquatic environment would be likely to affect their evolution, including virulence. Unlike other helminth parasites, gyrodactylids can directly reproduce asexually and sexually on fish hosts (Harris, 1989; Schelkle, Faria, Johnson, van Oosterhout, & Cable, 2012), transmit directly between hosts, and survive on dead hosts for a short time (Scott & Anderson, 1984). Gyrodactylid virulence is strongly related to the parasite's growth rate on an infected host. For example, strong positive correlations between the growth rate of parasite infections and parasite induced host death have been recorded in the interactions between *Gyrodactylus turnbullis* and guppies *Poecilia reticulata* (Scott & Anderson, 1984) and *Gyrodactylus salaris* and Atlantic salmon *Salmo salar* (Bakke & MacKenzie, 1993).

2 | MATERIALS AND METHODS

We quantified variation in virulence and the extent of local adaptation of the parasite to host populations, how virulence correlated with the pH of the lake from which the parasites originated, and the extent of local adaptation of parasites to that water. We use the term virulence (of parasite strains) to describe an index of the growth rate of infections ("total parasite count," see below) averaged over host strains (where possible), and susceptibility (of host strains) to describe the same measure averaged over parasite strains. Resistance is the reciprocal of susceptibility.

2.1 | Experimental design

Experiments involving stickleback were carried out under licence from the UK Home Office, PPL 40/3486. We carried out five experiments: (1) to quantify variation in virulence among parasite populations, strains of *Gyrodactylus* from four separate North Uist lakes (Obse, Reiv, Scad and Maga, Table S1) were used to infect laboratory-raised stickleback (N = 8, 8, 8, 6 respectively) from an allopatric (tester) population originating from a pond in Nottingham (Jubilee lake, ~880 km distant from N. Uist). See below for experimental infection details. (2) To estimate the extent of local adaptation, *Gyrodactylus* strains from three populations (Obse, Reiv and Scad) were used to infect laboratory-raised fish from the same populations, in a fully reciprocal design. Eight to 12 individual fish were infected in each host–parasite combination, and a further six individuals per fish population were included as uninfected controls. (3) To further explore variation in local adaptation and resistance of hosts, *Gyrodactylus* from Maga were used to infect laboratory-raised fish from Obse, Scad and Maga (N = 6 fish from each population). (4) To estimate the correlation between virulence and pH, *Gyrodactylus* strains from seven lakes with contrasting pH (Gill, Host, Maga, Obse, Reiv, Scad and Torm, Table S1) were sampled from infected wild fish and used to infect wild caught fish from Chru, a population in which natural infection with *Gyrodactylus* is almost absent, and fish are naturally susceptible. Eighty fish were divided into eight groups of 10 individuals and one group was monitored as uninfected controls. (5) To quantify local adaptation of the parasite to lake water, *Gyrodactylus* strains from seven lakes (same as experiment 4) were placed individually in water from their own and the other six populations in a reciprocal design. Twelve worms were exposed in each parasite population—lake water combination, in 100 μl of water in wells of 96 microwell plates. *Gyrodactylus* survival was recorded every 3 hr until all worms had died. Death was determined from lack of movement or muscular contractions.

2.2 | Study areas and fish sampling

North Uist is a small (300 km^2), relatively flat island in the Scottish Western Isles, with many isolated lakes and coastal saline lagoons. Due to variation in surface geology and connectedness to the sea, the

chemistry of these water bodies varies greatly in pH, alkaline metal concentration and salinity (MacColl et al., 2013). Most freshwater lakes are isolated from each other, although they may be connected to the sea by an outlet stream. Three-spined stickleback are resident in most water bodies, and lagoons are also visited in spring by breeding migratory stickleback which spend most of their lives at sea. Values of pH used in analyses were the means of two to six (mean = 5.3, *SD* = 1.50) annual measurements for each lake recorded in April or May between 2006 and 2014 using a calibrated electronic pH meter (Multi 340i; WTW, Weilheim, Germany).

For experiments 1–3 fish were collected using minnow traps ("Gees," Dynamic Aqua, Vancouver) during April–May 2013 from four geographically isolated lakes: Obse, Reiv, Scad and Maga. Minnow traps were set in pairs around lake shores in the morning, in water one to three metres deep and left overnight. The four lakes were chosen because of their contrasting environmental conditions, which represent the full range of variation on N. Uist (MacColl et al., 2013). Obse is connected with the sea at high tides and is saline, while the others are isolated freshwater lakes (Table S1). Fish for experiment 4 were collected in the same way in April 2014.

2.3 | Fish breeding and feeding

Approximately five fish families were raised for each of the Obse, Reiv, Scad, Maga and Jubilee fish populations. This was done by artificially crossing breeding males and gravid females of three-spined stickleback on North Uist as described in De Roij, Harris, and MacColl (2011). Fertilised eggs were transported on ice to the aquaria of the School of Life Sciences at the University of Nottingham and incubated until day 10 in oxygen saturated dechlorinated tap water with 2 ppt salt and methylene blue. At day 10, each clutch was separately moved into one half of a 100 L glass tank partitioned with fine mesh. Tanks were filled with dechlorinated Nottingham tap water (*c*. pH 7.5) and provided with a biological filter (Fluval, Askoll, Italy) and an air source under controlled temperature and photoperiod conditions mimicking the fish's natural habitat. After hatching, fry were fed on different regimes, starting with *Paramecium* until day 7 and then with a mixture of *Paramecium* and freshly hatched brine shrimp (*Artemia*) nauplii until day 14. After this stage, fry were fed on brine shrimp nauplii alone until day 30 and then changed to a mixture of brine shrimp and chopped bloodworm defrosted from frozen (gamma blister bloodworm, Tropical Marine Centre, UK) for 60 days. After that, fish were fed on whole blood worm, defrosted from frozen, until the end of the experiment.

2.4 | Parasite breeding and artificial infections

At the same time that fish were collected for crossing, stickleback were also collected to establish laboratory populations of *G. arcuatus*. The parasite strains were identified to species levels using morphological characteristics of the hard parts (opishaptor) and excretory system (Geets, Appleby, & Ollevier, 1999), and these identifications were checked by sequencing of ITS regions (S. Robertson, unpubl. data; A.K. Rahn, pers. comm.). The worms were passaged on naïve laboratory fish, until parasites were required for infection experiments.

For the first, second and fourth experiments each fish was infected with two *Gyrodactylus*, but in the third experiment three *Gyrodactylus* were used. At the start and end of the experiments, standard length and (wet) weight were measured for the fish. Total worm number (including the initial worms) on each fish was counted approximately every 4 days in the first experiment until day 36, every 3 days in the second to day 28, on days 5, 13 and 20 in the third experiment and every 3 days until day 24 in the fourth experiment. The procedures of infection and monitoring were carried out under gentle anaesthesia of the experimental fish in a weak concentration of MS222 (100 mg/L). Infected fish were housed individually in 3 L plastic tanks containing 2 L of dechlorinated tap water. For each tank, 50% of the water content was changed with clean water from the same source every 3 days. All the fish were housed in a room with controlled temperature (13.5 ± 1°C) and 16:8 of light/dark photoperiod mimicking the external conditions on North Uist. Infected fish were monitored twice daily and if a fish did not swim well or was not feeding properly, it was euthanised by overdose of anaesthetic and mechanical destruction of the brain. All remaining fish were euthanised at the end of the experiments and dissected for gender identification.

2.5 | Statistical analysis

In the four infection experiments, the response variable "total parasite count" for each fish was calculated as the total of all counts for that fish from day "0" to the last day of the experiment (De Roij et al., 2011). Total parasite count was analysed separately for each experiment using a generalised linear model (GLM) with gamma distribution and logarithm link function. Initially, we analysed data from artificial infection experiments using generalized linear mixed models (GLMMs) that included "family" or family nested within population (population. family) as a random term, depending on whether the experimental design was nested or not, but family never accounted for a significant proportion of the variance, and we reverted to the use of GLMs. Fish length and fish sex were included as independent variables in all analyses. For experiment 1, "parasite population" was the only other fixed factor. For experiment 2, data were analysed in two ways; first, excluding data for sympatric infections, with parasite population as the only explanatory variable to look at the effect of parasites' origin on their average performance on allopatric hosts and second, including all data, with parasite population, fish population and their interaction as explanatory variables to determine whether local adaptation was present (assessed from significance of the parasite population × fish population interaction). For experiment 3, fish population was included as a fixed factor, to assess variation in resistance. For experiment 4, parasite population was included as a fixed factor. Two-tailed Pearson correlations were used to assess the relationships between parasite virulence, estimated in experiment 4, and both the pH of lakewater from which the parasite originated and host resistance scores (estimated in experiment 2 by taking the inverse value of susceptibility (total worm count^{-1}) for three laboratory raised stickleback

populations (Obse, Reiv and Scad) to allopatric parasite strains in the reciprocal infections).

For experiment 5, the response variable "parasite survival time" (hr) was analysed with and without the saltwater parasite population (Obse), using a GLM with gamma distribution and log link function. Fixed factors "parasite population" and "lake water origin" were included in a fully factorial design. Also for this experiment, an unpaired-samples *t* test was used to compare the mean estimated survival time (hr) of all gyrodactylids when introduced into water from their own or from different lakes.

Effect size (*E*) of local adaptation was estimated using an approach developed by Rosenberg, Adams, and Gurevitch (2000) and used by other studies (Hoeksema & Forde, 2008; Konijnendijk, Raeymaekers, Vandeuren, Jacquemin, & Volckaert, 2013) to investigate parasite local adaptation. The effect size was measured as natural log ratio of X_S/X_A where X_S is the mean fitness measurement of a parasite strain on their sympatric host or in water from their local lake and X_A is the mean fitness measurement of the strain on allopatric hosts or in water from different lakes. Parasite fitness was inferred from "total worm count" on sympatric (X_S) and two allopatric hosts (X_A) in experiments 2 and 3 and from survival time (hr) in water from their local lake against six different lakes in experiment 5. If the mean of *E* values is positive, a parasite is said to be adapted to its local host or conditions and if *E* is negative a parasite is said to be maladapted.

For all the artificial infection experiments, fish which were euthanised during the course of infections were excluded from the analyses because they had incomplete data. Statistical tests were performed using the SPSS package (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, version 22.0. Armonk, NY: IBM Corp).

3 | RESULTS

In experiments in which laboratory raised fish were infected there was no evidence that the family that a fish came from made any important contribution to variation in infection dynamics. In GLMMs with "family" (experiments 1 and 2) or "population.family" (experiment 3) fitted as random terms, the variance component due to family was small in comparison to its standard error: 0.007 ± 0.017, 0.225 ± 0.197, 0.054 ± 0.085 and 0.00 ± 0.00 in GLMMs for experiments 1, 2 (allopatric), 2 (all infections) and 3 respectively. We therefore reverted to the use of GLMs because of their easier fitting and better diagnostics.

3.1 | Variation in virulence

In all three experiments in which it was possible to test the effect (1, 2 and 4), the "total worm count" on allopatric tester hosts differed significantly among parasite populations (Table 1i,ii.a). In experiment 1, Maga and Obse parasites attained significantly higher total worm count than Scad parasites (Figure 1a). In experiment 2, both Obse and Reiv parasites had significantly higher total worm counts than Scad parasites (Figure 1b). In experiment 4, multiple comparison tests showed that Scad and Gill parasites had significantly lower worm counts than Host, Maga, Obse and Reiv parasites (Table 1iv). In

TABLE 1 Statistical analysis of the five decribed experiments. GLMs of the total worm count for: (i) four parasite populations (Obse, Reiv, Scad and Maga) on one allopatric (Jubilee) host population in experiment 1, (ii) three parasite populations (Obse, Reiv and Scad) in a reciprocal cross infection between the parasites and their hosts in experiment 2, (iii) one parasite population (Maga) on its sympatric and two allopatric (Obse and Scad) host populations in experiment 3, (iv) seven parasite populations tested on one allopatric (Chru) host population in experiment 4 and (v) GLM of 'parasite survival time' (hr) measured for seven parasite strains (Gill, Host, Maga, Obse, Reiv, Scad and Torm) in experiment 5

Source of variation	*df*	χ^2	*p* value
(i) **Experiment 1**			
Parasite origin	3	10.1	.018
Fish sex	1	1.7	.187
Fish length	1	1.4	.245
(ii) **Experiment 2**			
(a) *For allopatric infections only*			
Parasite origin	2	25.3	<.001
Fish origin	2	6.7	.035
Fish sex	1	0.1	.769
Fish length	1	0.5	.489
Parasite origin × Fish origin	1	0.5	.495
(b) *For allopatric and sympatric infections*			
Parasite origin	2	24.4	<.001
Fish origin	2	19.2	<.001
Fish sex	1	1.8	.181
Fish length	1	1.9	.180
Parasite origin × Fish origin	4	16.4	.003
(iii) **Experiment 3**			
Fish population	2	57.2	<.001
Fish sex	1	0.03	.862
Fish length	1	0.54	.461
(iv) **Experiment 4**			
Parasite origin	6	20.8	.002
Fish sex	1	4.4	.036
Fish length	1	0.2	.621
(v) **Experiment 5**			
(a) *For all strains*			
Parasite origin	6	189.7	<.001
Water origin	6	1,007.4	<.001
Parasite origin × Water origin	36	644.4	<.001
(b) *For freshwater strains only*			
Parasite origin	5	48.4	<.001
Water origin	5	433.4	<.001
Parasite origin × Water origin	25	149.5	<.001

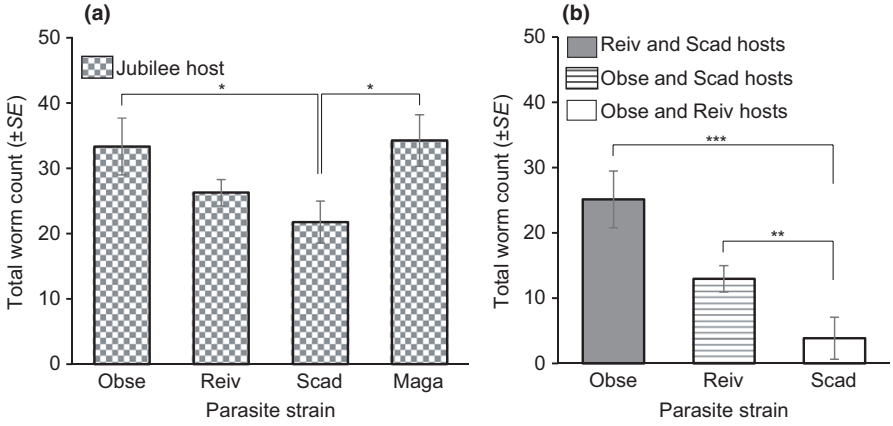

FIGURE 1 Virulence of parasite strains on allopatric hosts. (a) Mean total worm load of parasites from four different populations (Obse, Reiv, Scad and Maga) on hosts from a single allopatric stickleback population (Jubilee) in experiment 1. (b) Mean total worm load of parasite strains from Obse, Reiv and Scad on hosts from the two allopatric stickleback populations in experiment 2. In experiment 2, each of the three parasite populations was tested reciprocally on its sympatric and two allopatric hosts, but only their average measures on allopatric hosts are used in this figure (i.e. Obse on Reiv and Scad: shaded; Reiv on Obse and Scad: lined; Scad on Obse and Reiv: plain). Asterisks above the error bars represent results of post hoc (LSD) tests indicating the presence of significant differences (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$)

experiments 1 and 2, neither sex nor length of fish hosts had an effect on total worm counts (Table 1i and ii respectively). In experiment 4, total worm count was not affected by fish body size, but males had higher total worm counts than females (Table 1iv).

3.2 | Host–parasite local adaptation

In the reciprocal cross infection experiment 2 there was again significant variation in virulence among parasite populations (Table 1iib). Fish populations also differed consistently in the parasite counts recorded on them, indicating variation in resistance among host populations. Scad hosts supported the highest infection levels overall. The interaction between parasite population and fish population was significant, indicating local adaptation (Table 1iib). Parasites did best on their own host population, with the exception of Obse (the most virulent parasite population), which did best on Scad (the most susceptible host population). The total parasite count of Reiv and Scad parasite populations was significantly higher on sympatric than allopatric host populations (Figure 2a).

In experiment 3, the total worm count of parasites from Maga differed significantly among Maga, Obse and Scad fish populations (Table 1iii), and performance was better on sympatric Maga fish than allopatric Obse and Scad hosts (Figure 2b). Fish sex and size had no significant influence on worm count in this experiment.

In experiments 2 and 3, the three freshwater parasite populations (Reiv, Scad and Maga) consistently had positive values of effect size *E* measured for total worm count, but the Obse parasite had negative *E* values (Table 2a).

3.3 | Parasite performance and environment

In experiment 4, there was a strong positive correlation between total parasite counts and host resistance to allopatric parasite infection (i.e. taking the inverse value of total worm counts during infections in experiment 2), although this was for only three populations ($r = .99$, $N = 3$, $p = .037$, Figure 4a). Mean total worm counts for parasite strains in experiment 4 were strongly positively correlated with the pH of the water in the lake from which the worms originated ($r = .92$, $N = 7$, $p = .003$, Figure 4b). When the data from all experiments which used different parasite strains were combined in a single GLM, with total parasite counts as the response variable, and "experiment" (1, 2 and 4) and "pH" of lake of origin as explanatory variables, a significant positive relationship between parasite count and pH was again found (for "experiment", Wald $F_{2,10} = 31.7$, $p < .0001$; for "pH", Wald $F_{1,10} = 7.28$, $p = .022$).

In experiment 5, parasite survival time was generally higher in water from their own lakes than in water from different lakes (Figure 3a,b). The expected survival of detached *G. arcuatus* varied significantly among the seven parasite strains (including Obse, the saltwater strain (Table 1v.a) and this remained true when only data for freshwater strains were analysed (Table 1v.b). Survival of strains was also affected by the water to which they were exposed, such that the interaction between parasite strain and lakewater origin was significant (Table 1v.a). The interaction remained significant even after excluding the saltwater strain from the analysis (Table 1v.b). Most parasite strains (Host, Gill, Obse, Scad and Torm) had positive *E* measured for survival time, but two parasite strains (Maga and Reiv) had negative *E* values (Table 2b).

4 | DISCUSSION

We found clear evidence of variation among parasite populations in the growth rate of infections, which is likely to be associated with virulence (Bakke & MacKenzie, 1993; Scott & Anderson, 1984). This variation was strongly associated with the dominant axis of aquatic abiotic environmental variation across lakes, the pH. Host resistance

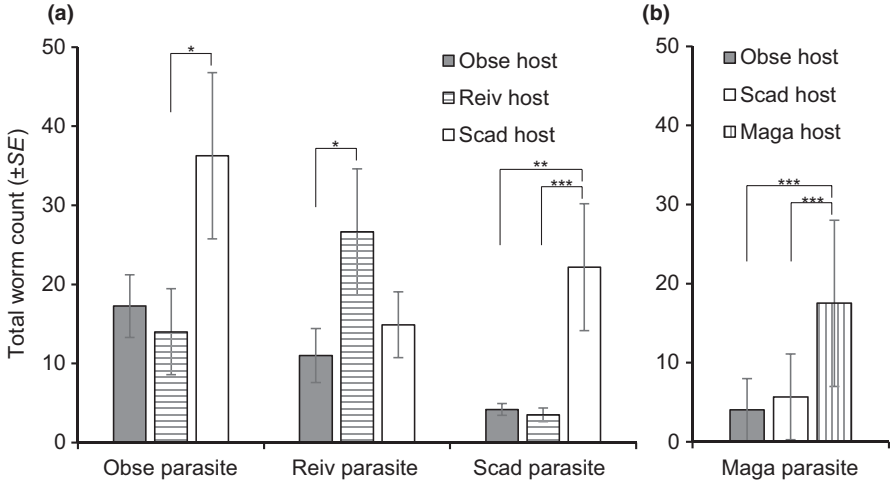

FIGURE 2 Differences in the total worm load measured for each parasite population on its sympatric and two allopatric host populations. (a) In experiment 2 each of Obse, Reiv and Scad parasites was tested on three fish populations (Obse: shaded; Reiv: horizontally lined and Scad: plain). (b) In experiment 3 Maga parasites were also tested on three fish populations (Obse: shaded; Scad: plain and Maga: vertically lined) (*$p \leq .05$, **$p \leq .01$, ***$p \leq .001$)

TABLE 2 Local adaptation effect size (*E*) for the parasite performance measured: (a) in situ using the formulae "ln (the average of total worm count on a sympatric host/the average of total worm count on two allopatric hosts)" in the second and third experiments and (b) in vitro using "ln (the average survival hours in water from own lake/the average survival hours in water from six different lakes)" for the fourth experiment

	Effect size (*E*)	
Parasite strain	**(a) Using total worm count from artificial infection**	**(b) Using survival time of detached worms**
Gill		0.213
Host		0.011
Maga	1.287	−0.280
Obse	−0.736	0.890
Scad	2.497	0.225
Torm		0.422
Reiv	0.867	−0.216

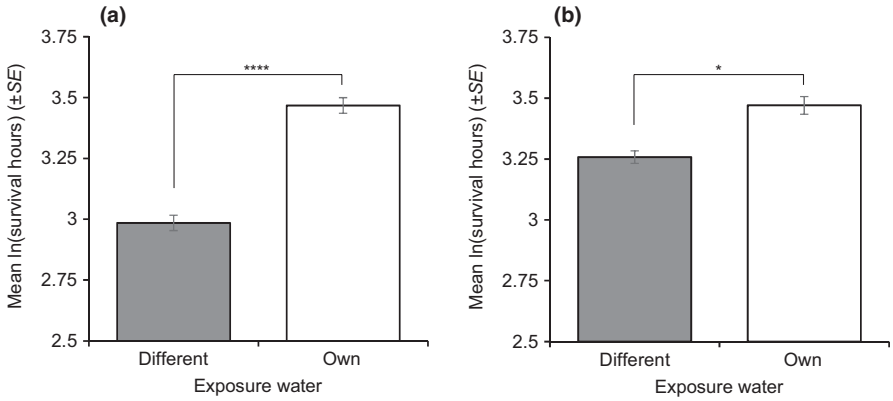

FIGURE 3 Difference in the log transformed mean survival time (hr) of detached gyrodactylids when incubated in water from their own (plain) and six different (shaded) lakes: (a) represents data from all seven strains (Gill, Host, Maga, Obse, Reiv, Scad and Torm) of the parasite while in (b), the saltwater strain (Obse) was excluded from the analysis (*$p \leq .05$, ****$p \leq .0001$)

also differed consistently across the four infection experiments, suggesting a geographic mosaic of co-evolution, in which parasites were generally locally adapted. *Gyrodactylus*, an ectoparasite continually immersed in its aquatic environment, exhibited local adaptation (higher survival) in the water from its own lake, consistent with the association between the pH of the water and variation in virulence.

There was a very strong relationship between the virulence of parasites in the lab and the pH of water in their natural environment. Since virulence was measured in common garden conditions (and sometimes after many generations of maintaining, or passaging, the parasites in the laboratory), it is likely that much of the variation is an evolved, genetic response. Given that *Gyrodactylus* is an ectoparasite, exposed to its environment, and that pH has many effects on organisms, it is quite possible that pH itself has driven divergent evolution of *Gyrodactylus* among North Uist lakes. However, in these lakes, pH is also strongly associated with the availability of alkaline (e.g. calcium, magnesium and sodium) and transition (e.g. zinc and copper) metals, and with overall water conductivity. Zinc in particular is known to have toxic effects on gyrodactylids (Gheorghiu, Cable, Marcogliese, & Scott, 2007). Therefore, pH may be a proxy for a wide range of water chemistry and resource conditions (MacColl et al., 2013). The association between environmental pH and parasite virulence could be a direct result of selection on the parasite or an indirect result of changes in the life-history traits of hosts, although the former seems more likely,

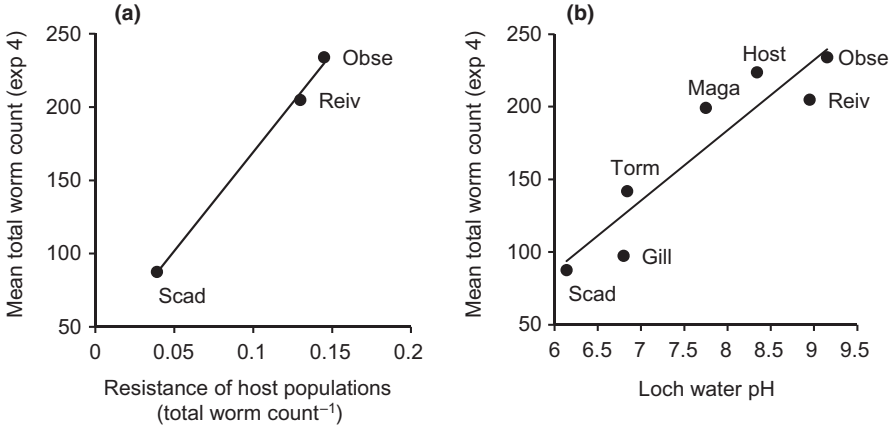

FIGURE 4 The relationship between the response variable "total worm count" measured for parasite populations in the laboratory (experiment 4) and (a) host resistance scores of three stickleback populations to two allopatric *Gyrodactylus* strains ("mean total worm count^{-1}" in experiment 2) and (b) lake-water pH for seven lakes on North Uist

given the strength of the relationship. Lakes with low pH probably have poorer resources for stickleback, and this may affect the evolution of the host–parasite relationship. For example, stickleback may mount a weaker immune response when resource stressed, favouring reduced virulence in *Gyrodactylus* (Allen & Little, 2011; Rauw, 2012).

The relationship between pH and virulence has consequences for our understanding of the effects on host–parasite interactions of environmental change, especially eutrophication and ocean acidification (Budria & Candolin, 2014; MacLeod & Poulin, 2012). Our results suggest that ocean acidification might lead to a reduction in the virulence of (especially) ectoparasites. The effects of euthrophication on virulence, which can result in oscillating pH, are harder to predict.

There has been very little investigation of the relationship between abiotic environmental variables and evolved virulence, although many parasites vary in abundance across gradients of e.g. temperature and moisture (Combes & Morand, 1999; Karvonen et al., 2013; Wolinska & King, 2009), and host–parasite dynamics are clearly affected by abiotic conditions (Wolinska & King, 2009). Associations between biotic variation and virulence have been investigated, making clear that virulence can respond to environmental circumstances, but this is still poorly understood. In a study of bird–malaria interactions, the parasite (*Plasmodium relictum*) was found to adapt to the nutritional conditions of its hosts and these were thought to shape parasite virulence (Cornet, Bichet, Larcombe, Faivre, & Sorci, 2014). De Roode, Pedersen, Hunter, and Altizer (2008) found that a protozoan parasite (*Ophryocystis elektroscirrha*) of monarch butterflies (*Danaus plexippus* L.) exhibited low virulence when the larvae of its host fed on a plant containing a toxic substance, possibly through a direct effect of toxicity on virulence, or because the longevity of the host was reduced by toxicity.

Our results suggest that *Gyrodactylus* are generally adapted to their local host fish population, although the most virulent parasite (Obse) did better on the weakest host (Scad) than on its sympatric host. The survival of detached *Gyrodactylus* also suggested local adaptation of the parasite to its aquatic environment. The majority of the parasite strains tested in this study had positive values of local adaptation effect size (*E*) measured for their performance on sympatric against allopatric hosts and for their survival time in water from their own against different lakes. Although parasite local adaptation is a common prediction of theoretical models of host–parasite coevolution, there have been few reports of it in experimental studies of vertebrate host–parasite interactions (Ballabeni & Ward, 1993; Voutilainen et al., 2009). Stickleback may provide a model system in this regard, since the isolation of many water bodies from one another may favour evolutionary divergence and local adaptation. Given the direct transmission of *G. arcuatus*, and its rapid reproductive strategy it is likely that gene flow between parasite populations will be higher than between host populations, and this may favour local adaptation of the parasite (Raeymaekers, Wegner, Huyse, & Volckaert, 2011).

Apparent lack of local adaptation in one of the parasite strains (Obse) has an obvious explanation. Two ecotypes of three-spined sticklebacks coexist in this saltwater lagoon which is flooded by the sea at spring tides. We used fish of (and parasites from) the 'resident' phenotype which inhabit this waterbody year-round. However, anadromous stickleback also enter this lagoon in the spring to breed. It seems likely that the gene flow between fish or parasites that surely results may disrupt the potential for local adaptation (Lively, 1999). In this regard, our results agree with previous studies on the evolutionary outcomes of fish parasite combinations from connected waterbodies. For example, Sasal, Durand, Faliex, and Morand (2000) used four strains of a digenean flatworm (*Labratrema minimus*) and *Pomatoschistus microps* hosts, Konijnendijk et al. (2013) used two strains of *Gyrodactylus gasterostei* and three-spined stickleback hosts and Perez-Jvostov, Hendry, Fussmann, and Scott (2015) used four isolates of *Gyrodactylus* sp. and their guppy populations. In the three studies, the parasite strains did not show quantitative differences between sympatric and allopatric host infections. In such scenarios parasite local adaptation could be absent because gene flow in hosts is expected to be higher than in the parasite (Konijnendijk et al., 2013).

The interaction between stickleback and *Gyrodactylus* appears to match the conditions necessary to be a geographic mosaic of coevolution (Gomulkiewicz et al., 2007; Thompson, 2005), at least in terms of pattern: traits (virulence and resistance) are spatially variable, and while there is some correlation between traits across populations (e.g. Figure 4a), implying reciprocal selection between virulence and resistance, there are also mismatches. For example, we have shown here that *Gyrodactylus* from Torm are of intermediate virulence, yet De Roij et al. (2011) found this to be the most resistant of the stickleback

populations they assayed. It follows that neither resistance nor virulence are species level traits (Gomulkiewicz et al., 2007).

It is more difficult to establish the necessary conditions for a geographic mosaic in terms of processes (Gomulkiewicz et al., 2007). However, it seems likely that there is geographic variation across the mosaic in the strength of interactions (hot and cold spots): for example in Torm we have never recorded more than one *Gyrodactylus* on an individual stickleback (N = 83, ADCM unpubl. data), while in Scad we have never recorded more than six (N = 154) and it seems unlikely that such low abundances can have substantial effects on the fitness of hosts. In contrast, stickleback in saltwater occasionally have *Gyrodactylus* abundances as high as 300! As discussed in the previous paragraph, it also seems likely that trait remixing is occurring in this system: some lakes are connected to each other in the same catchment, while those close to the sea also experience an influx of migratory stickleback (and their parasites) in the spring each year, making gene flow between both host and parasite populations likely. We cannot at this stage establish that there is a selection mosaic in the interaction between stickleback and *Gyrodactylus* (Gomulkiewicz et al., 2007), although it is possible to imagine individually based, quantitative genetic experiments that might make this possible.

In conclusion, our study suggests that the interaction between *Gyrodactylus* and stickleback can be described as a geographic mosaic of co-evolution, but that levels of virulence exhibited by parasites from different populations are more a result of the aquatic environment (pH) to which the parasite is exposed, than an emergent property of the host–parasite interaction. As both the hosts and their parasites used in some experiments were raised in the laboratory, the difference among populations is likely genetic and driven by differences in gene flow between the parasites and their hosts (Greischar & Koskella, 2007). Collectively, this body of work highlights the fact that environmental variables (especially water pH) can potentially alter the dynamic of this host–parasite interaction and may determine virulence levels (Lively et al., 2014).

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AUTHORS' CONTRIBUTIONS

M.A.M. conducted fieldwork, designed and carried out experiments, analysed data and contributed to writing the manuscript. J.E.B. contributed to project design and writing the manuscript. A.D.C.M. conceived the project, designed and supervised experiments, and contributed to data analysis and writing the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data from the reported experiments have been archived in the Dryad Digital Repository https://doi.org/10.5061/dryad.37ns0 (Mahmud, Bradley, & MacColl, 2017).

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