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

Muayad A Mahmud [1,2], Janette E Bradley [1] and Andrew DC MacColl [1,*]

[1]School of Life Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

[2]Current address: Research Centre, Erbil Polytechnic University, Erbil 44001, KRG-Iraq

*Corresponding author: andrew.maccoll@nottingham.ac.uk, tel: +441159513410.

Running headline: Abiotic environment drives virulence evolution

SUMMARY

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 coevolution is rarely considered.

2. Geographic mosaics are fertile systems for studying coevolution, 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 coevolution 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 coevolution. 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

coevolution, disease, Gasterosteus aculeatus, Gyrodactylus, local adaptation, three-spined stickleback, trematode

INTRODUCTION

The geographic mosaic of coevolution has provided an attractive, if controversial, metaphor for the study of spatial variation in the evolution of biotic interactions (Thompson 2005; Nuismer 2006; Gomulkiewicz *et al.* 2007). Numerous empirical studies interpreted in this way provide compelling examples of the possible diversity of evolutionary outcomes, especially when antagonistic coevolution is inferred (Benkman, Holimon & Smith 2001; Brodie, Ridenhour & Brodie 2002; Kraaijeveld, Ferrari & Godfray 2003; Berenbaum & Zangerl 2006). An implicit assumption of some of the best known examples has been that coevolutionary dynamism by itself, or related biotic interactions, are enough to account for the spatial diversity of outcomes (Benkman, Holimon & Smith 2001; Brodie, Ridenhour & Brodie 2002; Berenbaum & Zangerl 2006). 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 *et al.* 2014), although such relationships could have important consequences for our understanding of the consequences of global environmental change (MacLeod & Poulin 2012; Budria & Candolin 2014). 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 (Ebert 1994; Herre 1995; de Roode, Yates & Altizer 2008). 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 *et al.* 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 of 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 *et al.* 2008; Lopez Pascua, Gandon & Buckling 2012), and how they are likely to respond to environmental change (MacLeod & Poulin 2012; Budria & Candolin 2014).

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 (Waterston *et al.* 1979; MacColl, El Nagar & de Roij 2013; Magalhaes *et al.* 2016). 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 *et al.* 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 *G. turnbullis* and guppies *Poecilia reticulata* (Scott & Anderson 1984) and *G. salaris* and Atlantic salmon *Salmo salar* (Bakke & MacKenzie 1993).

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.

Experimental design

Experiments involving stickleback were carried out under licence from the U.K. 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 lab-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 lab-raised fish from the same populations, in a fully reciprocal design. Eight to twelve 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 lab-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 100µl of water in wells of 96 microwell plates. *Gyrodactylus* survival was recorded every three hours until all worms had died. Death was determined from lack of movement or muscular contractions.

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

134 breeding migratory stickleback which spend most of their lives at sea. Values of pH used in
135 analyses were the means of two to six (mean = 5.3, standard deviation = 1.50) annual
136 measurements for each lake recorded in April or May between 2006 and 2014 using a
137 calibrated electronic pH meter (Multi 340i, WTW, Weilheim, Germany).
138
139 For experiments (1) to (3) fish were collected using minnow traps ('Gees', Dynamic Aqua,
140 Vancouver) during April-May 2013 from four geographically isolated lakes: Obse, Reiv,
141 Scad and Maga. Minnow traps were set in pairs around lake shores in the morning, in water
142 one to three metres deep and left overnight. The four lakes were chosen because of their
143 contrasting environmental conditions, which represent the full range of variation on N. Uist
144 (MacColl et al 2013). Obse is connected with the sea at high tides and is saline, while the
145 others are isolated freshwater lakes (Table S1). Fish for experiment (4) were collected in the
146 same way in April 2014.
147
148 **Fish breeding and feeding**
149 Approximately five fish families were raised for each of the Obse, Reiv, Scad, Maga and
150 Jubilee fish populations. This was done by artificially crossing breeding males and gravid
151 females of three-spined stickleback on North Uist as described in de Roij, Harris and
152 MacColl (2011). Fertilised eggs were transported on ice to the aquaria of the School of Life
153 Sciences at the University of Nottingham and incubated until day 10 in oxygen saturated
154 dechlorinated tap water with 2 ppt salt and methylene blue. At day 10, each clutch was
155 separately moved into one half of a 100L glass tank partitioned with fine mesh. Tanks were
156 filled with dechlorinated Nottingham tap water (approx. pH 7.5) and provided with a
157 biological filter (Fluval, Askoll, Italy) and an air source under controlled temperature and
158 photoperiod conditions mimicking the fish's natural habitat. After hatching, fry were fed on

159 different regimes, starting with *Paramecium* until day 7 and then with a mixture of
160 *Paramecium* and freshly hatched brine shrimp (*Artemia*) nauplii until day 14. After this stage,
161 fry were fed on brine shrimp nauplii alone until day 30 and then changed to a mixture of
162 brine shrimp and chopped bloodworm defrosted from frozen (gamma blister bloodworm,
163 Tropical Marine Centre, UK) for 60 days. After that, fish were fed on whole blood worm,
164 defrosted from frozen, until the end of the experiment.
165
166 **Parasite breeding and artificial infections**
167 At the same time that fish were collected for crossing, stickleback were also collected to
168 establish lab populations of *G. arcuatus*. The parasite strains were identified to species levels
169 using morphological characteristics of the hard parts (opishaptor) and excretory system
170 (Geets, Appleby & Ollevier 1999), and these identifications were checked by sequencing of
171 ITS regions (S. Robertson, unpublished data; A.K. Rahn, personal communication). The
172 worms were passaged on naïve lab fish, until parasites were required for infection
173 experiments.
174
175 For the first, second and fourth experiments each fish was infected with two *Gyrodactylus*,
176 but in the third experiment three *Gyrodactylus* were used. At the start and end of the
177 experiments, standard length and (wet) weight were measured for the fish. Total worm
178 number (including the initial worms) on each fish was counted approximately every four days
179 in the first experiment until day 36, every three days in the second to day 28, on days 5, 13
180 and 20 in the third experiment and every three days until day 24 in the fourth experiment. The
181 procedures of infection and monitoring were carried out under gentle anaesthesia of the
182 experimental fish in a weak concentration of MS222 (100mg L^{-1}). Infected fish were housed
183 individually in 3L plastic tanks containing 2L of dechlorinated tap water. For each tank, 50%

of the water content was changed with clean water from the same source every three 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.

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, Harris & MacColl 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 x 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 lab raised stickleback populations (Obse, Reiv and Scad) to allopatric parasite strains in the reciprocal infections).

For experiment (5), the response variable 'parasite survival time' (hours) 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 (hours) 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 *et al.* 2013) to investigate parasite local adaptation. The effect size (E) was measured as natural log ratio of 'X_S/X_A' where 'X_S' is the mean fitness measurements of the parasite strains on their sympatric hosts or in water from their local lake and 'X_A' is the mean fitness measurements of the strains 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 (hours) in water from their local lake against six different lakes in experiment 5. If the mean value of 'E' value is positive, a parasite is said to be adapted to its local hosts 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).

RESULTS

In experiments in which lab 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.

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 1 (i, 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 1(iv)). In experiments 1 and 2, neither sex nor length of fish hosts had an effect on total worm counts (Table 1(i 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 1(iv)).

Host-parasite local adaptation

In the reciprocal cross infection experiment (2) there was again significant variation in virulence among parasite populations (Table 1(iib)). 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 effect of interaction between parasite population and fish population was significant, indicating local adaptation (Table 1(iib)). 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 (Fig. 2A).

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

In experiment 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).

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. by taking the inverse value of total worm counts during infections in exp. 2), although this was for only three populations ($r = 0.99$, $N = 3$, $P = 0.037$, Fig. 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 ($r = 0.92$, $N = 7$, $P = 0.003$, fig. 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 $F_{2,10} = 31.7$, $P < 0.0001$; for 'pH', Wald $F_{1,10} = 7.28$, $P = 0.022$).

In experiment 5, parasite survival time was generally higher in water from their own lakes than in water from different lakes (Fig. 3A, B). The expected survival of detached *G. arcuatus* varied significantly among the seven parasite strains (including Obse, the saltwater strain, (Table 1(v.a)) and this remained true when only data for freshwater strains were analysed (Table 1(v.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 1(v.a). The interaction remained significant even after excluding the saltwater strain from the analysis (Table 1(v.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).

DISCUSSION

We found clear evidence of variation among parasite populations in the growth rate of infections, which is likely to be associated with virulence (Scott & Anderson 1984; Bakke &

307 MacKenzie, 1993). This variation was strongly associated with the dominant axis of aquatic
308 abiotic environmental variation across lakes, the pH. Host resistance also differed
309 consistently across the four infection experiments, suggesting a geographic mosaic of
310 coevolution, in which parasites were generally locally adapted. *Gyrodactylus*, an ectoparasite
311 continually immersed in its aquatic environment, exhibited local adaptation (higher survival)
312 in the water from its own lake, consistent with the association between the pH of the water
313 and variation in virulence.
314
315 There was a very strong relationship between the virulence of parasites in the lab and the pH
316 of water in their natural environment. Since virulence was measured in common garden
317 conditions (and sometimes after many generations of maintaining, or passaging, the parasites
318 in the lab), it is likely that much of the variation is an evolved, genetic response. Given that
319 *Gyrodactylus* is an ectoparasite, exposed to its environment, and that pH has many effects on
320 organisms, it is quite possible that pH itself has driven divergent evolution of *Gyrodactylus*
321 among North Uist lakes. However, in these lakes, pH is also strongly associated with the
322 availability of alkaline (eg.calcium, magnesium and sodium) and transition (e.g. zinc and
323 copper) metals, and with overall water conductivity. Zinc in particular is known to have toxic
324 effects on gyrodactylids (Gheorghiu *et al.* 2007). Therefore, pH may be a proxy for a wide
325 range of water chemistry and resource conditions (MacColl, El Nagar & de Roij 2013). The
326 association between environmental pH and parasite virulence could be a direct result of
327 selection on the parasite or an indirect result of changes in the life history traits of hosts,
328 although the former seems more likely, given the strength of the relationship. Lakes with low
329 pH probably have poorer resources for stickleback, and this may affect the evolution of the
330 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 (MacLeod & Poulin 2012; Budria & Candolin 2014). 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; Wolinska & King 2009; Karvonen *et al.* 2013), 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 *et al.* 2014). de Roode *et al.* (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.

355 Our results suggest that *Gyrodactylus* are generally adapted to their local host fish population,
356 although the most virulent parasite (Obse) did better on the weakest host (Scad) than on its
357 sympatric host. The survival of detached *Gyrodactylus* also suggested local adaptation of the
358 parasite to its aquatic environment. The majority of the parasite strains tested in the current
359 study had positive values of local adaptation effect size (E) measured for their performance
360 on sympatric against allopatric hosts and for their survival time in water from their own
361 against different lakes. Although parasite local adaptation is a common prediction of
362 theoretical models of host-parasite coevolution, there have been few reports of it in
363 experimental studies of vertebrate host-parasite interactions (Ballabeni & Ward 1993;
364 Voutilainen *et al.* 2009). Stickleback may provide a model system in this regard, since the
365 isolation of many water bodies from one another may favour evolutionary divergence and
366 local adaptation. Given the direct transmission of *G. arcuatus*, and its rapid reproductive
367 strategy it is likely that gene flow between parasite populations will be higher than between
368 host populations, and this may favour local adaptation of the parasite (Raeymaekers *et al.*
369 2011).
370
371 Apparent lack of local adaptation in one of the parasite strains (Obse) has an obvious
372 explanation. Two ecotypes of three-spined sticklebacks coexist in this saltwater lagoon which
373 is flooded by the sea at spring tides. We used fish of (and parasites from) the 'resident'
374 phenotype which inhabit this waterbody year-round. However, anadromous stickleback also
375 enter this lagoon in the spring to breed. It seems likely that the gene flow between fish or
376 parasites that surely results may disrupt the potential for local adaptation (Lively 1999). In
377 this regard, our results agree with previous studies on the evolutionary outcomes of fish
378 parasite combinations from connected waterbodies. For example, Sasal *et al.* (2000) used
379 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 *et al.* (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 (Thompson, 2005; Gomulkiewicz et al. 2007), 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. Fig. 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 unpublished 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

405 trait remixing is occurring in this system: some lakes are connected to each other in the same
406 catchment, while those close to the sea also experience an influx of migratory stickleback
407 (and their parasites) in the spring each year, making gene flow between both host and parasite
408 populations likely. We cannot at this stage establish that there is a selection mosaic in the
409 interaction between stickleback and *Gyrodactylus* (Gomulkiewicz et al. 2007), although it is
410 possible to imagine individually based, quantitative genetic experiments that might make this
411 possible.
412
413 In conclusion, our study suggests that the interaction between *Gyrodactylus* and stickleback
414 can be described as a geographic mosaic of coevolution, but that levels of virulence exhibited
415 by parasites from different populations are more a result of the aquatic environment (pH) to
416 which the parasite is exposed, than an emergent property of the host-parasite interaction. As
417 both the hosts and their parasites used in some experiments were raised in the lab, the
418 difference among populations is likely genetic and driven by differences in gene flow
419 between the parasites and their hosts (Greischar & Koskella 2007). Collectively, this body of
420 work highlights the fact that environmental variables (especially water pH) can potentially
421 alter the dynamic of this host- parasite interactions and may determine virulence levels
422 (Lively *et al.* 2014).
423
424 **AUTHOR CONTRIBUTIONS**
425 M.A.M. conducted fieldwork, designed and carried out experiments, analysed data and
426 contributed to writing the manuscript. J.E.B. contributed to project design and writing the
427 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.

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DATA ACCESSIBILITY

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

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570 Table 1. Statistical analysis of the five decribed experiments. GLMs of the total worm count
571 for: (i) four parasite populations (Obse, Reiv, Scad and Maga) on one allopatric (Jubilee) host
572 population in experiment 1, (ii) three parasite populations (Obse, Reiv and Scad) in a
573 reciprocal cross infection between the parasites and their hosts in experiment 2, (iii) one
574 parasite population (Maga) on its sympatric and two allopatric (Obse and Scad) host
575 populations in experiment 3, (iv) seven worm populations tested on one allopatric (Chru) host
576 population in experiment 4 and (v) GLM of 'parasite survival time' (hours) measured for
577 seven parasite strains (Gill, Host, Maga, Obse, Reiv, Scad and Torm) in experiment 5.
578

579

Source of variation	DF	χ^2	*P* value
(i) *Experiment one*			
Parasite origin	3	10.1	0.018
Fish sex	1	1.7	0.187
Fish length	1	1.4	0.245
(ii) *Experiment two*			
(a) For allopatric infections only			
Parasite origin	2	25.3	< 0.001
Fish origin	2	6.7	0.035
Fish sex	1	0.1	0.769
Fish length	1	0.5	0.489
Parasite origin * Fish origin	1	0.5	0.495
(b) For allopatric and sympatric infections			
Parasite origin	2	24.4	< 0.001
Fish origin	2	19.2	< 0.001
Fish sex	1	1.8	0.181
Fish length	1	1.9	0.180
Parasite origin * Fish origin	4	16.4	0.003
(iii) *Experiment three*			
Fish population	2	57.2	< 0.001
Fish sex	1	0.03	0.862
Fish length	1	0.54	0.461
(iv) *Experiment four*			
Parasite origin	6	20.8	0.002

Fish sex	1	4.4	0.036
Fish length	1	0.2	0.621
(v) *Experiment five*			
(a) For all strains			
Parasite origin	6	189.7	< 0.001
Water origin	6	1007.4	< 0.001
Parasite origin * Water origin	36	644.4	< 0.001
(b) For freshwater strains only			
Parasite origin	5	48.4	< 0.001
Water origin	5	433.4	< 0.001
Parasite origin * Water origin	25	149.5	< 0.001

580

581

582 Table 2. Local adaptation effect size (E) for the parasite performance measured: (A) *in situ*
583 using the formulae 'ln (the average of total worm count on a sympatric host / the average of
584 total worm count on two allopatric hosts)' in the second and third experiments and (B) *in*
585 *vitro* using 'ln (the average survival hours in water from own lake/ the average survival hours
586 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

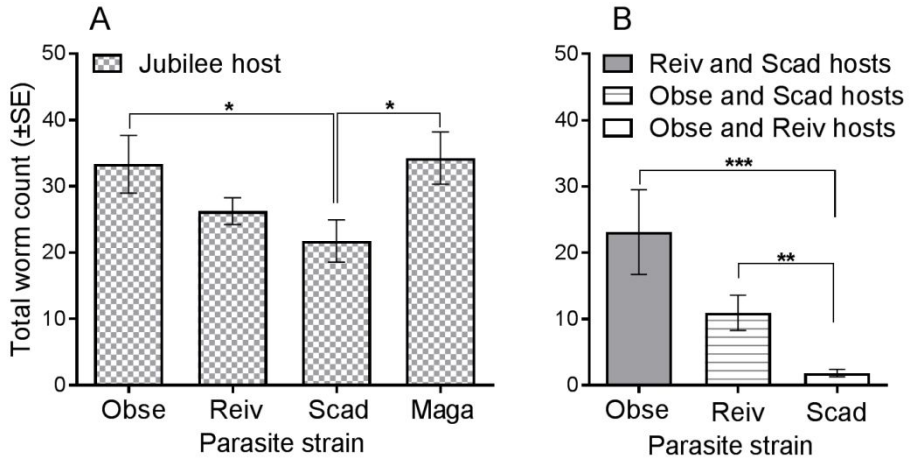
587

588 Figure 1. Virulence of parasite strains on allopatric hosts. (A) Mean total worm load of
589 parasites from four different populations (Obse, Reiv, Scad and Maga) on hosts from a single
590 allopatric stickleback population (Jubilee) in experiment 1. (B) Mean total worm load of
591 parasite strains from Obse, Reiv and Scad on hosts from the two allopatric stickleback
592 populations in experiment 2. In experiment 2, each of the three parasite populations was
593 tested reciprocally on its sympatric and two allopatric hosts, but only their average measures
594 on allopatric hosts are used in this figure (i.e. Obse on Reiv and Scad: shaded; Reiv on Obse
595 and Scad: lined; Scad on Obse and Reiv: plain). Asterisks above the error bars represent
596 results of post hoc (LSD) tests indicating the presence of significant differences (* = $P \leq$
597 0.05, ** = $P \leq 0.01$, *** = $P \leq 0.001$).
598
599 Figure 2. Differences in the total worm load measured for each parasite population on its
600 sympatric and two allopatric host populations. (A) In experiment 2 each of Obse, Reiv and
601 Scad parasites was tested on three fish populations (Obse: shaded; Reiv: horizontally lined
602 and Scad: plain). (B) In experiment 3 Maga parasites were also tested on three fish
603 populations (Obse: shaded; Scad: plain and Maga: vertically lined).
604
605 Figure 3. Difference in the log transformed mean survival time (hours) of detached
606 gyrodactylids when incubated in water from their own (plain) and six different (shaded)
607 lakes: (A) represents data from all seven strains (Gill, Host, Maga, Obse, Reiv, Scad and
608 Torm) of the parasite while in (B), the saltwater strain (Obse) was excluded from the
609 analysis.
610
611 Figure 4. The relationship between the response variable 'total worm count' measured for
612 parasite populations in the lab (experiment 4) and: (A) host resistance scores of three

613 stickleback populations to two allopatric *Gyrodactylus* strains ('mean total worm count $^{-1}$' in

614 experiment 2) and (B) lake-water pH for seven lakes on North Uist.

615 Fig. 1



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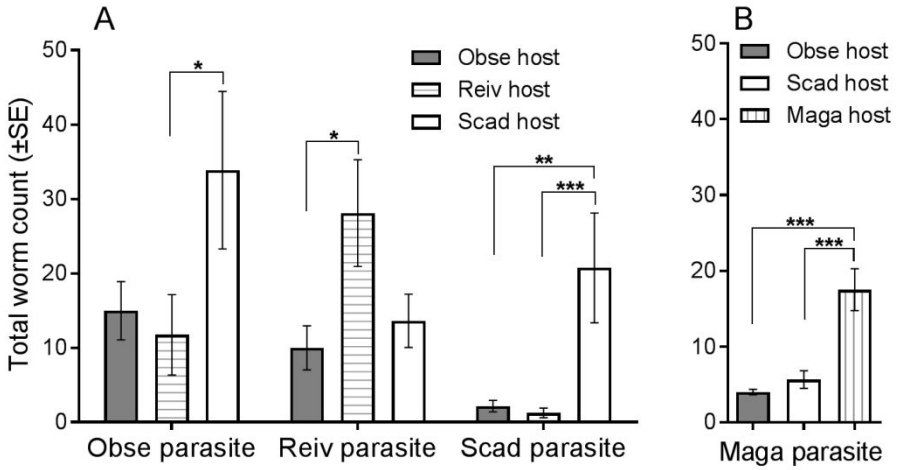
618 Fig. 2.



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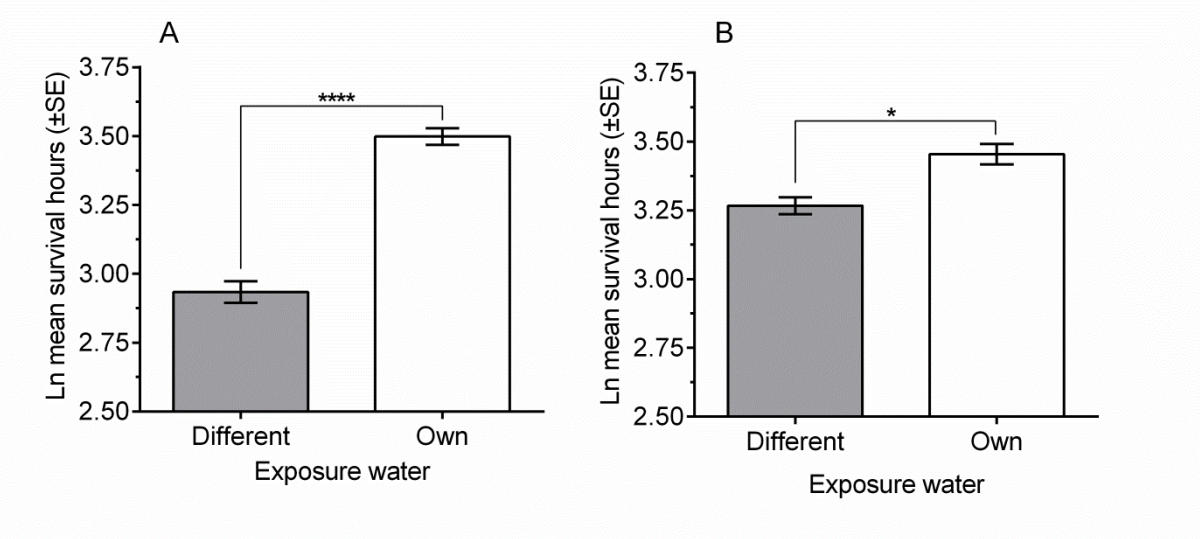
621 Fig. 3



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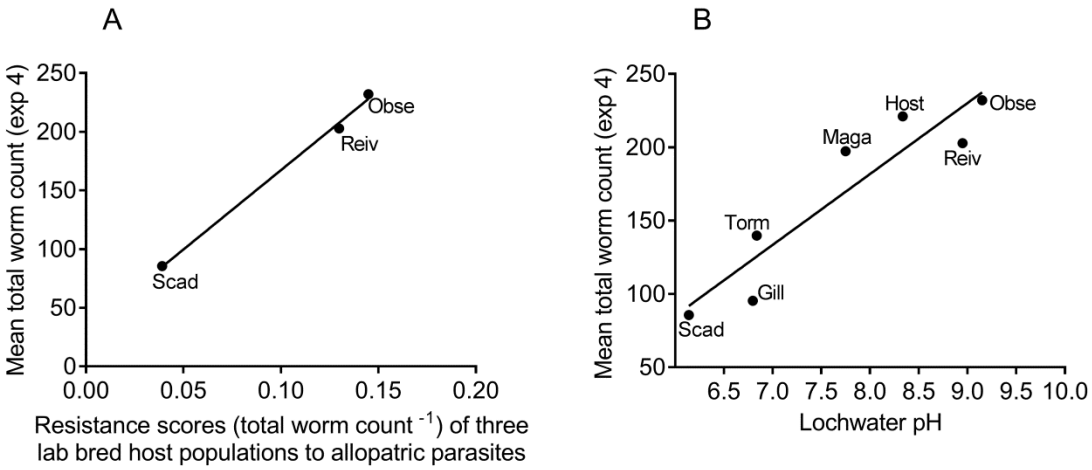
624 Fig. 4



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