Title: Functional immunogenetic variation, rather than local adaptation, predicts ectoparasite infection intensity in a model fish species

Running title: Immunogenetics predict infection intensity

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Keywords: host-parasite coevolution; major histocompatibility complex; *Poecilia reticulata*; *Gyrodactylus*; MHC supertypes

Abstract

Natural host populations differ in their susceptibility to infection by parasites, and these intra-population differences are still an incompletely understood component of host-parasite dynamics. In this study, we used controlled infection experiments with wild-caught guppies (*Poecilia reticulata*) and their ectoparasite *Gyrodactylus turnbulli* to investigate the roles of local adaptation and host genetic composition (immunogenetic and neutral) in explaining differences in susceptibility to infection. We found differences between our four study host populations that were consistent between two parasite source populations, with no indication of local adaptation by either host or parasite at two tested spatial scales. Greater host population genetic variability metrics broadly aligned with lower population mean infection intensity, with the best alignments associated with Major Histocompatibility Complex (MHC) 'supertypes'. Controlling for intra-population differences and potential inbreeding variance, we found a significant negative relationship between individual-level functional MHC variability and infection: fish carrying more MHC supertypes experienced infections of lower severity, with limited evidence for supertype-specific effects. We conclude that population-level differences in host infection susceptibility likely reflect variation in parasite selective pressure and/or host evolutionary potential, underpinned by functional immunogenetic variation.

Introduction

While hosts are under selection to combat pathogens, pathogens are under concurrent selection to overcome host defences (Woolhouse, Webster, Domingo, Charlesworth, & Levin, 2002; Schmid-Hempel, 2011). This interaction is ubiquitous (e.g., Poulin, 1999) and has consequences ranging across the evolution of host immune systems (Frank, 2002), epidemiology and the emergence of new infectious diseases (Ebert, 1994; Lively, 2016), adaptive radiation (Karvonen, 2012), the maintenance of sex (Morran, Schmidt, Gelarden, Parrish, & Lively, 2011), and conservation biology (Altizer, Harvell, & Friedle, 2003). Hosts within a population typically vary in their susceptibility to infection (Woolhouse et al., 2002), and, in spatially heterogeneous host-pathogen systems, host

50 populations often differ in their average observed susceptibility (Lively & Dybdahl, 2000; Eizaguirre &
51 Lenz, 2010; Brunner, Anaya-Rojas, Matthews, & Eizaguirre, 2017). Integrating these within- and
52 between-population interactions is important for understanding the evolutionary and
53 epidemiological consequences of these dynamic processes (Hess, 1996; Carlsson-Granér & Thrall,
54 2002; Smith, Ericson, & Burdon, 2003; Thompson, 2005; Soubeyrand, Laine, Hanski, & Penttinen,
55 2009; El Nagar & MacColl, 2016; Penczykowski, Laine, & Koskella, 2016; Brunner et al., 2017;
56 Schneider, Nilsson, Höjesjö, & Martin Österling, 2017). Host adaptation to local parasites, for
57 example, may constitute a barrier to gene flow between host populations, facilitating speciation (El
58 Nagar & MacColl, 2016), or increase host susceptibility to parasites transmitted from distant
59 populations or different species (Daszak, Cunningham, & Hyatt, 2000).
60 Less genetically variable host populations are often reported to be more susceptible to
61 infection (e.g., Gibson & Nguyen, 2020). Such associations may arise if genetically homogenous hosts
62 are easier for parasites to adapt to (reviewed in Radwan, Biedrzycka, & Babik, 2010; King & Lively,
63 2012) and/or because individuals with higher homozygosity – genome-wide or at immunity genes –
64 are more susceptible (e.g., Acevedo-Whitehouse, Gulland, Greig, & Amos, 2003; Ortego, Calabuig,
65 Cordero, & Aparicio, 2007; Luikart, Pilgrim, Visty, Ezenwa, & Schwartz, 2008). Differences between
66 hosts and parasites in the processes contributing to genetic diversity may thus play a critical role in
67 local coevolutionary outcomes. In turn, this may help explain why, while shorter generation times
68 and larger populations should give parasites the edge over hosts in local adaptation arms races
69 (Price, 1980; Gandon & Michalakis, 2002), the majority of reciprocal infection experiments report no
70 significant local adaptation by either parasite or host (approx. 56%; Greischar & Koskella, 2007).
71 Complementary tests of local reciprocal adaptation alongside data on immunogenetic markers thus
72 offer a potentially useful, but underutilised, approach for understanding patterns of infection within
73 and among host populations.
74 Here, we performed a controlled infection test for local adaptation using a model fish-
75 ectoparasite system, complemented with the study of two sets of highly polymorphic genetic

markers, one presumed to be neutral and the other known to be under intense selection from parasites. For the neutral marker set, we used microsatellites: a well-characterised and well-utilised set is available for our host species, and their poly-allelic nature makes them useful in direct comparisons with our marker under selection, the Major Histocompatibility Complex (MHC). Genes of the MHC encode molecules involved in immune responses in vertebrates (Klein, 1986), and decades of research has been devoted to the complex suite of selection pressures that maintain and promote the gene family's extreme polymorphism, which includes parasite-mediated selection (Snell, 1968; Spurgin & Richardson, 2010; Radwan, Babik, Kaufman, Lenz, & Winternitz, 2020), sexual selection (Penn & Potts, 1999; Ejsmond, Radwan, & Wilson, 2014), and selection acting on the MHC-linked sheltered load (van Oosterhout, 2009). The ecological pertinence of the MHC is well established, including numerous studies reporting associations between MHC alleles and resistance/susceptibility to parasites in wild systems (e.g., Schad, Ganzhorn, & Sommer, 2005; Fraser & Neff, 2010; Buczek, Okarma, Demiaszkiewicz, & Radwan, 2016; Kaufmann, Lenz, Kalbe, Milinski, & Eizaguirre, 2017). The role of MHC genes and MHC variability in causing differences in host resistance/susceptibility between populations is less well understood, with the hypothesis that populations with more MHC variants have lower parasite burdens supported by observational evidence (Meyer-Lucht & Sommer, 2009), mesocosm experiments (e.g., Eizaguirre, Lenz, Kalbe, & Milinski, 2012), and wild cage experiments (e.g., Bolnick & Stutz, 2017), but limited exposure-controlled experimental testing (but see Smallbone, Ellison, Poulton, van Oosterhout, & Cable, 2021). An important concept in the study of MHC evolution is that of 'supertypes' (STs), groups of MHC alleles that encode peptides with similar antigen-binding properties. STs may better capture the functional breadth of host defence than alleles or phylogenetic groupings. In ecological MHC studies, STs are usually assigned by statistical clustering (see Materials and Methods), but the concept is founded in laboratory immunology (Sidney, Grey, Kubo, & Sette, 1996; Sandberg, Eriksson, Jonsson, Sjöström, & Wold, 1998; Doytchinova & Flower, 2005).

Guppies (*Poecilia reticulata*) are tropical freshwater fish native to northern South America and the Caribbean, and have been an important model species in elucidating processes as diverse as sexual selection, predator-prey interactions, ecological competition, and, most relevant to the present study, host-parasite dynamics. Furthermore, their MHC has been well-characterised and well-studied (e.g., Fraser & Neff, 2010; Smallbone et al., 2021). Monogenean ectoparasites in the genus *Gyrodactylus* are widespread across wild guppy populations, but their prevalence varies greatly between and within populations, and through time (van Oosterhout, Harris, & Cable, 2003; Dargent, Scott, Hendry, & Fussmann, 2013; Stephenson, van Oosterhout, Mohammed, & Cable, 2015; Stephenson et al., 2017; Mohammed et al., 2020). The known pathogenicity of the parasites, coupled with the relative ease with which they can be maintained in a laboratory and used in exposure-controlled infection trials, make the guppy-*Gyrodactylus* system an excellent model for studying a wide range of host-parasite interactions, including the effects of parasitism on some of the processes described above (e.g., sexual selection, predator-prey; reviewed by Bakke, Cable, & Harris, 2007). In the present study, we use this highly tractable system to investigate (i) whether wild host populations show consistent differences in resistance across parasite strains, (ii) whether parasites are adapted to their local hosts and *vice versa*, (iii) what role inter-population variation in MHC traits may play in this dynamic, and (iv) whether MHC genotypes predict infection intensity at the individual level and if this varies between host populations/parasite lineages.

Cross infection methods and results

Methods

Host collection and rearing

We collected (hand seine) juvenile guppies (standard length 5-12 mm) from four wild populations in March 2016, two on Trinidad (Lopinot, ‘Lop’; Santa Cruz, ‘SC’) and two on Tobago (Scarborough Health Centre, ‘HC’; Roxborough, ‘Rox’; Table S1.1). Previous survey work of ours (unpublished) had shown that all sites had populations of gyrodactylids, and previous population genetic analyses have

127 shown significant neutral and MHC differentiation between all host population pairs, with
128 differentiation stronger between islands than within islands (Phillips et al., 2018; Herdegen-Radwan,
129 Phillips, Babik, Mohammed, & Radwan, 2021). At our field station in Tobago, we treated all fish with
130 salt water (15 ppt, 5 min) to kill any gyrodactylids (Schelkle, Doetjes, & Cable, 2011), confirmed by
131 briefly anaesthetising all fish (0.02% tricaine methanesulfonate; MS-222) and screening them under
132 a dissecting microscope with cold illumination multiple times over several days, according to
133 Schelkle et al. (2009). Each population was then reared in a separate aquarium (80 L, 50-100 fish per
134 aquarium) and fed daily with live *Artemia* nauplii and generic, pet-shop fish flakes (Aquarian®).
135
136 *Parasite collection and rearing*
137 In June 2016 we returned to each of the four sites and collected 50-60 guppies to act as gyrodactylid
138 donors for our experiment. The prevalence and intensity of gyrodactylid infections on these fish
139 were too low for our preferred protocol of infecting experimental fish with parasites straight from
140 the wild, and from sourcing parasites from all four populations, so we cultured *Gyrodactylus*
141 *turnbulli* from populations Lop and HC using fish from a gyrodactylid-free captive population. Details
142 of gyrodactylid species identification are in Appendix S1; see also (Cable & van Oosterhout, 2007a,b;
143 King, van Oosterhout, & Cable, 2009; Xavier et al., 2015). This 'farm' host population, maintained in
144 an 800L mesocosm, had been founded 18 months earlier by crossing captive-reared virgin females
145 from a Tobagonian population with males from a Trinidadian population (Appendix S1), and had
146 been verified as gyrodactylid-free at P, F1 and F2 generations. Neither founding population of the
147 farm stock features in the present study. Farm gyrodactylid (henceforth 'gyro') lineages were
148 established by briefly anaesthetising both a wild donor and a recipient fish, and, under a dissecting
149 microscope with cold illumination, bringing together their caudal fins (tail) until a single gyro moved
150 from the donor to the recipient. Any extra gyros that jumped were removed with watchmaker's
151 forceps. Donor and recipient were then separated and revived, and the recipient was moved to a
152 500 ml isolation container. After six days the procedure was repeated, using infected farm hosts to

153 make single-gyro infections on a fresh batch of farm recipients. Gyro cultures were subsequently
154 maintained by keeping 1-3 parasite-naïve recipients in an isolation container with an infected donor
155 for 3-4 hrs, then moving each new recipient to its own isolation container (after Stewart et al., 2017).
156 Farm hosts were fed fish flakes daily, with water changed every other day. All gyros used in
157 experimental infections could be traced to their original wild founder.
158
159 *Experimental design*
160 We performed exposure-controlled gyrodactylid infection trials (Cable & van Oosterhout, 2007a,b)
161 on the fish captured as juveniles in March 2016. Experimental infections were established by briefly
162 anaesthetising parasite donor (infected farm fish) and recipient and allowing two gyros to move to
163 the recipient (any extras were removed – see above). Recipients were measured (standard length)
164 before infection. All infected fish were females, with length ≥ 15.0 mm. Each experimental host was
165 kept in its own 500 ml isolation container at ambient shade temperature and fed with fish flakes
166 every other day. The day after infection (day 1), we anaesthetised each experimental host and
167 counted the number of gyros it carried and repeated this every other day thereafter until day 17 or
168 until the fish was observed to be gyro-free for five days. Each fish received a water change at the
169 time of screening. Every 12 hrs, we checked whether all fish were alive, increasing this to every 4 h if
170 a host's infection intensity rose above 70 gyros. Fish found dead were promptly preserved in 1 ml
171 absolute ethanol (changed as in Appendix S1). We preserved a fin clip (caudal fin, 2-4 mm^2; 0.3 ml
172 absolute ethanol) from all fish that survived the experiment. Any fish that cleared its infection within
173 the first seven days was re-infected 4-6 days after first being recorded as 'clear', as rapid clearance
174 may be a stochastic effect of gyro quality (further details below). We initiated experimental
175 infections in two blocks four days apart, balanced by fish and gyro population. All reinfections were
176 initiated six days after the second block.
177
178 *Genetic analyses*

We extracted genomic DNA (20-100 ng per sample) from guppy fin clips using MagJET Genomic DNA kits (Thermo Scientific). We then PCR amplified a 217 bp fragment of the Major Histocompatibility Complex (MHC) class II second exon, which codes for the highly polymorphic β-chain of the MHC molecule's antigen binding groove (primers, reagents, and PCR conditions as in Phillips et al. 2018). The PCR included the fusion primers required for DNA sequencing with an IonTorrent Personal Genome Machine (PGM; Life Technologies), as well as a unique combination of 6 bp tags (20 tags = 400 potential F×R combinations) for each amplicon (fish). After amplification, we pooled amplicons approximately equimolarly and sequenced the pool (PGM). We then used the adjustable clustering method of Biedrzycka *et al.* (2017), implemented in the software AmpliSAS (Sebastian, Herdegen, Migalska, & Radwan, 2016; parameters as Phillips et al., 2018), to turn raw sequence data into individual genotypes. We followed this up by allocating MHC alleles to the supertype (ST) groups of Herdegen-Radwan *et al.* (2021), based on physicochemical properties at positively selected sites reported by Lighten *et al.* (2017).

Copy number variation at the MHC means that the locus affinity (phasing) of alleles is rarely known, but an earlier cross-breeding experiment (Phillips et al., 2018) allowed us to phase all of the alleles in this study's focal populations. That earlier experiment reported a single linkage block of 1-3 alleles in a *de facto* single locus, though only 1-2 alleles per block feature in the present study. We use the term 'superhaplotype' to refer to haplotypes based on STs rather than alleles.

To provide a proxy for neutral genetic variation, all hosts in the experiment were genotyped at 15 microsatellite loci (Becher, Russell, & Magurran, 2002; Watanabe, Yoshida, Nakajima, & Taniguchi, 2003; Olendorf, Reudi, & Hughes, 2004; Shen, Yang, & Liao, 2007), of which eight were used in the main analyses (Appendix S2). These loci are routinely used in guppy behavioural ecology and population genetics, including for comparisons against MHC variability (e.g., Lighten et al., 2017; Herdegen-Radwan et al., 2021).

Statistical analyses

To assess how host population and parasite population affected the outcome of infection trials, without considering any explicitly genetic predictors, we first tested for biases in host death rate. For this, we used contingency table-based analyses (χ^2 and Fisher's exact tests), as we considered the death rate (5/113 fish, 4.4%) too low for logistic regression. We then tested for effects of host population and parasite population on the number of 'worm-days' experienced by fish that survived the experiment. Worm-days were calculated as the area under a fish's infection trajectory graph (number of gyros against time), and are both ecologically pertinent and statistically tractable – more worm-days can reasonably be considered a more intense infection, and the metric avoids needing to consider time series, temporal autocorrelation, zero inflation, or individual-level random effects (Phillips et al., 2018). For fish that were re-infected, we retained the infection that reached the highest peak intensity (details in Appendix S3).

Worm-days were analysed using general linear models (LMs; Gaussian errors) on $\log_e$-transformed worm-days, and multi-model inference implemented in the *MuMIn* package (Bartoń, 2016) of the statistical software 'R' (R Development Core Team, 2016). We opted for LMs over the generalised linear models with negative binomial errors used by Phillips *et al.* (2018) because the interpretation of LMs is usually more intuitive (e.g., use of R^2 to quantify the proportion of explained variation), their *post hoc* options are more tractable and more widely known, and because, in the present study, LMs tended to produce residuals that slightly better reflected a normal distribution. In the supplementary material, we show that no interpretive differences would have arisen had we used negative binomial models (Appendix S4). We used corrected Akaike Information Criterion (AIC_C) to rank models with all combinations of the following parameters: host population (factor, four levels); parasite population (factor, two levels); the interaction between host and parasite population; temperature (factor, three levels corresponding to date of infection; empirical temperature data and rationale for factor in Appendix S5); and fish size (standard length; continuous, z-transformed). If the top-ranked model was more than two AIC_C units clear of the second model, we deemed it the nominal best model and examined its coefficients for values

231 significantly different from zero. If more than one model comprised the top two units of AIC_C, we
232 used AIC_C-weighted model averaging to estimate coefficients and their significance, implemented in
233 *MuMIn*. We inspected LM assumptions for all models by adding five LM diagnostic statistics to the
234 summary tables produced by *MuMIn*: skewness and kurtosis of residuals; Kappa and maximum
235 variance inflation factor to assess multicollinearity; and the maximum Cook's distance value to check
236 for influential data points.

237 As alternatives to using host population and parasite population, we tested two predictors
238 based on host-parasite allopatry/sympatry: one in which infections were considered 'sympatric' if
239 the host and parasite came from the same stream, and the other in which infections were
240 considered 'sympatric' if the host and parasite came from the same island (i.e. Trinidad or Tobago).
241 In exploratory analyses, we did not find a significant effect of parasite lineage within source location
242 (Appendix S6).

243 As an indicator of possible relationships between population-level genetic variability and
244 worm-days, we calculated the r_SP_c ordered heterogeneity statistic (Rice & Gaines, 1994) for five
245 population-level genetic diversity metrics: phased and unphased diversity for MHC alleles and STs,
246 and mean microsatellite diversity. Significant r_SP_c values suggest an effect of a categorical predictor
247 that is directional with respect to the ranking of categories by a third variable. Metrics were not
248 derived from the present study's genotyped fish but from the much larger population genetics
249 dataset of Herdegen-Radwan et al. (2021). We assess additional population variability metrics in
250 Appendix S7.

251 Using all host populations and restricting data to amplicons with ≥ 300 MHC sequence reads,
252 we tested for individual-level effects of number of MHC alleles and STs, and included interactions
253 with host and parasite population in the model ranking procedure. As a measure of background
254 individual genetic variability, we used 1 - [homozygosity-by-loci] (henceforth 'HL'). This weights loci
255 by their individual expected heterozygosity (H_E) when calculating multilocus heterozygosity, which
256 may better capture background genetic variability when a microsatellite panel is small (Aparicio,

Ortego, & Cordero, 2006) (in Appendix S8 we have repeated the analyses with alternative microsatellite metrics that apply different weightings, out of which HL produced the better fits). HL was calculated separately for each fish population, using allele frequencies from the experiment's genotyped fish. No population showed significant identity disequilibrium across the eight loci ($g_2 \leq 0.07$, $P \geq 0.40$; Table S2.3; g_2 tests performed in *inbreedR* (Stoffel et al., 2016), meaning there was not significant variance in individual hetero-/homozygosity. Similarly, HL was not a significant predictor of MHC heterozygosity, although the relationship was positive (logistic regression: 1.67, SE = 1.57, z = 1.07, $P = 0.29$; note that we refer here to Mendelian heterozygosity of phased haplotypes, and not, as is often the case in the MHC literature, to the number of MHC variants that an individual carries).

To test whether any difference in effect size between MHC alleles and STs could be an artefact of aggregating a large number of alleles into a smaller number of STs, we compared the observed coefficient for number of STs to a simulated distribution derived from repeating the model after reallocating alleles to STs at random (adapted from Lighten et al., 2017; Herdegen-Radwan et al., 2021). We used the 335 alleles and 15 STs of Herdegen-Radwan *et al.* (2021), and randomised ST membership size from a Dirichlet-multinomial distribution (all $\alpha = 1$; Appendix S9). To test whether the interpretation of HL was disproportionately influenced by any one microsatellite locus, we re-ran the best model to include HL with jack-knife removal of each locus.

Finally, we tested for effects of specific supertypes on individual infection intensity. If a variant had at least three occurrences in more than one host population, we tested for an across-population general effect, as well as the respective interaction. Population-specific analyses were performed for all variants with 3+ instances in a population. If a variant had at least two occurrences for each gyro source within a host population, we tested the interaction. We did not analyse death rate with respect to individual MHC genotype, as only two dead fish gave MHC amplicons that met our genotyping quality criteria (details below).

Ethics statement

This experiment was conducted in accordance with Cardiff University's UK Home Office Licence PPL 303424. Tobago-sourced wild fish were collected under Tobago House of Assembly Permit #004/2014. Permission to collect fish in Trinidad was granted by the Fisheries Division of the Ministry of Food Production and Fisheries, and fish were collected only from areas where guppies were previously identified as abundant.

Results

We successfully infected 113 guppies with *Gyrodactylus turnbulli*, with 12-16 fish in each experimental block (four fish sources × two gyro sources; Fig. 1). Overall mortality was low (five fish, 4.4%), and was entirely accounted for by fish from the Tobagonian populations of Scarborough Health Centre ('HC'; 2/32, 6.3%) and Roxborough ('Rox'; 3/27; 11.1%), with no fish dying from the Trinidadian populations Lopinot ('Lop'; 0/27) and Santa Cruz ('SC'; 0/27). Mortality was not significantly biased by either host population (bootstrap χ^2: $\chi^2 = 5.61$, reps = 100k, $P = 0.13$) or host island (Fisher's exact test: $P = 0.06$). Mortality by parasite source was evenly split (HC = 2/54, 3.7%; Lop = 3/59, 5.1%), and did not differ significantly across the two affected host populations (Fisher's Exact test: $P = 1$).

The top-ranked of worm-days among the 108 fish that survived the experiment, excluding genetic predictors, contained fish source, fish standard length, and an interaction between the two. Together, these explained 84% of variance (Tables 1a-c). All pairwise differences between fish populations were significant in a *post hoc* test ($P \leq 0.001$; 'glht' function of R package *multcomp*; Hothorn, Bretz, & Westfall, 2008), with the rank order, in increasing infection intensity, of SC, Lop, HC, Rox (Fig. 2A; Tables 1b-c; infection trajectories in Appendix S11). Larger fish experienced significantly more worm-days than smaller fish, though the slope differed significantly between populations. Although fish in SC were significantly smaller than the other three populations, multicollinearity was not problematic (Appendix S12). The only other model in the top two units of

309 AIC_C (ΔAIC_C = +1.85) added parasite source but with a non-significant coefficient (*P* = 0.47; Fig. 2B;
310 Table 1b). There was poor support for any effect of temperature (ΔAIC_C = +3.59) or for any model
311 that might indicate local adaptation by host or parasites ($\Delta AIC_C \geq +3.37$; Table 1a). Restricting local
312 adaptation analyses to the two host sources from which parasites were collected (HC and Lop)
313 changed the composition of the top set of models but did not produce a qualitative change in
314 interpretation (Appendix S13).

315 All population-level genetic diversity metrics had negative $r_S P_c$ values against population
316 mean worm-days, meaning populations with higher genetic diversity tended to have lower mean
317 infection intensity (Table 2). The effect for microsatellite diversity was weaker than for STs (Table 2).
318 Tests of additional genetic variability metrics are given in Appendix S7.

319 For the analysis of the impact of individual-level genetic predictors on infection intensity, we
320 used the MHC and microsatellite genotypes of 93/108 fish (86.1%) that survived the experiment and
321 met our genotype quality criteria. We recognise the theoretical possibility for the untyped fish to be
322 a biased subset of genotypes, but the two supplementary analyses in Appendix S14 suggest that if
323 such a bias exists, it is not problematic for our interpretation. The number of MHC STs was in all
324 three models comprising the new top model set (Table 3a). Its model-averaged coefficient was
325 significantly lower than zero (Tables 3a-b), meaning individuals carrying more STs experienced
326 infections of lower intensity. Microsatellite HL (our measure for overall genetic variation) was
327 present in the second model (ΔAIC_C = +0.44; Table 3a), with a non-significant negative coefficient
328 (Table 3b). Differences between populations in mean infection intensity were consistent with those
329 of the non-genetic analysis (Tables 1a-b; see also Appendix S14). Fish length retained its net positive
330 relationship with worm-days, but its interaction with fish source was no longer in all top-set models
331 (AIC_C weight = 0.247; Table 3a). Gyro source was not in the top set, i.e. excluding it produced better
332 fits. The first model to include number of MHC alleles had ΔAIC_C = +4.65.

333 The stronger effect of number of STs relative to number of alleles was significantly greater
334 than expected from randomised grouping of alleles (obs. coef. = -0.36, exp. = -0.15 ± 0.09 [SD], n.

randomisations = 10k, $P = 0.011$; details in Appendix S9), meaning it is unlikely to be a side-effect of aggregating alleles into STs. No microsatellite locus exerted a disproportionate influence on HL in jack-knife removal (Appendix S10).

Five supertypes were carried by 3+ fish in at least two populations (Tables 4-5) and were thus available for cross-population testing for effects of specific STs on infection intensity. Three of these STs were in their respective top model set and two were in their top-ranked model, but none were present in all top-set models or had a significant coefficient in their best model (Table 5; see also Appendix S15). There is thus no strong support for any particular ST having a general resistant/susceptible effect.

For population-specific tests for effects of particular supertypes, there were 17 testable instances (3+ carriers in a population; 2-6 STs per population; Table 5). Of these, six were in their population's top model set (Table 6), of which only one was present in all models of a top set or had a significant coefficient. Lop hosts carrying ST12 experienced significantly fewer worm-days, but only when infected with Lop (i.e., local) gyros. However, this result should be treated with caution, as the interaction is based on the minimum allowable sample size of two carriers per gyro source.

Discussion

In our controlled parasite infection trials, we found no evidence for local host or parasite adaptation. This contrasts with evidence for local adaptation to parasites in other fish systems (El Nagar & MacColl, 2016; Bolnick & Stutz, 2017), but is not out of keeping with the multi-taxa meta-analysis of Greischar & Koskella (2007) in which studies reporting no significant overall local adaptation slightly outnumber (56%) those that do. However, host population, but not parasite population, was a strong, significant predictor of the intensity of infection experienced by hosts. Comparable results of consistent differences between host populations have been reported in other fish species (e.g. Konijnendijk, Raeymaekers, Vandeuren, Jacquemin, & Volckaert, 2013; Pérez-Jvostov, Hendry, Fussmann, & Scott, 2015; Weber et al., 2017), as well as in laboratory guppies infected with the

same parasite species (Smallbone et al., 2021). The precise reasons for such differences are usually unclear, though populations of stickleback (*Gasterosteus aculeatus*) have been shown to differ in gene expression profiles in response to *Gyrodactylus* spp. infection (Brunner et al., 2017; Robertson, Bradley, & MacColl, 2017). In our study, infection intensity was associated with both population- and individual-level genetic variability of hosts. Contrasts between the effects of MHC physicochemical supertypes (STs), MHC alleles, and microsatellites indicate a functional role of the MHC in explaining these patterns, whilst also suggesting the MHC is not the whole story.

Higher host population genetic diversity was associated with lower population mean infection intensity. These associations were significant for MHC STs and microsatellites, but not for MHC alleles. Several previous studies have reported evidence for positive correlations between pathogen diversity and MHC polymorphism and/or positive selection in cyprinids (Šimková, Ottová, & Morand, 2006), birds (Minias, Pikus, Whittingham, & Dunn, 2018), rodents (de Bellocq, Charbonnel, & Morand, 2008), and primates (Garamszegi & Nunn, 2011). Intraspecies, inter-population examples include MHC polymorphism correlating positively with pathogen vector and ectoparasite abundance in the lizard *Ctenophorus ornatus* (see Radwan, Kuduk, Levy, LeBas, & Babik, 2014), and with length of time in which rabies has been present in racoon populations (*Procyon lotor*) (see Kyle et al., 2014). However, these examples should be compared to our study with caution, as all are observational/ecological rather than exposure-controlled experiments, and all focus on MHC alleles without a parallel assessment of STs. The significant relationship for MHC STs, which agrees with another recent guppy-gyrodactylid-MHC study (Smallbone et al., 2021), suggests a history of stronger selection on functional MHC. Our observed relationship could also result from population demographic histories affecting diversity by drift, with which the significant alignment with microsatellite diversity would be consistent. Drift and selection, though, are not mutually exclusive (e.g., *C. ornatus* ; see Radwan et al., 2014), and the much wider range of ST diversity relative to microsatellite diversity (0.14-0.84 v. 0.47-0.53 respectively) is hard to reconcile with drift being dominant over selection on MHC functionality. Moreover, MHC allele diversity (range 0.74-

387 0.91), which is known to be sensitive to drift (Radwan et al., 2010; McMullan & van Oosterhout,
388 2012; Lighten et al., 2017; Herdegen-Radwan et al., 2021), was not significantly aligned with
389 infection load.

390 Further evidence supporting the role of functional MHC diversity comes from our analyses of
391 individual infection intensities. Stronger selection by parasites should not only maintain more
392 variants in a population, but should also select for more variants expressed by an individual. The
393 latter will be determined by both zygosity and the number of MHC genes in haplotypes (Minias,
394 Wojczulanis-Jakubas, Rutkowski, & Kaczmarek, 2015; Bentkowski & Radwan, 2019), as more variants
395 should widen the range of antigens that can be detected and responded to. Such effects have been
396 reported in numerous other studies (Carrington et al., 1999; Penn, Damjanovich, & Potts, 2002;
397 Oliver, Telfer, & Piertney, 2009; Radwan et al., 2012; Pierini & Lenz, 2018), but others have found no
398 such effect (Phillips et al., 2018), some have found the reverse (Ilmonen et al., 2007; Schwensow,
399 Fietz, Dausmann, & Sommer, 2007; Sepil, Lachish, Hinks, & Sheldon, 2013), and, in species with high
400 numbers of duplicated MHC loci, some have found intermediate numbers of MHC alleles to be
401 associated with the lowest infection burdens (Wegner, Kalbe, Kurtz, Reusch, & Milinski, 2003;
402 Wegner, Reusch, & Kalbe, 2003; Madsen & Ujvari, 2006; Wegner, Kalbe, Milinski, & Reusch, 2008;
403 Kloch, Babik, Bajer, Sinski, & Radwan, 2010). In our experiment, individuals carrying more MHC STs
404 experienced significantly fewer worm-days: back-transformed from the $\log_e$ scale (Table 3b), one
405 extra ST predicts a reduction in worm-days over the study period by 29.6%. This effect was not
406 confounded by differences in ST diversity between host populations – it applied within each
407 population, and without a significant interaction. Moreover, it did not interact with parasite
408 population, and, importantly, was independent of multilocus neutral (microsatellite) heterozygosity.
409 In contrast, the effect of number of MHC alleles was relatively weak (19.0% worm-day reduction,
410 and non-significant), producing a poorer fit than a model with no genetic predictors. The ST effect is
411 unlikely to be a consequence of aggregating alleles into STs, as the coefficient is significantly more
412 negative than expected if alleles are clustered into STs at random. Collectively, this suggests that the

413 relationship between the number of STs and infection intensity may be causal rather than
414 correlational. If gyrodactylid infections select for individual-level functional MHC variability in
415 guppies, our analysis of individual-level predictors supports a role for differences in past selection by
416 parasites in causing differences between host populations in susceptibility to infection.
417 Interestingly, microsatellite variability itself also showed a negative effect, which, though
418 weak, produced an improvement of fit when added to a model that already included number of STs.
419 Moreover, the addition of microsatellite variability neither weakened the effect of number of STs
420 nor produced problematic multicollinearity. As with number of STs, the effect was not confounded
421 by between-population differences in variability. This suggests a general heterozygosity-fitness
422 correlation (HFC), of which there are numerous examples relating to infection susceptibility in both
423 genome-wide terms (e.g., Acevedo-Whitehouse et al., 2003; Luikart et al., 2008; Eastwood et al.,
424 2017) and with respect to other families of immune system genes (e.g. Hellgren, Sheldon, &
425 Buckling, 2010; Lara et al., 2020; Levy et al., 2020). The relative weakness of this effect (an entirely
426 heterozygous individual would experience 1.7% fewer worm-days than an entirely homozygous
427 individual) may be due to limited within-population variance in genome-wide heterozygosity (non-
428 significant identity disequilibrium; David, Pujol, Viard, Castella, & Goudet, 2007) coupled with a small
429 number of loci. However, neither of these caveats justifies outright dismissal of our observed effect:
430 although g_2 correlates with HFC effect size (e.g., Miller & Coltman, 2014), HFCs can reach significance
431 before g_2 does (Szulkin, Bierne, & David, 2010), and weak microsatellite HFCs can hint at effects that
432 become much stronger when large panels of neutral SNPs are available (e.g., Hoffman et al., 2014).
433 That we found a strong effect of the number of MHC STs but only weak evidence for effects
434 of particular STs is likely a result of our limited power to detect the latter. Few MHC variants were
435 shared among populations at high enough frequency to carry out meaningful analyses against the
436 relatively small sample sizes (n = 12-16) of each host × parasite treatment block. Previous work has
437 shown that the guppy-gyrodactylid system is capable of producing such effects, but also that those
438 effects are context-dependent (Smallbone et al., 2021). The nominal best evidence in our study is

439 also context-dependent – ST12 (which was not one of those with a significant effect in Smallbone et
440 al. 2021) conferred resistance to Lopinot parasites, but in Lopinot hosts and not Santa Cruz hosts.
441 The lack of a significant effect of parasite source population, or of any interaction with host
442 population, appears inconsistent with previous work on this study system showing that MHC
443 variants that were novel to a given host population conferred a significant advantage in resisting
444 local parasites (Phillips et al., 2018). That finding, which used replicated population crosses to
445 control for non-MHC genetic background, and which did not find significant interactions associated
446 with those crosses, hinges on parasites being adapted to their local host's MHC composition. Host
447 populations likely segregate at other gene families reported to be involved in fish immune responses
448 to ectoparasites (Lindenstrøm, Secombes, & Buchmann, 2004; Skugor, Glover, Nilsen, & Krasnov,
449 2008; Zhou, Lin, Pang, Shan, & Wang, 2018; Konczal et al., 2020), and effects of these loci may mask
450 any effect of MHC novelty in the present study. The results of a recent translocation study on
451 sticklebacks imply such background effects (Bolnick & Stutz, 2017). While MHC alleles that were
452 common in a local host population were more susceptible to local parasites (consistent with an
453 advantage from introgressing MHC alleles), immigrant fish experienced higher parasite loads when
454 MHC genotype was controlled for (Bolnick & Stutz, 2017). The lack of signal of local adaptation in our
455 study may thus result from opposing signals of MHC and other genomic regions affecting resistance
456 to gyrodactylids.
457 An alternative explanation for the lack of a significant effect of parasite source population in
458 our study is the culturing of parasites ('gyro farming'). HC and Lop gyrodactylids were both cultured
459 on a single, separate lineage of hosts for 18 days (3-4 farm cycles; potentially 9+ parasite
460 generations) in order to obtain sufficient numbers for the experimental infections. This may have
461 caused artificial selection, either on ability to infect novel hosts or to a specific set of host immune
462 genotypes. However, we think this explanation is unlikely, as it would require the effective erasure
463 of many generations of local adaptation in two large, natural populations of parasites, and it would

require that this be achieved in a short time, with limited starting genetic variance (each culture lineage was founded from a single animal), on parasite-naïve hosts.

Host mortality in the experiment was low and restricted to the two Tobago fish populations, and was not significantly biased by gyro origin. Low host mortality likely reflects our experiment's relatively benign conditions. In the wild, gyrodactylid infections expose guppies to a suite of additional selection pressures, including other infections, anything requiring efficient swimming (e.g., escape from predators and surviving flood events; van Oosterhout et al., 2007; Stephenson, Kinsella, Cable, & van Oosterhout, 2016), and reduced reproductive opportunities (Kennedy, Endler, Poynton, & McMinn, 1987).

Overall, our results suggest that pathogens may select for higher numbers of MHC supertypes at an individual level, and previous work implies that it can also promote MHC polymorphism within populations, independently of benefits derived from simply carrying more variants (Phillips et al., 2018). Both of these effects will lead to differences between populations in functional immunogenetic diversity. The wider genomic implications of such selection (e.g., interactions with other immunity genes, effects on neutral genetic variability, and the shaping of MHC phylogenetics) requires further investigation. While this is a considerable effort, our results highlight that our understanding of infection dynamics will remain incomplete unless we appreciate the differences in the history of selection imposed by pathogens. A lack of such understanding may limit our ability to predict consequences of emergent diseased threatening humans and wildlife (Altizer et al., 2003; Penczykowski et al., 2016; Stephenson et al., 2017; Chabas et al., 2018; Ekroth, Rafaluk-Mohr, & King, 2019), and further research in this area should underpin the One Health approach (Daszak et al., 2000) in the coming decades.

Acknowledgements

We thank the staff of the Environmental Research Institute Charlotteville ('ERIC', Tobago) and Marcin Radwan for support with guppy catching and rearing, Pat Turpin for renting us the field

station, Julia Olechnowicz and Jarek Raubic for assistance with molecular work, and Joshka Kaufmann for feedback on earlier manuscript drafts. This project was funded by a Polish National Science Centre Harmony grant to JR, CvO and JC (grant number UMO-2013/10/M/NZ8/00253). KPP is supported by Science Foundation Ireland.

Author contributions

JR, CvO and JC conceived the study, with input from KPP and RSM on experimental design. JR and RSM collected the fish, which RSM reared; RSM and KPP collected and reared parasites; KPP and SC performed infection trials, with assistance from KJP and RSM; SC and KJP assisted molecular work; KPP analysed the data; JR and KPP wrote the manuscript, with input from all co-authors. The authors declare no competing interests.

Data accessibility

All data and scripts will be made available in accordance with the publisher's requirements.

List of supplementary material

Appendix S1. Origins of guppy and gyrodactylid populations used in the cross-infection experiment.

Appendix S2. Details of microsatellite genotyping, including locus summary statistics, Hardy-Weinberg equilibrium and linkage disequilibrium tests, and identity disequilibrium test.

Appendix S3. Tests for biases in hosts requiring re-infection.

Appendix S4. Demonstration of comparability between linear models of $\log_e$-transformed worm-days and generalised linear models of untransformed worm-days with negative binomial errors.

Appendix S5. Summaries of empirical water temperature data during the experiment, and rationale for modelling temperature as a three-level factor in the main analyses.

Appendix S6. Assessment of potential effect of within-river parasite lineage.

Appendix S7. Additional population-level genetic variability metrics tested as predictors of population mean infection intensity.

Appendix S8. Expansion of analyses regarding the relationship between individual-level genetic variability and infection intensity using alternative metrics of microsatellite variability.

Appendix S9. Expanded description of randomisation procedure used to test if the difference in effect size between number of supertypes and number of alleles can be accounted for by diversity reduction.

Appendix S10. Jack-knife tests for over-influential microsatellite loci.

Appendix S11. Visualisation of average gyrodactylid infection trajectories by host source (Fig. S11.1) and parasite source (Fig. S11.2).

Appendix S12. Exploration of the implications of collinearity between fish population and length.

Appendix S13. Repeating main cross-infection analysis on only hosts from the two populations from which parasites were sourced (Lopinot and Health Centre).

Appendix S14. Are individuals that were not successfully genotyped a biased subset of the infection intensity data?

Appendix S15. Multi-model inference tables for tests of specific MHC supertypes, as referred to by Tables 4-6 in main document.

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Table 1a. Selected models of the number of 'worm-days' experienced by guppies infected with *Gyrodactylus turnbulli* during our experiment, ranked by corrected Akaike Information Criterion (AIC_C). Model ranking was conducted without genetic predictors and includes four fish source populations (see Table S13.1a for version restricting analysis to the two fish sources from which the experiment's gyros came). Selected models are: anything in the top two units of AIC_C (lowest AIC_C); the first model outside of this top set; the best model to include an interaction between fish source and gyro source; the best model not to include length; and the best model not to include fish source. Models are general linear models (Gaussian error distribution) of $\log_e$-transformed worm-days. For continuous predictors, we give the regression slope when the parameter is present without an interaction, and '+' when it is part of an interaction; for categorical predictors and interactions, we indicate inclusion with '+'. Coefficients for fish length are for z-transformed data. Importance is by Akaike weight and applies only to models in the top two units of AIC_C. 'Res. skew.' and 'Res. kurt.' are skewness and kurtosis of model residuals (Gaussian distribution has 0 and 3 respectively); 'Max. Cook' is the maximum Cook's distance value, an indicator of over-influential data points; 'Max. VIF' (maximum variance inflation factor) and 'Kappa' are multicollinearity diagnostics. Model-averaged coefficients are in Table 1b. *Post hoc* pairwise comparisons between all levels of fish source in the top-ranked model are in Table 1c.

Intercept	Fish source	Length	Gyro source	Fish × length	Fish × gyro	R^2	Res. skew.	Res. kurt.	Max. Cook	Max. VIF	Kappa	df	AIC_C	Weight
2.47	+	+	-	+	-	0.835	-0.19	2.81	0.24	5.45	13.64	9	230.47	0.716
2.43	+	+	+	+	-	0.834	-0.20	2.82	0.23	5.51	13.26	10	+1.85	0.284
2.75	+	0.34	-	-	-	0.825	-0.16	2.90	0.14	1.41	9.31	6	+2.14	-
2.59	+	0.35	+	-	+	0.832	-0.13	2.96	0.12	19.41	20.53	10	+3.37	-
2.46	+	-	-	-	-	0.796	0.24	3.77	0.10	-	5.05	5	+17.90	-
4.34	-	0.60	-	-	-	0.127	0.45	2.41	0.06	-	1.00	3	+172.50	-
Sum of weights	1.00	1.00	0.28	1.00	-									

Table 1b. Model-averaged coefficients for models comprising the top two units of AIC_C model ranking of predictors of the number of worm-days experienced by guppies infected with *Gyrodactylus turnbulli* during our experiment, in models without genetic predictors and including four fish source populations (Table 1a). Fish source coefficients are given in reference to Santa Cruz. The interaction between fish length (z-transformed) and fish source was present in all models contributing to the model averaging, and is also given with Santa Cruz as the reference level. The gyro source coefficient is given with reference to Lopinot. Sum of weights (a.k.a. 'importance') is sum of Akaike weight. All *P*-values are two-tailed. *Post hoc* pairwise comparisons between all levels of fish source are in Table 1c.

Term	Slope	SE	z	*P*	Sum of weights
Intercept	2.46	0.18	13.37	<0.001	-
Fish source					
Lopinot	1.06	0.24	4.51	<0.001	1
HealthCentre	2.22	0.23	9.79	<0.001	1
Roxborough	4.08	0.23	17.90	<0.001	1
Fish length (× fish source)					

Fish length (SantaCruz)	0.01	0.15	0.04	0.969	1
× Lopinot	0.26	0.24	1.07	0.286	1
× HealthCentre	0.53	0.19	2.77	0.006	1
× Roxborough	0.49	0.24	2.09	0.037	1
Gyro source					
HealthCentre	0.10	0.13	0.72	0.469	0.284

Table 1c. *Post hoc* pairwise comparisons of number of worm-days ($\log_e$-transformed) between all levels of fish source in the top-ranked model of Table 1a.

Term	Mean diff.	SE	t	*P*
Lopinot - SantaCruz	1.06	0.23	4.58	<0.001
HealthCentre - SantaCruz	2.22	0.22	9.93	<0.001
Roxborough - SantaCruz	4.08	0.22	18.13	<0.001
HealthCentre - Lopinot	1.16	0.2	5.75	<0.001
Roxborough - Lopinot	3.01	0.2	14.85	<0.001
Roxborough - HealthCentre	1.85	0.19	9.56	<0.001

Table 2. Ordered heterogeneity testing by r_SP_c (Rice & Gaines, 1994) of host population-level genetic diversity metrics against population mean number of worm-days. r_SP_c is calculated as the Spearman correlation coefficient (r_S) multiplied by 1 - (*P*-value for the overall effect of host population). *P*-values for r_SP_c are for a four-level factor. The host population columns (SantaCruz-Roxborough) are ordered to that mean worm-days increases reading left to right (see also Tables 1a, 1c). Additional metrics are tested in Appendix S7. All *P*-values are two-tailed.

Metric	Marker class	MHC phasing	SantaCruz	Lopinot	HealthCentre	Roxborough	r_SP_c	*P*
ST div.	MHC ST	Unphased	0.84	0.83	0.72	0.14	-1.00	<0.001
Msat div.(8)	Microsat.	-	0.53	0.52	0.51	0.47	-1.00	<0.001
S.hap. div.	MHC ST	Phased	0.84	0.84	0.72	0.14	-0.95	0.005
Haplo. div.	MHC allele	Phased	0.88	0.90	0.74	0.79	-0.60	0.098
Allele div.	MHC allele	Unphased	0.89	0.91	0.74	0.79	-0.60	0.098

Table 3a. Models comprising the top two units of corrected Akaike Information Criterion (AIC_C) model ranking of predictors of the number of 'worm-days' experienced by guppies infected with *Gyrodactylus turnbulli* during our experiment, assessing models that were allowed to include an MHC and a microsatellite individual-level

genetic variability metric in addition to fish source (four-level factor). To provide additional context, we include: the first model outside the top set; the first model to include each genetic predictor not present in the top set; and the first model to include no genetic predictors. Models are general linear models (Gaussian errors) of $\log_e$-transformed data. For continuous predictors, we give the regression slope when the parameter is present; for categorical predictors, we indicate presence with '+'. The coefficient for fish length is for z-transformed data. Sum of weights (a.k.a. 'importance') is by Akaike weight and applies only to models in the top two units of AIC_C. Model-averaged coefficients are in Table 3b.

Intercept	Fish source	Length	N MHC STs	N MHC als	Msat HL	Gyro source	Fish × length	R^2	Res. skew.	Res. kurt.	Max. Cook	Max. VIF	Kappa	df	AIC_C	Weight
2.82	+	0.42	-0.36	-	-	-	-	0.810	-0.23	2.91	0.12	1.66	9.07	7	202.85	0.417
2.85	+	0.44	-0.37	-	-0.10	-	-	0.812	-0.17	2.93	0.11	1.71	10.76	8	+0.44	0.336
2.56	+	+	-0.32	-	-	-	+	0.816	-0.22	2.77	0.22	5.55	14.11	10	+1.05	0.247
2.60	+	+	-0.33	-	-0.08	-	+	0.817	-0.19	2.82	0.21	5.60	14.99	11	+2.14	-
2.82	+	0.42	-0.36	-	-	+	-	0.808	-0.23	2.91	0.11	1.70	8.63	8	+2.40	-
2.48	+	+	-	-	-	-	+	0.808	-0.16	2.73	0.25	5.24	12.84	9	+3.65	-
2.76	+	0.39	-	-0.21	-	-	-	0.800	-0.17	2.90	0.11	1.51	8.74	7	+4.65	-
Sum of weights	1.00	1.00	1.00	-	0.34	-	0.25									

Table 3b. Model-averaged coefficients for models comprising the top two units of AIC_C model ranking of predictors of the number of worm-days experienced by guppies infected with *Gyrodactylus turnbulli* during our experiment, in models that were allowed to include an MHC and a microsatellite individual-level genetic variability metric in addition to fish source (Table 3a). Models are general linear models (Gaussian error distribution) of $\log_e$-transformed worm-days. Fish source coefficients are given in reference to Santa Cruz. Fish length (z-transformed) was present in all models in the top set but interacted with fish source in 1/3 models, and we present this in the table as if length-without-interaction and length-with-interaction (the latter with Santa Cruz as the reference level) were two different predictors. Sum of weights (a.k.a. 'importance') is sum of Akaike weight. The coefficient for fish length is for z-transformed data. All *P*-values are two-tailed.

Term	Slope	SE	z	*P*	Sum of weights
Intercept	2.77	0.21	13.44	<0.001	-
Fish source					
Lopinot	0.81	0.26	3.13	0.002	1
HealthCentre	2.01	0.25	8.15	<0.001	1
Roxborough	3.51	0.29	12.18	<0.001	1
Microsats					
Msat HL	-0.10	0.07	1.33	0.182	0.336
MHC STs					
N MHC STs	-0.35	0.15	2.39	0.017	1
Fish length (× fish source)					
Fish length (no interaction)	0.43	0.09	4.90	<0.001	0.753

Fish length (SantaCruz)	0.10	0.16	0.62	0.537	0.247
× Lopinot	0.27	0.25	1.05	0.297	0.247
× HealthCentre	0.45	0.20	2.22	0.029	0.247
× Roxborough	0.49	0.26	1.92	0.059	0.247

Table 4. Per-population counts of carriers of each MHC supertype (ST) among genotyped fish in our experiment. ST groupings are those of Herdegen-Radwan et al. (2020), and STs 4, 8, and 9 were not detected in the present experiment's sample. Instances of STs with 3+ carriers in a given population were tested for within-population resistance/susceptibility effects: † = ST with an effect present in the top two units of its population's AIC_C-ranked general linear models of worm-days (see Table 6 for details of such instances); ‡ = ST not present in its population's top model set; § = ST carried by every genotyped individual in a population. Tests of supertypes with 3+ carriers in 2+ populations are in Table 5.

MHC ST	Santa Cruz	Lopinot	Health Centre	Roxborough
ST01	2	5‡	0	0
ST02	1	10‡	9†	0
ST03	1	13‡	0	0
ST05	0	0	21‡	0
ST06	6‡	2	0	0
ST07	18‡	6‡	5†	0
ST10	3†	12‡	0	4†
ST11	1	1	0	0
ST12	10‡	4†	0	0
ST13	2	0	0	0
ST14	3†	0	0	0
ST15	0	2	21‡	30§

Table 5. Descriptions of effects associated with single MHC supertypes (STs) in multiple populations (3+ carriers in 2+ populations) in AIC_C-ranked general linear models of worm-days. Res./susc. = whether carrying the ST is associated with reduced or increased infection intensities ('resistant' or 'susceptible') among the top two AIC_C units of a focal ST's ranked models. Numerical values in 'First model' are ΔAIC_C values relative to the top-ranked model. Sum of weights = sum of Akaike weights of models containing the ST within the focal top model set (a.k.a. 'importance'). Min. *P* = lowest *P*-value for the ST's effect among the top model set. 'Interactions' indicates whether there are interactions between the ST and fish/gyro source. See Table S15.2 for remarks on each test.

MHC ST	Populations	Res./susc.	First model	Sum of weights	Min. *P*	Interactions	See also
ST02	Lop, HC	Res.	Top-ranked model	0.394	0.132	None	Table S15.4
ST07	SC, Lop, HC	Susc.	+0.24	0.588	0.153	Some	Table S15.5
ST10	SC, Lop, Rox	-	+2.32	-	-	-	-
ST10	SC, Lop	-	+2.04	-	-	-	-
ST12	SC, Lop	Res.	Top-ranked model	0.487	0.058	None	Table S15.6

	ST15	HC, Rox	-	-	-	-	-	-
	al387	HC, Rox	-	+2.30	-	-	-	-

Table 6. Descriptions of single MHC supertypes (STs) with effects present in the top two units of a single population's AIC_C-ranked general linear models of worm-days. An ST required 3+ carriers to be tested. Res./susc. = whether carrying the ST is associated with reduced or increased infection intensities ('resistant' or 'susceptible'). Numerical values in 'First model' are ΔAIC_C values relative to the top-ranked model. Sum of weights = sum of Akaike weights of models containing the ST within the focal top model set (a.k.a. 'importance'). Min. *P* = lowest *P*-value for the ST's effect among the top model set. 'Interaction' indicates whether the ST interacts with gyro source. See Table 4 for other tested STs, and Table S15.3 for remarks on each test.

MHC ST	Populations	Res./susc.	First model	Sum of weights	Min. *P*	Interaction	See also
ST02	HC	Resistant	+1.61	0.174	0.320	No	Table S15.7
ST07	HC	Susceptible	+1.12	0.221	0.234	No	Table S15.7
ST10	SC	Resistant	+1.16	0.111	0.143	No	Table S15.8
ST10	Rox	Resistant	+0.07	0.388	0.099	No	-
ST12	Lop	Interaction	Top-ranked model	1.000	0.037	Yes	Table S15.9
ST14	SC	Resistant	Top-ranked model	0.470	0.070	No	Table S15.8

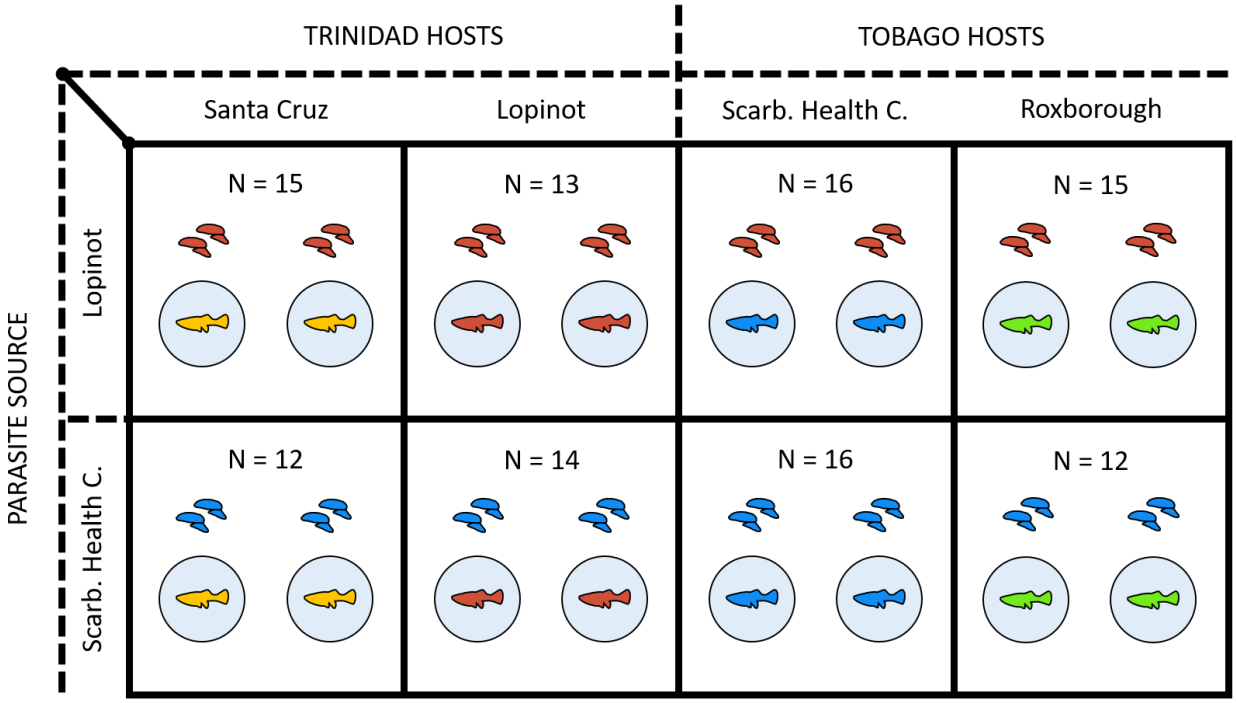

Figure 1. Design of cross-infection experiment, with four host (guppy) populations and two parasite (gyrodactylid) populations sourced from two different islands (Trinidad vs Tobago). Sample sizes refer to numbers of infected hosts per treatment block, with each host receiving two gyrodactylid worms.

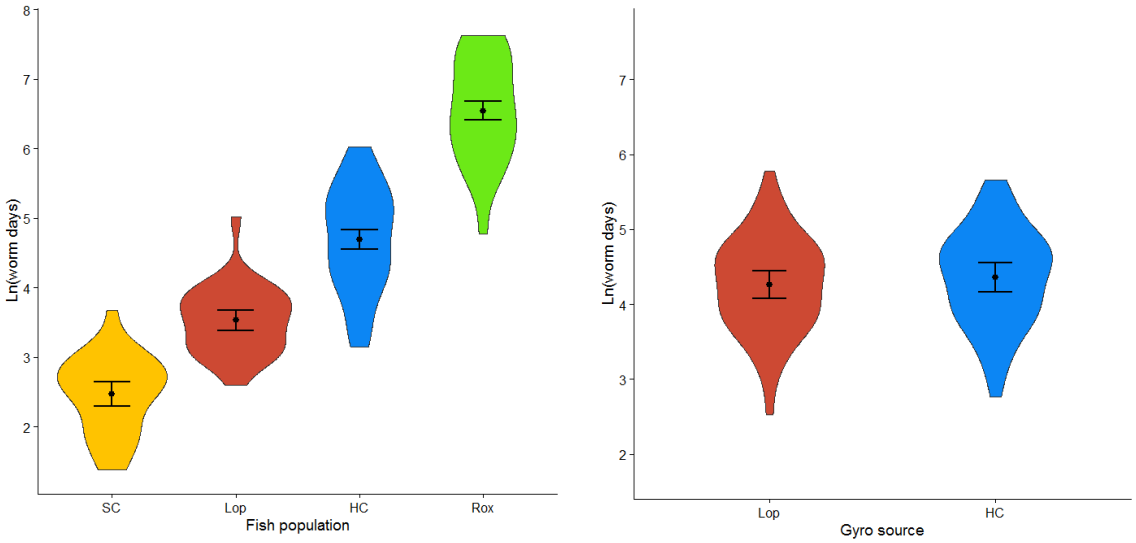

Figure 2. Differences in worm-days ($\log_e$) between (A) hosts (fish) from four populations, and (B) parasites (gyros) from two populations. Points are fitted means, errors bars are SEs for the means, and violins show partial residuals from the top-ranked model to include the focal predictor. See Appendix S11 for average expected infection trajectories.