Artificial Selection and the Skin Microbiome Independently Predict Parasite Resistance

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Abstract

1. Host responses to parasite infection involve several interacting systems. Host genetics determine much of the response, but it is increasingly clear that the host-associated microbiome also plays a role. Genetically determined systems and the microbiome can also interact; for example, the microbiome can modulate the host immune response, and *vice versa*. However, it remains unclear how such interactions between the host immune system and the microbiome may influence the host's overall response to parasites.
2. Host sex is an important source of variation in both immune response and the microbiome across systems, so interactions between them may also therefore differ between males and females, potentially explaining sex-specificity in response to parasites.
3. To investigate how host genetics and the microbiome interact to shape responses to parasites, we imposed truncation selection on the response of Trinidadian guppies (*Poecilia reticulata*) to the specialist ectoparasite *Gyrodactylus turnbulli*. Following experimental infection, we used the 30% of guppies with the lowest parasite load to found the resistant

line, the 30% with the highest load to found the susceptible line, and randomly chosen uninfected fish to found the control line. After 3-6 generations of breeding without parasites, we sampled the skin-associated microbiome and then infected fish from each line. We applied machine-learning to identify bacterial community types across lines and evaluated how selection line and community type explained variations in infection severity.

4. Our findings showed that among females, the resistant line had significantly lower infection severity, while the susceptible line had higher infection severity than other lines. Interestingly, females in the resistant line lost more mass per parasite, i.e., exhibited lower parasite tolerance, than in the control or susceptible lines. Among males, the susceptible line experienced higher infection severity than the other lines, but there was no difference between the resistant and control lines.
5. We found that the microbiome and host genetics independently explain similar variation in infection severity, highlighting the microbiome's active role in host-parasite interactions. Our results also indicate sex specificity in the evolution of host defenses.

1. Introduction

Hosts use multiple strategies to defend themselves against parasites and pathogens (parasites hereafter). Host genetic background is commonly credited with much of the defense against parasites through the immune system, behavior, or other genetic/protein alterations, e.g., thalassemia (Labro, 2022). A recently highlighted alternative defense, that conferred by the host-associated microbial communities or microbiome, may act indirectly through the host immune system or directly via niche occupation or secreting anti-parasitic compounds (Bernardo-Cravo et al., 2020), and may also exhibit heritable components. However, it remains unclear whether the

50 microbiome plays an active role in host-parasite infection or merely reflects the host's
51 health/immune status (Trevelline et al., 2020). While there is abundant evidence that the microbial
52 community can have a direct or indirect role in host-parasite interactions (Becker et al., 2009;
53 Nelson & May, 2020), there is also evidence that the microbiome is merely a bystander in some
54 diseases and a reflection of the host's genetics, diet, and general health (Ni et al., 2017).
55
56 Host genetics and the microbiome interact throughout host development, with complex feedbacks
57 on traits like parasite defense. On one hand, the host immune system relies on early microbial
58 colonization for proper development and the microbiome ultimately shapes immune cell
59 composition (Amenyogbe et al., 2017). On the other hand, hosts exert control over their symbionts,
60 shaping their associated microbiomes, via immune responses, barrier function, and physiological
61 regulation (Wilde et al., 2024). The host's genetic makeup therefore influences microbiome
62 composition, and specific microbial communities may therefore become heritable (Bordenstein &
63 Theis, 2015). However, distinguishing host genetic effects from factors such as priority effects,
64 drift, and other stochastic elements, is an ongoing challenge (Debray et al., 2022). Direct
65 manipulation of host genetics in traits like parasite resistance, where the microbiome plays a
66 known role, thus provides a useful tool in disentangling host genetic effects from microbial ones.
67
68 Both the host-associated microbiome and the immune system commonly differ between host sexes
69 (Markle & Fish, 2014). These differences are likely due to other correlated differences, for
70 example, high testosterone levels in the blood both suppress the immune system and significantly
71 alter gut microbial communities (Santos-Marcos et al., 2023), and both the immune system and
72 microbiome change during female reproductive cycles and pregnancy (Chen et al., 2023; Gorczyca

et al., 2022). Given that host sexes across taxa also differ in their response to parasites (Morrow, 2015), it is essential to test for sex-specific interactions between genetic resistance factors, the microbiome, and parasites.

Trinidadian guppies (*Poecilia reticulata*) and their specialist parasite *Gyrodactylus turnbulli* provide a rich context to study host-parasite dynamics. *Gyrodactylus* spp. feed and reproduce (both sexually and asexually, generation time 24hrs) on host skin. Guppies show a large variation in parasite resistance across populations and ecological contexts (Dargent et al., 2016; Phillips et al., 2021; Stephenson, et al., 2015; Van Oosterhout et al., 2003). Guppy resistance is at least partly genetically determined and heritable, with sex differences (Dargent et al., 2016; Van Oosterhout et al., 2003; Weiler et al., 2024). Innate and acquired immunity are important for *G. turnbulli* resistance, although the underlying mechanisms remain unclear (Cable & Van Oosterhout, 2007; Mohammed et al., 2016). Research has largely focused on the major histocompatibility complex (MHC) for genetic-based resistance to *G. turnbulli* (Fraser et al., 2010; Fraser & Neff, 2009; Phillips et al., 2018). Notably, skin bacterial alpha diversity may correlate with MHC heterozygosity in frogs (Cortazar-Chinarro et al., 2024). If also true for fish, this covariation sets up a clear avenue for the skin microbiome and host genetics to interactively shape parasite resistance in our system.

Here, we tested how host genetics and host-associated microbiomes affect the interaction between guppies and *G. turnbulli*. We imposed truncation selection for host parasite resistance and susceptibility, quantified as the mean number of parasites over infection. Additionally, while we did not specifically select for tissue-specific tolerance, or the ability to minimize the fitness losses

caused by a given parasite load ('tolerance' hereafter; (Stephenson & Adelman, 2022)), we compared tolerance between our lines. Previous literature has demonstrated that tolerance is an essential factor to consider in guppy response to *Gyrodactylus* parasites (Jog et al., 2022; Stephenson, van Oosterhout, & Cable, 2015; Tadiri et al., 2021), and the heritability of tolerance requires additional empirical test (Hayward et al., 2014; Råberg, 2014). Our results highlight the complex interplay among host genetics, the microbiome, and sex-specific responses to infections.

2. Materials and methods

2.1 Does guppy resistance to Gyrodactylus turnbulli respond to selection?

2.1.1 Selection experiment

We used laboratory-bred descendants of wild guppies from a high-predation population on the Caura River, Trinidad (UTM: 20 P 679527.7 m E, 1180376.4 m N; elevation 112 m). Wild guppies (n ~600) were transported to Cardiff University in June 2012 (Cefas APB authorization number CW054-D-187A) and treated prophylactically with Binox® (Nitrofurazone). The site was previously confirmed *Gyrodactylus* spp.-free by previous surveys, and no *Gyrodactylus* spp. were found in a subset of 80 fish examined. We collected fin clips from 66 parental-generation fish for microsatellite genotyping. Fish were housed in 70 L mixed-sex tanks under a 12h light:12h dark cycle at 24±1°C and fed daily with Aquarian® tropical fish flakes, supplemented with Artemia and bloodworm. Fry were transferred to juvenile tanks soon after birth, checked for ectoparasitic infection, and reared until sex determination (6-8 weeks). They were then kept in sex-specific tanks for at least another month before use, ensuring all fish were sexually mature and unmated.

We infected 100 male and 100 female F1 guppies to evaluate their resistance and select founders for our 'resistant' and 'susceptible' lines. To initiate infections, a heavily infected fish from our parasite culture was euthanized with MS222 (0.02% tricaine methanesulfonate) and placed next to an anesthetized experimental fish until two *G. turnbulli* parasites transmitted, as observed under a dissecting scope. Experimental fish were housed individually in 1 L tanks, and parasites were counted every other day under anaesthetic. After nine days, all fish were treated with Levamisole (0.002g per L) and screened under anesthetic several times to confirm parasite clearance. Fin clips were collected for genotyping. The 30 males and 30 females with the highest (lowest) mean number of worms founded the 'susceptible' (‘resistant’) line, and randomly selected uninfected F1 fish (30 of each sex) founded the control line. Each line was allowed to breed freely in separate 120 L tanks. Tanks were monitored for fry, which were moved to F2 rearing tanks. The F2 generation was split into two subpopulations, maintaining 60 fish per tank. New tanks were set up for each subsequent generation. Population size was kept consistent across the six subpopulations, with new tanks set up for each subsequent generation as above.

To evaluate response to selection, 88 guppies 3-7 generations post selection were experimentally infected with *G. turnbulli* in five batches between August and November 2017, following the same procedure as for the F1 generation, described above. 14 guppies from each line were ‘sham’ infected and were used in microbiome analysis.

2.1.2 Data analysis

We used R v.4.3.2 (R Core Team, 2020) and provide our code, output, and validation in the Supporting Information (SI). We used ggplot2 (Wickham, 2016) to plot all the figures. For all

models, we assessed collinearity among predictor variables using the pairs function (Zuur et al., 2009), validated model fits using the DHARMa package (Hartig, 2022), and used Type II Wald chi-square tests to test for significance using the *Anova()* function from the 'car' package (Fox & Weisberg, 2019).

We quantified infection resistance/susceptibility as the area under the curve of parasite number over time, or "infection severity." To test response to selection, we used infection severity as the response variable in a generalized linear mixed model (GLMM) in the glmmTMB package, v. 1.1.2.3 (Brooks et al., 2017) (Gamma error family and logit link function). As fixed effects, we included selection line (resistant n=30, control n=28, susceptible n=29), fish sex, length (pre-infection, using residuals from a regression on sex to control for sex differences), body condition (scaled mass index; (Peig & Green, 2009)), generation, number of parasites establishing the infection (dose), and trial date (Julian date). Length and body condition were calculated including sham infected fish (resistant n=14, control n=14, susceptible n=14). We also included the interaction between sex and line.

We quantified tolerance as the host's relative per-parasite percentage mass change during infection, calculated by dividing the percentage mass change from day 0 to day 20 by the maximum number of worms on the fish, relative to the highest observed percentage change. To assess the impact of selection on tolerance, we used tolerance as a response variable in a GLMM, including the fixed effects of line, sex, length, dose, and Julian date. With interaction between line and sex. We excluded scaled mass index due to its overlap with mass change. What about interaction?

We additionally tested for a tradeoff between resistance and tolerance using a quadratic polynomial regression model to account for non-linear effects. We used tolerance as the response variable and log-transformed infection severity (our resistance metric), length, and generation as fixed effects. We did not include infection severity due to high collinearity with other fixed effects.

2.1.4 Calculating heritability

We calculated the narrow-sense heritability of resistance and tolerance following the breeder's equation (Lush, 1937). As our resistance metric, we used the average number of parasites between 1-9 days of infection, which was the metric we originally used in our truncation selection. We used the same tolerance metric described above. Both metrics were centered to a mean of zero to account for the differences in worm growth between the infections of the F1 and subsequent generations.

P_b: Average starting phenotype (mean phenotype of the population before selection)

P_s: Average selected phenotype (mean phenotype of the selected parents)

P_r: Average response phenotype (mean phenotype of the offspring generation)

h^2: Narrow-sense heritability

$h^2 = P_r - P_b P_s - P_b$

2.1.5 Do differences in inbreeding contribute to line differences?

We evaluated the genetic diversity and variance in inbreeding within the wild-caught populations, F1, and F3-F6 generations, to test if the observed difference between the resistant and susceptible lines was due to differences in inbreeding (Smallbone et al., 2016). We extracted DNA from fin

clips with the HotSHOT method: using 50 µl of alkaline lysis reagent and neutralizing solution followed by incubation at 95 °C for 30 minutes (Truett et al., 2000). We used two multiplex assays to amplify eight previously designed microsatellite markers (Becher et al., 2002; Nater et al., 2008; Paterson et al., 2005; van Oosterhout et al., 2006; Watanabe et al., 2005) at the Genetic Diversity Centre (GDC), ETH Zurich. Samples were genotyped on an ABI Genetic Analyzer 3730 (Applied Biosystems, USA), scored using GeneMarker (Version 2.6.4; Softgenetics), and binned using the package MSatAllele (Alberto, 2013) in R ((R Core Team, 2020); see SI).

We tested allelic richness (A_R), gene diversity (H_S), $F_{IS,}$ and identity disequilibrium (g2). Allelic richness is the first population genetic metric to reflect bottleneck effects, which decrease the effective population size and facilitate reductions in heterozygosity due to inbreeding (Nei et al., 1975). Significant differences in allelic richness among populations are indicative of variation in the probability that inbreeding may occur. Gene diversity measures the mean heterozygosity per individual per population. Under inbreeding conditions homozygosity increases but gene diversity is not immediately lost (Charlesworth, 2003). Positive values in F_{IS} can be generated by inbreeding but may also be artificially elevated by large allele drop out or null alleles (De Meeûs, 2018; Nei et al., 1975). We therefore also estimated identity disequilibrium (g_2), the correlation of heterozygosity across loci resulting from inbreeding, which is robust in the presence of null alleles (David et al., 2007). Patterns across several of these population genetic metrics consistent with inbreeding would indicate that phenotypic differences between lines are attributable to inbreeding. Allelic richness (A_R) (rarefied to the lowest population sample size), gene diversity (H_S) and F_{IS}, were calculated in FSTAT 2.39 (Goudet, 01 2001). Significance levels for F_{IS} were assessed in GENEPOP (web version 4.2;(Raymond & Rousset, 1995) with 5000 iterations followed by a Bonferroni correction for multiple comparisons. Tests for statistically significant differences

allelic richness (A_R), gene diversity (H_S) and F_{IS} among groups were calculated in FSTAT with 2000 permutations. Identity disequilibrium (g^2) is the difference between joint identity by descent and the product of the separate probabilities of identity by decent for two loci. g^2 is estimated with microsatellite markers as the excess of double heterozygous genotypes at all pairs of loci relative to a random distribution of heterozygous genotypes among individuals at each locus (Cockerham & Weir, 1973; David et al., 2007). g_2 values significantly greater than 0 are indicative of identity disequilibrium (ID). ID was calculated in inbreedR (Stoffel et al., 2016).

2.2 Does the host-associated microbiome contribute to guppy resistance to Gyrodactylus infection?

2.2.1 Microbial inventory

Before the F3-F7 infections began, we collected samples from 51 fish split between the infection and sham-infection treatment groups: control (infected n=10, sham n=5), Resistant (infected n=12, sham n=6), and Susceptible (infected n=9, sham n=8). We used the methods previously published to collect and inventory the microbial communities on the fish skin.

Table 1: Replication Statement-Counts of Artificial selection lines in sham, infection, and microbiome experiment

Scale of inference	**Scale at which the factor of interest is applied**	**Number of replicates at the appropriate scale**
Uninfected fish	Three selection lines (Resistant, Susceptible, Control)	Resistant: 14, Control: 14, Susceptible: 14
Infected fish	Three selection lines (Resistant, Susceptible, Control)	Resistant: 30, Control: 28, Susceptible: 29
Microbial community types	Skin swabs across lines	Control: 19, Resistant: 15, Susceptible: 16

2.2.2 Microbial alpha and beta diversity

Raw sequence data were processed using QIIME2 version 2019.7 (Bolyen et al., 2019). We rarefied the resulting ASV table to 3,654 sequences per sample which resulted in excluding one sample and kit controls, and used the DADA2 pipeline to calculate the unweighted and weighted UniFrac distances (Lozupone & Knight, 2005) pairwise between all samples to create distance matrices. We used a PERMANOVA from the adonis package in R (Anderson, 2001) to test for significant differences in microbial community across fish line, sex, length, and body condition. We used QIIME2 to calculate the number observed number of ASVs, Shannon's diversity index, and Faith's phylogenetic diversity, and compare the mean values of these metrics across line and sex using Pairwise Kruskal-Wallis tests.

2.2.3 Bacterial community typing

We used Dirichlet multinomial mixture models (DMM) (Holmes et al., 2012) in the Dirichlet Multinomial v1.16.0 package (Morgan, 2023) to assign all samples (n=50) to three different bacterial community types based on the relative frequency of taxa. We compared the Laplace approximation of the negative log models to identify the number of Dirichlet components that resulted in the best model fit (see SI).

2.2.4 Does bacterial community type predict the severity of Gyrodactylus *infection?*

To test if the bacterial community type significantly predicted infection severity, we used bacterial community type as a fixed effect in the GLMM model used for the selection line resistance analysis described in section 2.1.2, with the data subset to just swabbed fish.

2.3 What is the relative importance of host genetics and the microbiome to guppy resistance to Gyrodactylus infection?

To compare how well our GLMM models with a) bacterial community type only, b) artificial selection line only, and c) both factors explained variation in infection severity, we used the *anova*() function. All three models shared the same fixed effects, differing only in whether line or bacterial community type or both were included as a fixed effect, and were run on the reduced dataset using only the infected and swabbed fish (n=31).

3. Results

3.1 Guppy resistance to, and tolerance of, Gyrodactylus infection responded to selection

3.1.1 Resistance responded to selection

Artificial selection line was a significant predictor of infection severity ($\chi^2(2) = 37.74$, p=6.4e-09), but the effect differed between sexes (Figure 1; line:sex interaction: $\chi^2(2) = 13.51$, p=0.0012). Females had significantly more severe infections than males ($\chi^2(1) = 24.29$, p=8.3e-07) and high starting worm 'dose' resulted in higher infection severity ($\chi^2(1) = 5.11$, p=0.024). Post hoc tests to evaluate whether selection line significantly explained infection severity among each sex found that artificial selection line was still significant in both sexes (female; $\chi^2(2) = 43.69$, p=5.2e-06 & male; $\chi^2(2) = 16.10$, p=0.00032). Post hoc Tukey tests also revealed that the female guppies from the susceptible line experienced significantly higher (Cohen's d=3.49, p<0.001), and from the resistant line experienced significantly lower infection severity (Cohen's d=2.74, p=0.016) than the control. Male guppies from the susceptible line experienced significantly higher infection severity than control line males (Cohen's d=4.19, p<0.001), but resistant line males did not differ

274 significantly from control line males (Cohen's d=1.86, p=0.15). The average number of parasites
275 throughout infection was highly heritable (susceptible line h^2=0.79; resistant line h^2=0.41).
276
277 *3.1.2 Tolerance responded to selection and trades off with resistance*
278 The artificial selection lines significantly differed in tolerance ($\chi^2(2)$=14.56, p=0.00069) again
279 depending on host sex (Figure 2; $\chi^2(2)$=10.28, p=0.00586). Larger fish were more tolerant
280 ($\chi^2(1)$=5.94, p=0.014). Post hoc testing found artificial selection line was only significant in
281 females ($\chi^2(2)$=23.81, p=6.7e-06), and smaller females were less tolerant ($\chi^2(2)$ =6.96, p=0.0083).
282 Post hoc Tukey tests revealed that resistant line females were significantly less tolerant than control
283 and susceptible line females (control; Cohen's d=7.04, p=0.0003; susceptible; Cohen's d=8.54,
284 p<0.00016). No fixed effect explained variation in tolerance among males. Bacterial community
285 types did not predict tolerance. Tolerance was heritable (susceptible line: h^2=0.52; resistant line:
286 h^2=0.24).
287
288 The polynomial regression model revealed a tradeoff between tolerance and resistance: higher
289 tolerance was correlated with lower resistance (Figure S1; F= 62.64, p<2e-16). No other fixed
290 effects explained significant variation in tolerance.
291
292 *3.1.3 Selection lines did not differ in the extent of inbreeding*
293 We saw a significant reduction in allelic richness among all experimental infection groups relative
294 to the wild-caught founders, likely reflecting a post-capture reduction in population size (Table
295 S1). However, we found no significant difference in AR (p= 0.589), Hs (p=0.73), FIS (p=0.42), or
296 g2 (Table S2) among the three lines.

297

298 ***3.2 The host-associated microbiome and host genetics independently underly guppy resistance***

299 ***to Gyrodactylus infection***

300 DMM modeling found that the lowest Laplace approximation model fit was three bacterial clusters

301 (*Figure S2*). All samples had a posterior probability of 98% or higher. Each bacterial community

302 type contained 10.0–66.8% of all samples (n=5-33), and no samples were undefined. The three

303 bacterial community types (A, B, and C) in our fish skin samples were distinct (PERMANOVA:

304 F = 7.37, p = 0.0003, Figures 3a & S3-4). Males and females were balanced among the bacterial

305 community types A and B, and bacterial community types clustered evenly across the artificial

306 selection lines (Figure 3a; Fisher's exact test, p=0.8). Additionally, we found no interaction

307 between line and bacterial community types in our model ($\chi2(4)=3.07$, p=0.546). All skin swab

308 samples were assigned to a community type with the greatest posterior probability. For top

309 contributing bacterial genera, see *Figure S3*.

310

311 The artificial selection lines did not significantly differ in any skin microbial diversity metrics

312 tested. The microbiomes associated with females had significantly higher alpha diversity than

313 those associated with males (Kruskal–Wallis pairwise test, Shannon's index: H=4.06, q=0.04;

314 observed ASVs: H=4.88, q=0.03). Consistent with Kramp et al. (2022), the microbiomes

315 associated with the two sexes also had significantly different beta diversity (unweighted unifrac

316 PERMANOVA; F=1.56, p=0.006).

317

318 The skin bacterial community type A had significantly reduced infection severity compared to

319 bacterial community type B & C ($\chi^2(2) = 27.54$, p=1e-6, Figure 3b). A post-hoc test showed that a

model including both artificial selection line and bacterial community type is significantly better at explaining variation in infection severity than models with either variable alone (Figure 4; $\chi^2(14)=15.00$, $p=0.00055$).

4. Discussion

Our selection lines demonstrated striking heritability of parasite resistance and tolerance. Bacterial community type predicted infection severity independently from selection line. We also observed notable differences between males and females in skin microbiome, and in response to selection. We discuss each of these results in turn.

We found that guppy resistance and susceptibility to *G. turnbulli* responded readily to a single generation of artificial selection. We found no notable differences in the three selection lines in our genetic metrics, indicating that the differences were not due to differences in inbreeding among the lines. Our narrow-sense heritability estimates for resistance and tolerance were high, particularly for our susceptible lines. However, our values are still comparable to heritability estimates in previous studies (Weiler et al. 2024) and to male guppy ornaments (Morris et al. 2020), which are correlated with *G. turnbulli* resistance (Stephenson et al. 2020).

Our results suggest that susceptibility to parasite infection responds more readily to selection than does resistance. This may be because response to selection could involve an increase in tolerance, or a loss of resistance. Our experimental design favored tolerance in the susceptible line, as only individuals surviving severe infections contributed to future generations, and indeed, susceptible line fish trended towards higher tolerance. Tolerance and resistance likely have different genetic

bases, and hosts appear to invest in one strategy at the expense of the other (Råberg, Sim, and Read 2007). This theory is supported by the fact that the resistant line was less tolerant than control and susceptible lines, and that we found a resistance-tolerance tradeoff at the individual level. However, these tradeoffs do not always occur, and both tolerance and resistance traits can coexist within an individual host or population (Hardy et al. 2024; Pagán and García-Arenal 2018).

Understanding how resistance, susceptibility, and tolerance of *Gyrodactylus* spp. infection respond to selection is crucial for understanding how Trinidadian guppy behavioral and immunological defenses interact with parasite spread and evolution (Walsman, Lambe, and Stephenson 2024; Walsman et al. 2022). Our heritability estimates suggest an important genetic contribution to traits influencing infection outcomes, which may allow natural selection to act rapidly under high parasite pressures. In some populations, infection prevalence is very high (>90%; (Clark et al., 2023; Stephenson, van Oosterhout, Mohammed, et al., 2015)), potentially selecting for increased infection tolerance and decreased resistance, as 'resistance is futile' (Walsman et al. 2023). In concert, transgenerational immune priming may reduce the expression of parasite susceptibility under natural conditions in this system Weiler et al bred fish for several generations in the absence of parasites to remove such effects. Future work should take into account the full complexity of eco-evolutionary feedbacks, as well as the heritability estimates we present here (Weiler et al., 2025).

Despite differences in their infection phenotypes, we did not find that microbiome composition, alpha diversity, or bacterial community type differed significantly between lines, nor did the bacterial communities predict tolerance. Nevertheless, we found a strong correlation between

366 bacterial community and infection severity, pointing to important interactions between the skin
367 microbiome and parasites, supporting previous studies (Scheifler et al. 2022; Speer 2022). Our
368 findings therefore suggest that host genetics and host-associated skin microbiome significantly
369 predict parasite infection severity, but not tolerance, and that they do so independently and
370 additively to host genetics. This result appears contrary to previous findings that host immune
371 system, metabolism, and growth rate correlate with the microbiome (Zheng, Liwinski, and Elinav
372 2020; Speer 2022). Indeed, artificial selection for body size in guppies significantly altered the
373 host-associated microbiome, and the fast-growing line had greater parasite colonization resistance
374 (Piazzon et al. 2020), and life history and size selection experiments indirectly affect guppy
375 resistance to *G. turnbulli* (Bartuseviciute et al. 2022), potentially mediated through the
376 microbiome. This difference may be due to the fact that we used skin microbiome, and fish skin
377 microbiomes are highly variable and may not reflect the host's genetics but drift due to stochasticity
378 (Jones et al. 2022; Wang et al. 2023).
379
380 Our results add to the growing body of work demonstrating that the microbiome affects parasite
381 resistance in several taxa: pre-infection microbiome composition, alpha diversity, or microbiome
382 function is a powerful tool to predict individuals that are most susceptible to parasites within a
383 population (Bates et al. 2022; Lee et al. 2019). Further investigation of our bacterial community
384 findings revealed similarities to a recent study of skin microbial metabolism function in amphibian
385 chytridiomycosis (Bates et al. 2022): we found five of the eight ASVs reported to be indicator taxa
386 of epizootic dynamics; *Burkholderiales*, *Pseudomonadales*, *Sphingomonadales*, *Rhizobiales*,
387 *Flavobacteriales* were also key contributing taxa in our bacterial community types (see Figure S2).
388

In alignment with those of Dargent et al. (2016), our results indicate that parasite resistance evolved more slowly in males than females. This may be due to a pre-existing higher level of, and less genetic variation in, resistance in males due to sexual selection. This lack of variation in male resistance may explain why we found evidence for a trade-off between resistance and tolerance among females, but not among males. Indeed, males in our experiment had less severe infections, and males are less likely, and less heavily infected in surveys of natural populations (Clark et al., 2023; Gotanda et al., 2013; Stephenson, van Oosterhout, Mohammed, et al., 2015). Females in many populations prefer males with larger orange ornaments (Endler et al., 2001; Houde, 1988, 1997), with implications for disease dynamics (Rovenolt et al., 2024). Larger orange ornaments indicate resistance to this parasite (Stephenson et al., 2020). Our experimental design allowed female preference to affect male reproductive success, potentially resulting in the overall high and invariable resistance we observe in males.

Importantly, the apparently sex-specific evolutionary trajectory of parasite resistance we observe highlights the role of something other than the MHC, which has been a focus in this system to date (Fraser et al., 2010; Fraser & Neff, 2009; Phillips et al., 2018): MHC resistance is not sex based but a mixture of both parents' MHC genes (Cruz-Tapias et al., 2013). Our results suggest that the microbiome may contribute somewhat to the sex-specificity we observe: the sexes differed in both alpha and beta diversity metrics of bacterial communities, similar to the skin microbiome in other species (Rodríguez-Barreto et al. 2023). More broadly, our findings highlight potential co-evolutionary interactions between host sexes and their associated microbiomes in relation to parasite resistance. These possibilities will hopefully inspire exciting and novel exploration of the evolutionary dynamics of resistance and tolerance between the sexes.

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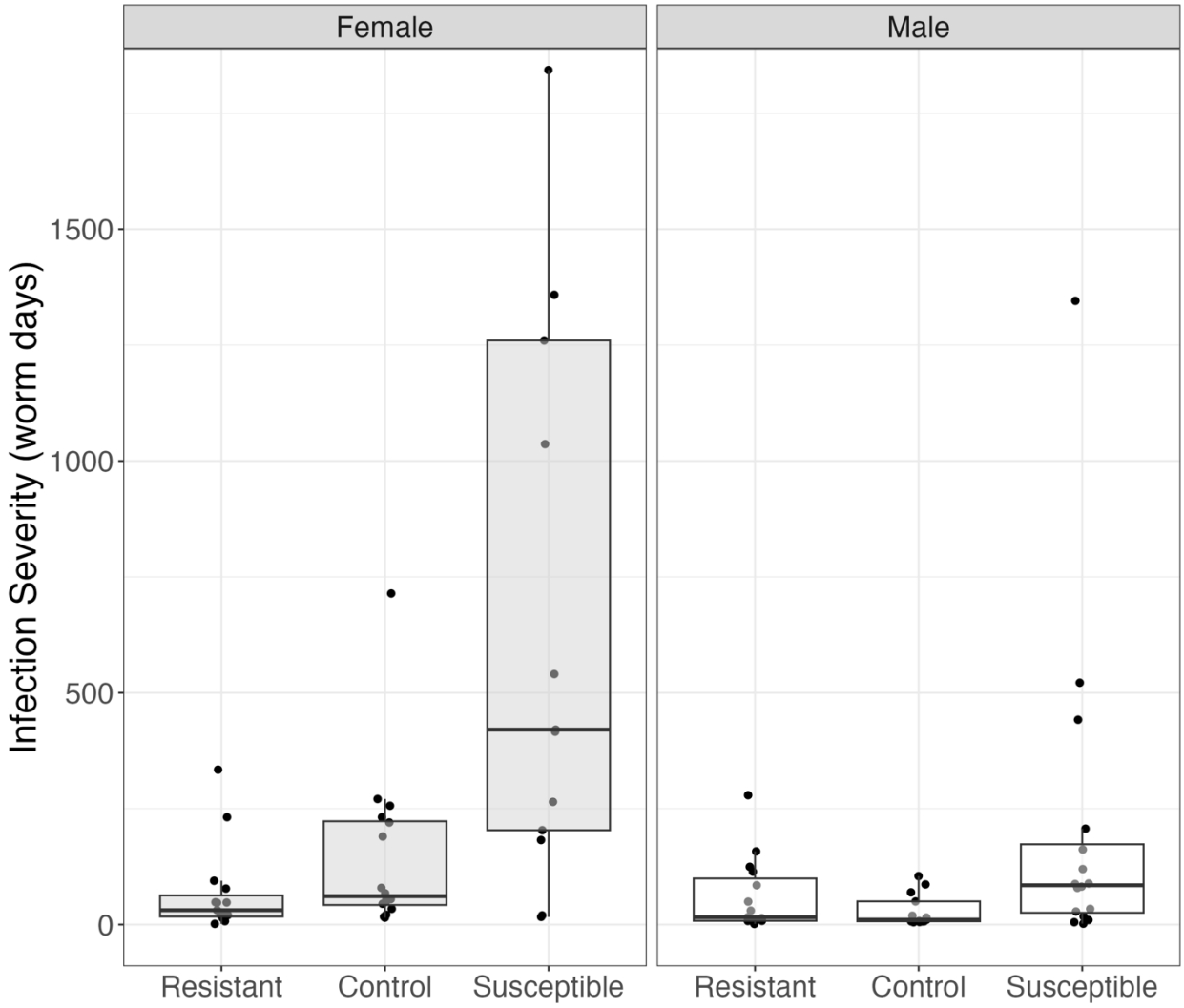
Figures



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655 ***Figure 1. Artificial selection lines significantly predicted G. turnbulli infection severity in***

656 ***Trinidadian guppies***. Infection severity (worm days) reflects the area under the infection load

657 curve. Female guppies (n=44) in susceptible and resistant lines differed from the control line.

658 Male guppies (n=43) only showed differences in the susceptible line. Box plots illustrate median,

659 interquartile range, and values within 1.5× the interquartile range of infection severity.

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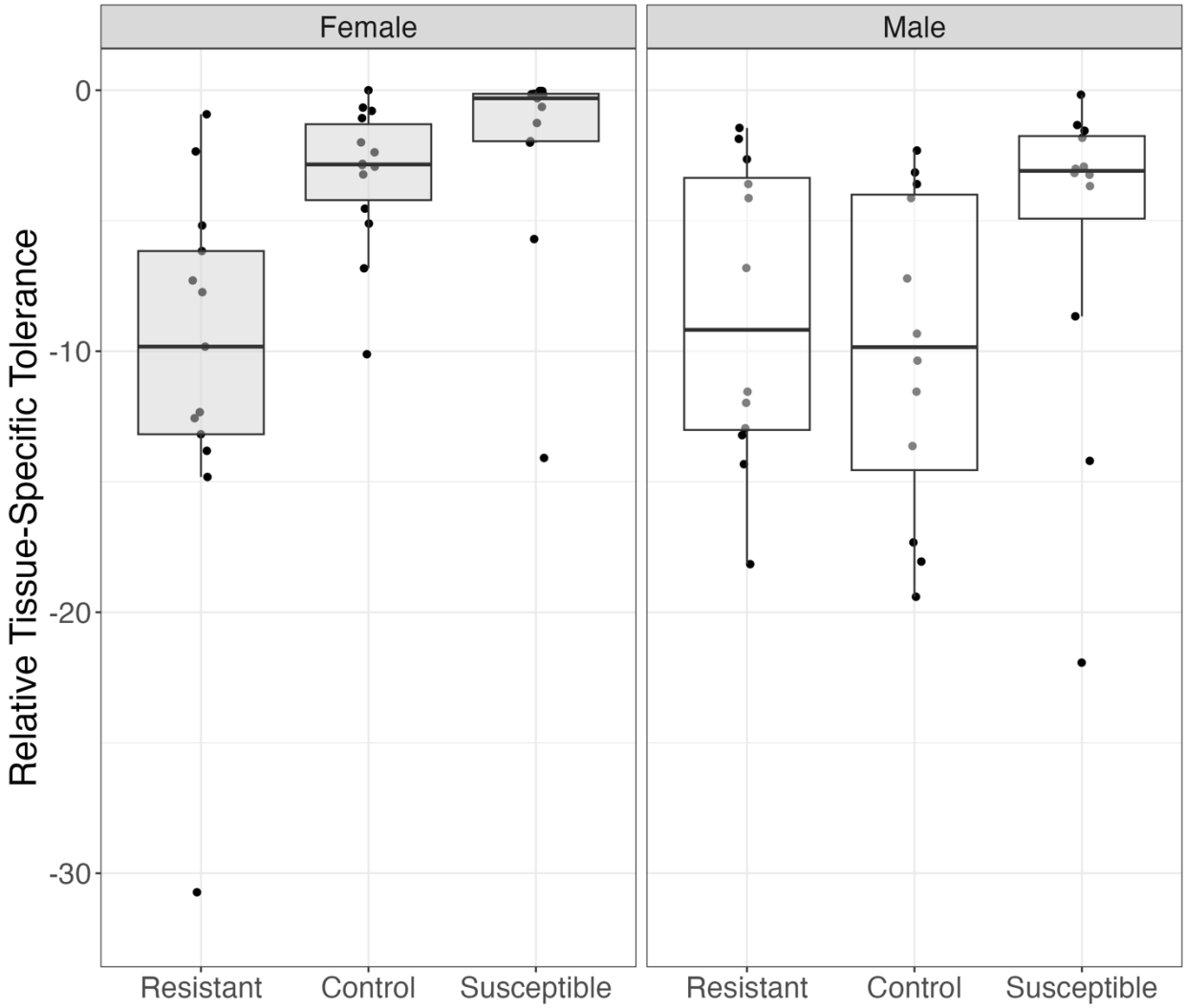

Figure 2. Artificial selection lines also predicted relative tissue-specific tolerance in Trinidadian Guppies. Tolerance measured as per-parasite mass change across infection minus max tolerance. Female guppies (n=44) in resistant lines significantly differed from control and susceptible lines. Male guppies (n=43) did not show significant differences. Box plots illustrate the same metrics as in Figure 1.

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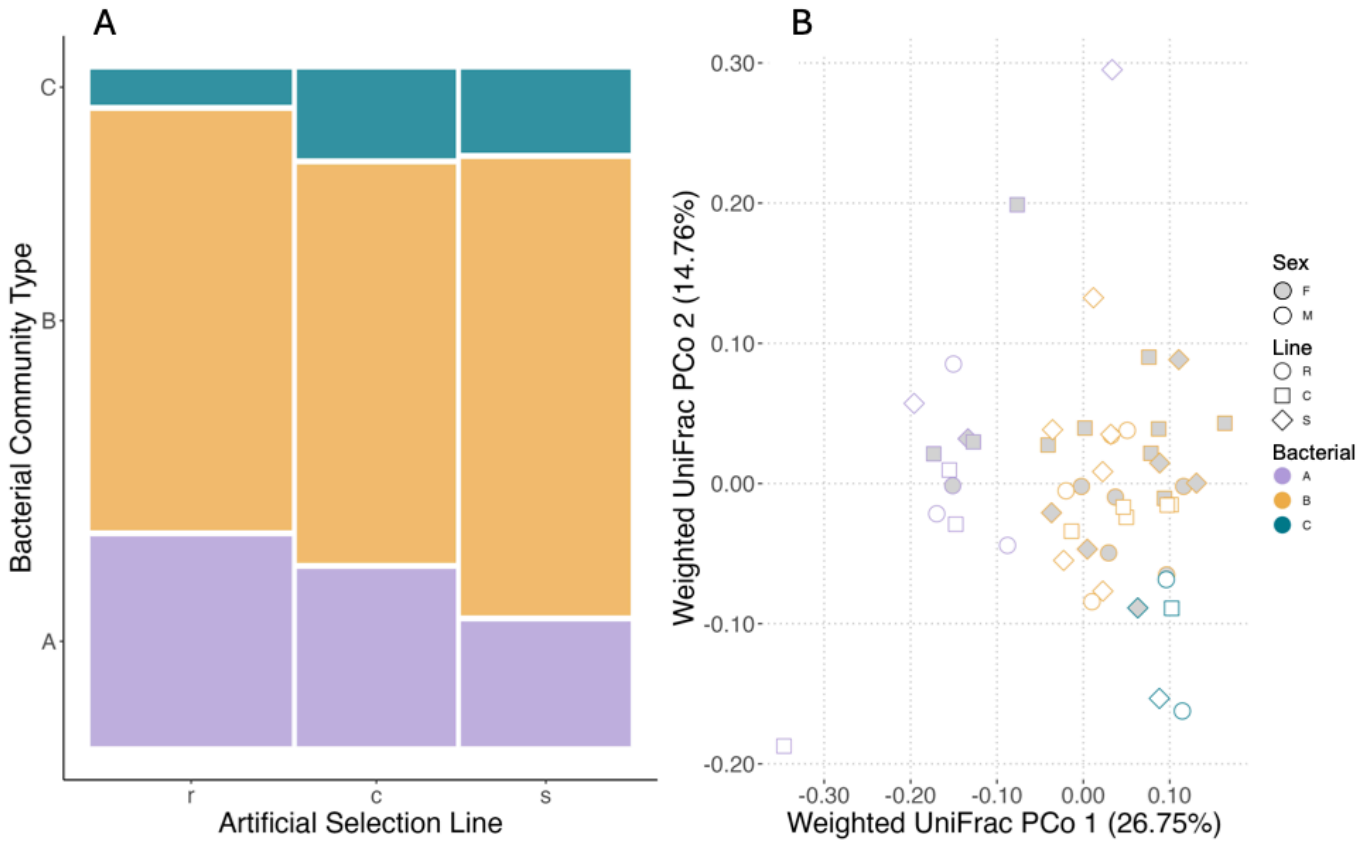

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678 ***Figure 3. The distribution of skin bacterial community types across guppy selection lines***

679 ***showed no significant association.*** A. mosaic plot presents bacterial community types (A, B,

680 C) across control (c), resistant (r), and susceptible (s) lines, with no significant distribution

681 differences (Fisher's exact test, p=0.8). B. PCoA analysis (n=52) indicated bacterial community

682 type was significant in variation (PERMANOVA: F=7.37, P=0.0003), but no significant clustering

683 was detected for selection lines (PERMANOVA: F=0.68, P=0.89).

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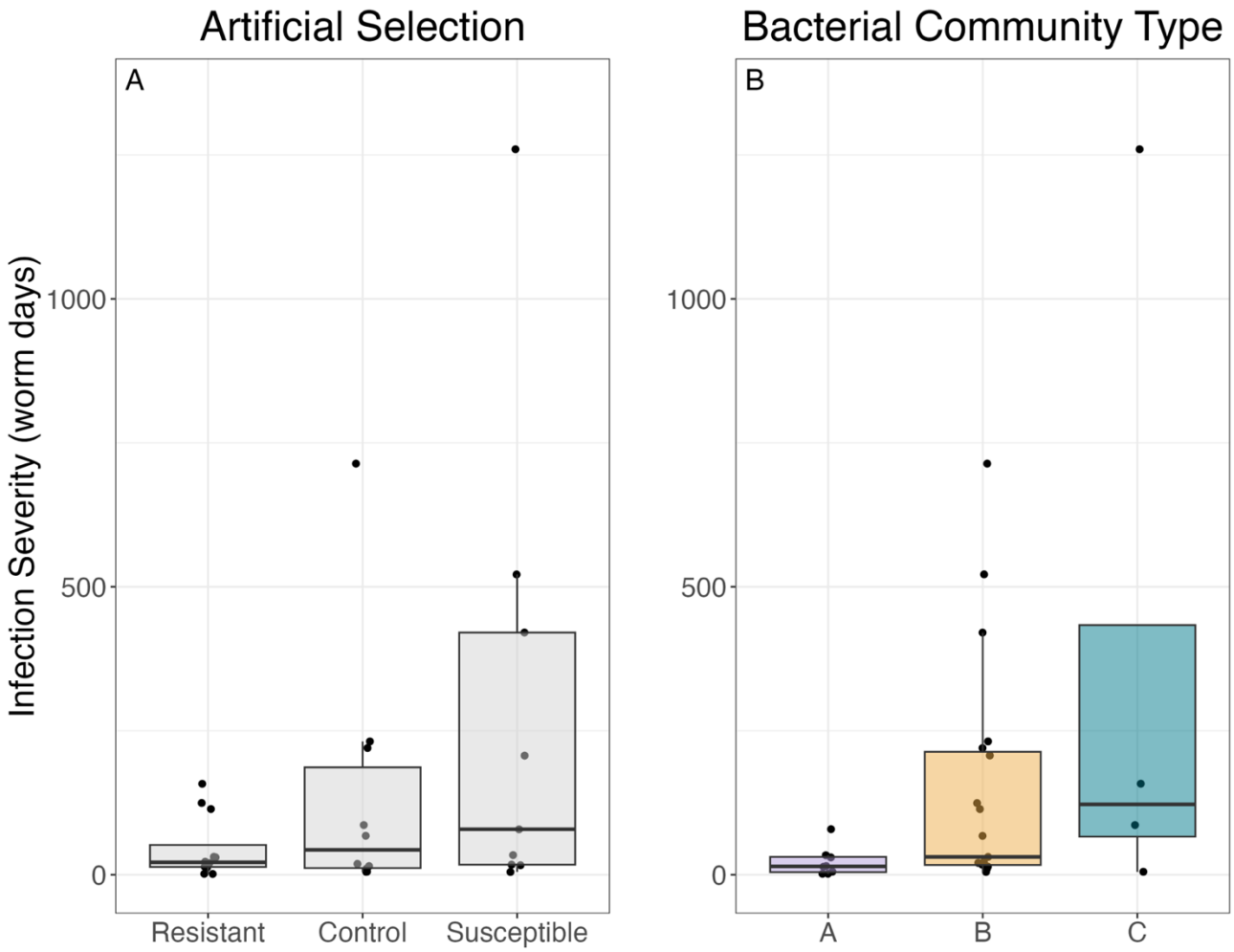

Figure 4. Selection lines and bacterial community types predicted G. turnbulli infection severity. A. Lines significantly predicted severity in swabbed fish (n=31; ANOVA: χ^2=9.39, P=0.009). B. Community type A had lower infection severity than types B and C (A-B, z=2.59, P=0.0034; A-C, z=4.92, P<0.001). No interactions between community type and selection line were detected (ANOVA: χ^2=7.4, P=0.116). A combined model of line and bacterial types explained infection severity better than either factor alone (χ^2=13.40, P=0.001).