Artificial Selection and the Skin Microbiome Independently Predict Parasite Resistance

Rachael D. Kramp[1*], Mary J. Janecka[1,5], Nadine Tardent[2,3], Jukka Jokela[2,3], Kevin D. Kohl[1], Jessica F. Stephenson[1,2,3,4]

1. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

2. Department of Aquatic Ecology, EAWAG, Swiss Federal Institute of Aquatic Sci & Tech, Dübendorf 8600, Switzerland.

3. Institute for Integrative Biology, ETH Zürich, Zürich 8092, Switzerland.

4. Center for Adaptation to a Changing Environment, ETH Zürich, Zürich 8092, Switzerland.

5. Department of Biological Sciences, University of Texas at El Paso, El Paso, TX 79968, USA

**Author for correspondence: rdkramp@gmail.com*

1. It is well established that host genetics determine much of hosts response to parasites, but recent research has highlighted that the host microbiome plays a role in defense against parasites. Interactions between host resistance to parasites and the microbiome are widely acknowledged; for example, the microbiome can modulate the host immune response, and *vice versa*. However, it remains unclear how the host immune system and the microbiome may together influence the host's overall response to parasites.
2. In many species males and females differ in both their genetically-based parasite resistance and the composition and structure of their associated microbiomes. We may therefore expect interactions between host resistance and the microbiome to differ between males and females, potentially explaining the often-observed variation in their response to parasites.

3. To investigate how host genetics and the microbiome interact to shape host response to parasites, we imposed truncation selection on Trinidadian guppies (*Poecilia reticulata*) for their response to the specialist ectoparasite *Gyrodactylus turnbulli*. The 30% of guppies with the lowest number of parasites, "parasite load", during experimental infection founded the resistant line, and the 30% with the highest parasite load founded the susceptible line, while randomly chosen uninfected fish founded the control line. After 3-6 generations of breeding in the absence of parasites, we sampled the skin-associated microbiome of fish from these lines and then infected them with *G. turnbulli*. We used Dirichlet multinomial modeling (DMM) machine learning to identify the bacterial community types across lines and evaluated the importance of selection line and bacterial community type in explaining variation between fish in parasite load over time 'infection severity".
4. Among females, the resistant line developed significantly lower infection severity, and the susceptible line developed significantly higher infection severity, than any other treatment. Among males, however, while the susceptible line developed significantly higher infection severity than the other lines, there was no difference between the resistant and control lines. We also found that female fish in the resistant line had significantly lower tolerance compared to the control or susceptible line females. This apparent tradeoff between resistance and tolerance was also supported by analysis of the individual-level data.
5. We found that host-associated microbiomes explain as much of the variation in infection severity as host genetics, and that these two factors appear independent and additive in their effects on infection severity. Furthermore, 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. Our results therefore suggest that the microbiome plays an active role in the interaction between host and parasite and is not a passive reflection of host genetics.

Keywords: Trinidadian guppies, ectoparasite, sex-specific differences, host-parasite interactions, artificial selection, parasite resistance, skin microbiome, pre-infection microbiome, bacterial community, parasite-tolerance

Introduction

Hosts use multiple strategies to defend themselves against parasites and pathogens (parasites hereafter). Host genetic background is commonly credited to determine much of the defense against parasites through immune system, behavior, or other genetic/protein alterations, e.g., thalassemia (Hawley et al., 2011; Janecka et al., 2021; Luzzi et al., 1991; Råberg et al., 2007). However, a more recently highlighted defense, the host-associated microbial communities or microbiome, may also influence parasite defense indirectly through the host immune system or directly via niche occupation or secreting anti-parasitic compounds (Nelson & May, 2020; Speer, 2022). Indeed, the host-associated microbiome can dramatically impact host survival and defense to parasites (Hopkins et al., 2017). For example, chytrid fungus, which is currently decimating amphibian biodiversity, can be suppressed by the addition of a natural skin microbe, *Janthinobacterium lividum* (Becker et al., 2009; Brucker et al., 2008). Similarly, the disruption of the gut microbiome alerts the host immune system to parasitic invasion of *Toxoplasma gondii*, and thus improves disease outcomes (Benson et al., 2009). Moreover, the "pre-infection" microbiome can be a powerful tool to predict individuals that are most susceptible to parasites within a population (Nasal: (Lee et al., 2019)& Skin:(Bates et al., 2022)). However, the question of whether the microbiome is playing an active role in host-parasite infection or is merely a reflection of the host's health/immune status is commonly posed (de Vos et al., 2022; Dheilly et al., 2019; Hanage, 2014; Reverter et al., 2020; Trevelline et al., 2020). While there is abundant evidence that the microbial community can have a direct or indirect role in host-parasite interactions (Abt et al., 2012; Becker et al., 2009; Ichinohe et al., 2011; Le Sage et al., 2021; Nelson & May, 2020; Ost & Round, 2017), there is also evidence that the microbiome is merely a bystander in some diseases and a reflection of the host's genetics, diet, and general health (Ni et al., 2017; Shanahan, 2012).

To fully understand the interplay between host genetics and microbiome, it is essential to consider how hosts influence microbial communities and their colonization. Hosts exert control over their symbionts via immune responses, barrier function, physiological regulation, and other mechanisms, enabling them to shape both the ecology and evolution of their microbiota (Wilde et al., 2024). This host-driven control creates natural selection for microbial traits that benefit the host, which ultimately could affect traits like parasite resistance. The host's genetic makeup influences microbiome composition, leading to selection for specific microbial communities that can be

inherited across generations (Bordenstein & Theis, 2015; Kohl, 2020; Lim & Bordenstein, 2020; Mazel et al., 2018). For example, siblings display more similar microbiomes than cohoused non-siblings, highlighting the heritable aspect of these communities (Garrigues et al., 2022; Hand et al., 2013; Robertson et al., 2019). This dynamic interaction between host genetics and microbiome suggests that hosts are not merely passive environments but are actively shaping their microbial inhabitants through selective pressures. However, disentangling host genetic effects from microbial influence presents challenges. These ecological communities are subject to dispersal, priority effects, ecological drift, and other elements of stochasticity (J. Z. Chen et al., 2024; Debray et al., 2022; Jones et al., 2022). Further, host immune systems rely on early microbial colonization for proper immune development (Amenyogbe et al., 2017; Mazel et al., 2018), creating a feedback loop that complicates the ability to fully isolate genetic versus microbial contributions. Despite the heritability of microbiomes, exact mechanisms governing these processes remain under investigation, and the influence of external factors such as environment and temporal changes must be carefully considered (Kohl, 2020). Direct manipulation of the host genetics in traits like parasite resistance, where the microbiome plays a known role—will aid in disentangling genetic effects from microbial ones.

Adding further complexity, the microbiome and the host immune system are both commonly cited to differ between the host sexes (Kramp et al., 2022; Sylvia et al., 2017). The microbiome can be altered by physiological factors that commonly differ between sexes. For example, high levels of testosterone in the blood are known to repress the immune system and significantly alter the microbial communities present within the gut (Santos-Marcos et al., 2023). Similar changes in both immune system and microbial community are observed during pregnancy and menstruation (Chen et al., 2023; Gorczyca et al., 2022). Investigating the immune system and the microbiome in a sex-specific manner thus provides a more holistic understanding of host-parasite interactions in natural systems, and it can also be a powerful tool to examine how genetic mechanisms of parasite resistance may co-evolve with microbiomes to meet differing fitness demands between the sexes.

We artificially selected Trinidadian guppies, *Poecilia reticulata*, for resistance or susceptibility to their specialist parasite *Gyrodactylus turnbulli*, with resistant fish having lower worm burdens (indication of parasite suppression) and susceptible fish having higher worm burdens. Additionally, while we did not specifically select for tissue-specific tolerance (tolerance hereafter), or the ability to minimize the fitness losses caused by a given

parasite load (Stephenson & Adelman, 2022), we did examine the levels of tolerance within our lines. Previous evidence indicates that guppy resistance or susceptibility to *Gyrodactylus turnbulli* is genetically linked and heritable, with potential sex differences (Cable & Van Oosterhout, 2007; Dargent et al., 2016; Fraser & Neff, 2009; Madhavi & Anderson, 1985). The major histocompatibility complex (MHC) appears to be part of genetic-based resistance to *G. turnbulli*, which functions by presenting peptide fragments from parasites to host immune cells, and subsequent immune recognition and response (Fraser et al., 2010; Fraser & Neff, 2009; Phillips et al., 2018). However, both innate and acquired immune responses likely contribute to *G. turnbulli* resistance (Cable & Van Oosterhout, 2007). Interestingly, skin bacterial alpha diversity has been shown to be positively correlated with expected MHC heterozygosity and negatively with MHC nucleotide diversity in frogs (Cortazar-Chinarro et al., 2024). If the same is true for fish, this covariation sets up a clear avenue for the skin microbiome and host genetics to interactively shape parasite resistance phenotypes within our system.

We found that both host genetics and the microbiome significantly predicted the severity of parasite infection, measured as the area under the curve of infection load throughout an individual's infection. However, the microbiome did not significantly differ across our three selection lines. Interestingly, parasite resistance and tolerance showed different patterns between the artificially selected male and females. These results suggest that the microbiome plays an active role in host-parasite interactions, rather than simply reflecting the host's genetics. Overall, our findings highlight the complex interplay between host genetics, the microbiome, and sex-specific responses to parasite infection.

Materials and methods

Experimental design and sample collection

Host origin and maintenance

We used laboratory-bred descendants of wild guppies from a high-predation population located at an undeveloped, rural site on the Caura River, Trinidad (UTM: 20 P 679527.7 m E, 1180376.4 m N based on WGS84 Datum; elevation 112m). Wild guppies (n ~ 600) were transported to Cardiff University in June 2012 (Cefas APB

125 authorization number CW054-D-187A), where they were prophylactically treated for infection using Binox®
126 (Nitrofurazone; Jungle Laboratories Corporation®, Cibolo, Texas). This site was found *Gyrodactylus* spp.-free in
127 a previous survey (Stephenson, van Oosterhout, Mohammed, et al., 2015), and we did not find any *Gyrodactylus*
128 spp. during the examination of a subset of 80 of the collected wild fish. Fin clips were taken from the caudal fin of
129 66 wild-caught, parental generation fish for microsatellite genotyping. Fish were housed in 70 L mixed sex stock
130 tanks on a 12h light: 12h dark lighting regime (overhead fluorescent lighting) at 24±1°C and fed daily with
131 Aquarian® tropical fish flakes supplemented with *Artemia* and bloodworm. Fry were removed from the breeding
132 tanks soon after birth, confirmed free of ectoparasitic infection under a microscope, and reared in one of four
133 juvenile tanks until sex determination was possible (~6-8 weeks; males identified by the initiation of the
134 modification of the anal fin, females by dark pigmentation at the base of the anal fin), and then reared in sex-specific
135 tanks for at least a further month before use. All fish used to initiate the selected lines were, therefore, sexually
136 mature and unmated.
137
138 *Selection experiment*
139 We experimentally infected 100 male and 100 female F1 fish, scored their resistance, and either assigned them to a
140 breeding population ('resistant' or 'susceptible'), or euthanized them. There was only one round of artificial
141 selection on our lines. To initiate experimental infections, a heavily infected fish "culture fish" from a laboratory-
142 maintained culture of *Gyrodactylus turnbulli* was killed with an overdose of MS222 (0.02% tricaine
143 methanesulfonate; PHARMAQ Ltd., Fordingbridge, UK) followed by pithing. This culture fish was then placed
144 next to an anaesthetized experimental fish until two parasites had transmitted, as observed under a dissecting
145 microscope and fiber optic illumination. The experimental fish were revived and housed individually in 1 L tanks,
146 and the number of parasites infecting each was counted under anesthetic every other day. All tanks were maintained
147 under the same conditions as the breeding tanks and received 100% water exchanges every other day. After nine
148 days of infection, all fish were treated with Levamisole and screened three times with at least four days between
149 screenings to confirm they were free of infection. Fin clips were taken from the caudal fin of all fish and stored in
150 100% ethanol for microsatellite genotyping.

The 30 males and 30 females who maintained the highest mean number of worms throughout their nine-day infection were assigned to the 'susceptible' line and allowed to breed freely in a single 120 L tank. The 30 males and 30 females who maintained the lowest mean number of worms over the nine-day infection were assigned to the 'resistant' line and allowed to breed freely in a second 120 L tank. As a control line, we randomly selected 30 males and 30 females from the uninfected F1 sexually mature virgin fish and allowed them to breed in a third 120 L tank. All three tanks were checked at least twice weekly for fry, and these were moved to F2 rearing tanks. In the F2 generation, each line was split into two (CA, CB, RA, RB, SA, SB), again with a population size of 60 fish per tank (30 males, 30 females). This population size was kept consistent across the six subpopulations, with new tanks set up for each subsequent generation as above.

Experimental infection

In August 2015, all fish in each of the three lines were transported from Cardiff, UK, to Zürich, Switzerland. In Zürich, guppies were housed at densities of 1-2 fish per L in 4.5 L tanks in a recirculating system at 24±1°C, on a 12 h light: 12 h dark lighting schedule (overhead fluorescent lighting) and fed daily on commercial flake food supplemented with *Artemia*. The fish were maintained and bred within the six subpopulations under these conditions until the start of the experimental infections, for which 130 fish were infected in five experimental batches between August and November 2017. All fish used for the experimental infections were born in Zürich and represented generations 3-6 after the imposed selection. Allowing fish to breed in the absence of parasites for multiple generations may eliminate potential parental and grand-parental effects, which recent research indicates are important in this system (TGIP MANUSCRIPT CITE). *G. turnbulli* were collected from Qualipet Glatt Zentrum in June 2017, and an isogenic line was initiated by infecting a parasite-naïve ornamental guppy with a single parasite individual. This line was subsequently maintained on parasite-naïve ornamental guppies. Experimental infections were initiated following the protocol described above. The experimental fish were revived and housed individually in 1 L tanks, and the number of parasites infecting each was counted under anesthetic every other day. All tanks were maintained under the same conditions as the breeding tanks and received 100% water exchanges every other

176 day. After 17 days of infection, all fish were fin-clipped (stored in 100% ethanol), treated and screened clear of
177 infection as above, and placed in a breeding stock tank.
178
179 *Microsatellite genotyping*
180 We evaluated the genetic diversity and variance in inbreeding within the wild-caught populations, F1, F3-F6
181 generations to determine if the observed difference in response to infection between the resistant and susceptible
182 lines could be attributed to differences in inbreeding between the groups. DNA was isolated from fin clips with the
183 HotSHOT method using 50 µl of alkaline lysis reagent and neutralizing solution followed by incubation at 95 °C
184 for 30 minutes (Truett et al., 2000). Eight previously designed microsatellite markers were amplified in two
185 multiplex assays (Becher et al., 2002; Nater et al., 2008; Paterson et al., 2005; van Oosterhout et al., 2006; Watanabe
186 et al., 2005). Microsatellites samples were generated in collaboration with the Genetic Diversity Centre (GDC),
187 ETH Zurich. Samples were genotyped on an ABI Genetic Analyzer 3730 (Applied Biosystems, USA). Samples
188 were scored using GeneMarker (Version 2.6.4; Softgenetics) and binned using the package MSatAllele (Alberto,
189 2013) in R (R Core Team, 2020). For more detailed microsatellite PCR prep see supplemental materials.
190
191 *Microbial inventory*
192 Before infection began, we collected samples from a total of 51 fish split between the infection and sham-infection
193 treatment groups: Control (n=10, sham n=5), Resistant (n=12, sham n=6), Susceptible (n=9, sham n=8). The
194 collection and inventory of the microbial communities on the fish skin we used the methods found in (Kramp et al.,
195 2022).
196
197 ***Data analyses***
198 *Infection of 3rd-6th generation offspring*
199 We quantified infection resistance/susceptibility as the area under the curve (AUC) of worm number over day of
200 infection or "infection severity". To test the outcome of our artificial selection, we used the infection severity AUC
201 as the response variable in a generalized linear mixed models (glmm) in the glmmTMB package, v. 1.1.2.3 (Brooks

202 et al., 2017) (Gamma error family and logit link function within R v.4.3.2 ((R Core Team, 2020); see supplementary
203 material). As fixed main effects, we included artificial selection (line; resistant n=19, control n=15, susceptible
204 n=16), fish sex, length (On day 0 and controlling for sex differences, see supplementary material), body condition
205 (scaled mass index (Peig & Green, 2009)), generation (gen), number of parasite starting infection (dose), and trial
206 date (Julien date). We also included a two-way interaction between sex and line based on lower AIC. We used
207 ggplot2 (Wickham, 2016) for plotting all figures. For all models, we assessed collinearity among predictor variables
208 using the pairs function (Zuur et al., 2009). Model validation was performed with the 'DHARMa' package (Hartig,
209 2022) and AIC criteria. To test for significance across models, we applied Type II Wald chi-square tests using the
210 Anova function from the 'car' package (Fox & Weisberg, 2019).
211
212 *Tissue-specific tolerance of 3rd-6th generation offspring*
213 We quantified tissue-specific tolerance "ppmass" as the relative per-parasite percentage mass change of the host
214 during infection by dividing the percentage mass change between day 0 and day 20 by the maximum number of
215 worms observed on the fish during its infection (Jog et al., 2022). We used "relative" per-parasite percentage mass
216 change because we do not expect zero percent body mass change throughout the experiment, e.g., a perfectly
217 tolerant individual would likely gain weight during the experiment. Therefore, we scale all percentage body mass
218 change relative to the highest percent body mass change observed in the experiment. To test the effect of artificial
219 selection on tolerance of infection we used tolerance as a response variable in a glmm in the glmmTMB package,
220 v. 1.1.2.3 (Brooks et al., 2017) (Gaussian error family and logit link function within R v.4.3.2 ((R Core Team,
221 2020); see supplementary material). As fixed main effects, we included artificial selection (line), fish sex, length
222 (On day 0 and controlling for sex differences, see supplementary material), generation (gen), number of parasite
223 starting infection (dose), and trial date (Julien date). We excluded body condition (scaled mass index) as a fixed
224 effect due to the response variable incorporating mass changes in its calculations. Furthermore, within the
225 microbiome we tested if the bacterial community types predicted tolerance by adding it as a fixed effect.
226
227 *Resistance-tolerance Tradeoff*

To investigate the relationship between tissue-specific tolerance and resistance we ran a polynomial regression model of the second degree (quadratic) to account for the non-linear effects. The model was fit using the lm() function in R v.4.3.2 ((R Core Team, 2020); see supplementary material). We used Tolerance as the response variable and log transformed AUC (our resistance metric), fish length, and generation as fixed effects. We did not include AUC within our main tolerance model, described above, due to high collinearity with other fixed effects within the model, and fit issues due the nonlinear relationship between AUC and ppmass. This model included fixed effects without collinearity with AUC.

Calculating Heritability

We calculated narrow-sense heritability (assuming that the response to selection is primarily due to additive genetic variance) of our artificial selection for mean worm and our calculated tolerance metric for susceptible and resistant lines following the breeder's equation (Lush, 1937). For mean worm, we used the average number of parasites between 1-9 days of infection, rescaled to a mean of zero to account for the differences in worm growth between the infections of the parental and offspring generations. For tolerance, we rescaled using the 'rescale' package (Gelman, 2008) which takes the variable to center (subtract mean) and scale (center and then divides by standard deviation).

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

$$h^2 = \frac{P_r - P_b}{P_s - P_b}$$

Host genetic analysis

We tested allelic richness (A_R), gene diversity (H_S), $F_{IS,}$ and identity disequilibrium (g2) among our artificial selection lines to evaluate whether the differences in phenotype were attributable to variation in inbreeding

253 (Smallbone et al., 2016). Allelic richness is the first population genetic metric to reflect bottleneck effects, which
254 decrease the effective population size and facilitate reductions in heterozygosity due to inbreeding (Nei et al., 1975).
255 Significant differences in allelic richness among populations are indicative of variation in increases in the
256 probability that inbreeding may occur. Gene diversity measures the mean heterozygosity per individual per
257 population, under inbreeding conditions homozygosity increases but gene diversity is not immediately lost
258 (Charlesworth, 2003). Positive values in F_{IS} can be generated by inbreeding but may also be artificially elevated by
259 technical artifacts such as large allele drop out or null alleles, which can artificially inflate a deficiency of
260 heterozygotes (De Meeûs, 2018; Nei et al., 1975). We therefore also estimated identity disequilibrium (g_2), the
261 correlation of heterozygosity across loci resulting from inbreeding, which is robust in the presence of null alleles
262 (David et al., 2007). Concurrence among population genetic metrics consistent with patterns inbreeding are needed
263 to determine if phenotypic differences among populations are attributable to inbreeding. Allelic richness (A_R)
264 (rarefied to the lowest population sample size), gene diversity (H_S) and F_{IS}, was calculated in FSTAT 2.39 (Goudet,
265 01 2001). Significance levels for F_{IS} were assessed in GENEPOP (web version 4.2;(Raymond & Rousset, 1995)
266 with 5000 iterations followed by a Bonferroni correction for multiple comparisons. Tests for statistically significant
267 differences allelic richness (A_R), gene diversity (H_S) and F_{IS} among groups were calculated in FSTAT with 2000
268 permutations. Identity disequilibrium (g^2) is the difference between joint identity by descent and the product of the
269 separate probabilities of identity by decent for two loci. g^2 is estimated with microsatellite markers as the excess of
270 double heterozygous genotypes at all pairs of loci relative to a random distribution of heterozygous genotypes
271 among individuals at each locus (Cockerham & Weir, 1973; David et al., 2007). g_2 values significantly greater than
272 0 are indicative of identity disequilibrium (ID). ID was calculated in inbreedR (Stoffel et al., 2016).
273
274 *Microbial alpha and beta diversity*
275 Raw sequence data were processed using the bioinformatics program QIIME2 version 2019.7 (Bolyen et
276 al., 2019). The resulting ASV table was rarefied to 3,654 sequences per sample, which retained all but 1 sample and
277 excluded kit controls. Within QIIME2, the DADA2 pipeline was used to calculate the unweighted and weighted
278 UniFrac distances (Lozupone & Knight, 2005) in a pairwise fashion between all pairs of samples to create distance

matrices. Significant differences in microbial community composition/membership across line, sex, length, and body condition were tested using a PERMANOVA for the adonis package in R (Anderson, 2001). We used Principal Coordinate Analysis (PCoA) plots with generated in R version 3.4.3. (R Core Team, 2020) to visualize all factors tested. We also calculated the following alpha diversity indices using QIIME2: the number observed number of ASVs, Shannon diversity, and Faith's phylogenetic diversity. We compared mean values of these metrics using Pairwise Kruskal-Wallis preformed on QIIME2 for line and sex.

Bacterial community typing

We used Dirichlet multinomial mixture models (DMM) (Holmes et al., 2012) in R v4.0.0 and the Dirichlet Multinomial v1.16.0 package (Morgan, 2023) to assign all samples (n=51) to three different bacterial community types based on the relative frequency of taxa. We determined the number of community types by comparing the Laplace approximation of the negative log models and identifying the point at Dirichlet components that resulted in the best model fit (see supplemental materials). All formal statistical inferences are based on the models relating community types to our outcomes of interest, with any findings being statistically supported by the data.

Infection analysis with bacterial community types

To test if the bacterial community types significantly predicted infection severity, we included bacterial community types as a fixed effect in the same glmm model used for artificial selection lines analysis, subsetting to only swabbed fish. We used AUC as the response variable in a glmm in the glmmTMB package, v. 1.1.2.3 (Brooks et al., 2017) (Gamma error family and logit link function within R v.4.3.2 ((R Core Team, 2020); see supplementary material). As fixed main effects, we included bacterial community type, artificial selection (line), fish sex, length (On day 0 and controlling for sex differences, see supplementary material), body condition (scaled mass index (Peig & Green, 2009)), generation (gen), number of parasites starting infection (dose), and trial date (Julien date). We also included a two-way interaction between sex and line based on lower AIC.

Comparing the Predictive Power in our GLMM Models

To compare how well our two GLMM models—one with bacterial community types and the other with the artificial selection lines only—explained variation in the response variable (AUC), we conducted an ANOVA-like comparison using the anova function. All three models shared the same fixed effects, differing only in whether "line" or "bacterial community type" or both was included as a fixed effect (see supplementary material). All models used the same subset of swabbed fish (n=31) to allow for a fair comparison.

Results

Infection of 3rd-6th generation offspring

The artificial selection lines significantly predicted infection severity (***Figure 1***; χ^2=37.74, P =6.4e-09), and had a significant interaction with sex (χ^2=13.51, P =0.0012). Additionally, sex and dose were both important to infection severity in this overall model (sex; χ^2= 24.29, P =8.3e-07 & dose; χ^2= 5.11, P =0.024), but length, scaled mass index, JulianDate, and generation were not. As a post hoc test to evaluate whether the effect of line was significant among both sexes, we subset the data by sex and re-ran the model. We found that artificial selection line was still significant in female and male guppies (female; χ^2=24.33, P =5.2e-06 & male; χ^2=17.57, P =0.00015). A post hoc Tukey test revealed that the female guppies from susceptible and resistant artificial selection lines differed significantly from control line, with susceptible female having high infection severities and resistant females lower compared to control (Susceptible; z=3.49, P<0.001; Resistant; z =-2.74, P=0.016). The male guppies from the susceptible artificial selection line, but not the resistant line, significantly differed compared to the control line (Susceptible: z=4.19, P<0.001; Resistant: z =1.86, P=0.15).

Tissue-specific tolerance of 3rd-6th generation offspring

The artificial selection lines significantly differed in relative tolerance (***Figure 2***; χ^2=14.56, P =0.00069), with a significant interaction with sex (χ^2=10.28, P =0.0059). Additionally, sex and length were both important to tolerance (length; χ^2=5.94, P =0.015). As a post hoc test to evaluate whether this correlation was significantly different from 0 among either sex, we subset the data by sex and re-ran the model. We found that artificial selection line was only

330 significant in female guppies (female; χ^2=23.81, P =6.7e-06) and smaller females were less tolerant (length, female;
331 χ^2=6.96, P =0.0083). However, no fixed effect was significant in the male subset. JulianDate was not significant in
332 explaining variation within our models. A post hoc Tukey test revealed that resistant line females differed
333 significantly from control and susceptible line females, with resistant females having lower tolerance and compared
334 to the other lines (control; z=-3.92, P=0.0003; susceptible; z =4.05, P=0.00016). We did not find that the bacterial
335 community types significantly predicted tolerance within our study.
336
337 *Resistance-tolerance Tradeoff*
338 The polynomial regression model revealed a significant relationship between tissue-specific tolerance and infection
339 severity (lower infection severity indicates higher resistance). AUC explained a substantial proportion of the
340 variation in tissue-specific tolerance (logAUC: F = 61.95, p < 4e-16, see supplement). No other fixed effects
341 included in our model were significant.
342
343 *Host genetic analysis*
344 We tested gene diversity (H_s), allelic richness (A_R), inbreeding coefficient (F_{IS}), , and identity disequilibrium (g^2)
345 among our artificial selection lines to evaluate whether the difference in phenotype were attributable to variation in
346 inbreeding. In this case the reduction in population size post-capture can be seen clearly in the decrease in allelic
347 richness among all of the experimental infection groups. The reduction in allelic richness among all experimental
348 infection groups relative to the wild caught founders likely reflects this post-capture reduction (*Supplement*
349 *materials; Table 1*). We found no significant difference in A_R, (p= 0.589), H_s (p=0.73) or F_{IS} (p=0.42) among the
350 three lines. We found no significant identity disequilibrium in any of the three lines (*Supplemental 1, Table 2*).
351
352 *Narrow sense heritability of resistance and tolerance*

353 We found that the heritability of 'meanworm' (the metric used for parental selection, i.e., average number of
354 parasites throughout infection) was h^2=0.79 in the susceptible line, and for the resistant lines h^2=0.41. We found
355 that heritability of tolerance was h^2=0.88 in the susceptible line, and h^2=0.77 for the resistant line.
356
357 *Bacterial community types*
358 All skin swab samples were assigned to a community type with the greatest posterior probability. All samples had
359 a posterior probability of 98% or higher. Each bacterial community type contained between 10.0–66.8% of all
360 samples (n = 5-33), and no samples were undefined. For top contributing bacterial genera see ***Figure S2*** in
361 supplemental material.
362
363 *Skin Microbiome Variation*
364 The artificial selection lines did not significantly differ any skin microbial diversity metrics tested (see
365 supplemental materials). Interestingly, the sex of the fish was significant in explaining Shannon's index and
366 observed ASVs, with females having significantly higher alpha diversities. (Kruskal–Wallis pairwise test, SI; H =
367 4.06, q = 0.04 & ASVs; H = 4.88, q = 0.03). Consistent with Kramp *et al*. 2022, female and male fish had
368 significantly different Unweighted Unifrac beta diversity (PERMANOVA; F=1.56, Pr=0.006).
369
370 *Infection outcomes with bacterial community types*
371 DMM modeling found that the lowest Laplace approximation model fit was three bacterial clusters (See
372 supplemental materials). To confirm that the three bacterial community types (named A, B, and C) within our fish
373 skin samples were distinct community types, we ran a PERMANOVA (PERMANOVA: F = 7.85, p = 0.001, ***Figure***
374 ***3a***). Male and female fish numbers were balanced among the bacterial community types A and B, and bacterial
375 community types clustered evenly across the artificial selection lines (***Figure 3a***; Fisher's exact test, p = 0.8).
376 Additionally, we found no interaction between line and bacterial community types in our model ($\chi 2$ = 7.4 p=0.116).
377 We found that bacterial community type was a significant in explaining AUC ($\chi 2$ = 19.83, p=4.9e-5, ***Figure 3b).***
378 We found that the bacterial community type A of the skin microbiome on Trinidadian guppies had significantly

379 reduced infection severity compared to bacterial community type B & C(B: z=3.23, p=0.0034 & C: z=4.92, p=1e-
380 04). A post-hoc test showed that a model including both artificial selection line and bacterial community type is
381 significantly better at explaining variation in infection severity than models with either variable alone (***Figure 4***;
382 Chisq=13.4, Pr=0.0012), see supplement materials.
383
384 **Discussion**
385 Our artificial selection lines demonstrated the striking heritability of parasite infection resistance and
386 tolerance. We did not find that the microbiome is correlated or differed significantly between our three selection
387 lines. Surprisingly, we did find that the microbiome community types predicted infection severity independently
388 from host lines through bacterial community types found by DMM machine learning, though the microbiome did
389 not predict metrics of host tolerance. We also observed notable differences between males and females, both in the
390 response to artificial selection and in the skin microbiome. Below, we explore each of these findings in turn. We
391 first analyze the implications of the guppies' responses to artificial selection, followed by a discussion on the
392 independence of the microbiome in predicting resistance. Finally, we delve into the observed differences between
393 males and females and their potential impact on parasite resistance and microbiome interactions.
394 Our study is the first, to our knowledge, to use artificial selection to select for guppies that are susceptible
395 or resistant to their specialist parasite, *G. turnbulli*. Previous studies have examined parasite resistance evolution
396 through natural population variation (Dargent et al., 2013; van Oosterhout et al., 2003). It appears that selection for
397 both resistance and susceptibility traits can be realized within a single generation. Our study expands past
398 investigations by considering susceptibility selection, and not resistance alone, we revealed that both susceptible
399 male and female guppies were significantly different to control line fish. Selection for susceptible fish could have
400 multiple genetic mechanisms, one being tolerance and other being loss of resistance, and evidence for evolved
401 tolerance has been shown in wild guppy populations (Stephenson, van Oosterhout, & Cable, 2015). Our
402 experimental design could have imposed selection for tolerance, particularly among the susceptible line, as only
403 those individuals that are able to survive and successfully reproduce after experiencing severe infection loads
404 contributed to subsequent generations (Medzhitov et al., 2012; Råberg et al., 2007). Tolerance and resistance likely

405 have different genetic basis, and tradeoffs between the traits have been demonstrated (Mikaberidze & McDonald,
406 2020; Råberg et al., 2007). Consistent with such a tradeoff, and that our experimental design imposed selection for
407 tolerance in the susceptible line, we found that resistant line fish had lower tolerance compared to control and
408 susceptible line fish. Also, we found that this resistance-tolerance tradeoff exists among all fish sampled: the more
409 resistant an individual is the lower tolerance the individual appears to have. However, it is important to note that
410 research has shown that these tradeoffs do not always occur and both tolerance and resistance traits can coexist
411 within a single individual host, and within a population (Hardy et al., 2024; Pagán & García-Arenal, 2018).
412 Additionally, tolerance can have multiple mechanisms, which we do not touch on, but have profound effects on
413 parasite virulence (Miller et al., 2006).

414 Our estimates of narrow-sense heritability for 'meanworm' and tolerance were high, particularly for our
415 susceptible lines, though our values are still comparable to heritability estimates to our previous studies (Weiler et
416 al., 2024), and to male guppy ornaments (Morris et al., 2020; van der Bijl et al., 2023), which are correlated with
417 *G. turnbulli* resistance (Stephenson et al., 2020). The greater heritability of susceptibility, and the indication of their
418 higher tolerance may be important for understanding how the behavioral and immunological defenses of
419 Trinidadian guppies interact with the spread and evolution of parasites (Walsman et al., 2022, 2024). In some guppy
420 populations, the prevalence of infection is very high (>90%; (Clark et al., 2023; Stephenson, van Oosterhout,
421 Mohammed, et al., 2015)), selecting for higher tolerance of infection and lower resistance to infection via the
422 'resistance is futile' effect which can resemble the evolution of tolerance (Walsman et al., 2023). It is important to
423 note that we did not find any notable differences in the three selections line in our genetic metrics: F_{IS} (inbreeding
424 coefficient) or g^2 (identity disequilibrium). These results taken together indicate that the differences we found within
425 our selection lines were not due to inbreeding and appear to be heritable.

426 Host characteristics such as immune system, metabolism, and growth rate have been shown to interact with
427 the microbiome (Hosseini et al., 2016; Linehan et al., 2018; Speer, 2022; Zheng et al., 2020). More recent evidence
428 has shown that life history and size selection experiments have an indirect effect on guppy resistance to *G. turnbulli*
429 (Bartuseviciute et al., 2022), suggesting more unknown complexities of parasite resistance interacting with

430 metabolism and growth rates of the host. However, we did not find that the microbiome composition, alpha
431 diversity, or bacterial community types differed significantly between the resistance, susceptibility, or control lines,
432 nor did the bacterial communities predict tolerance within our fish. Therefore, our findings suggest that selection
433 line host genetics and host-associated skin microbiome significantly predict parasite infection severity, but not
434 tolerance, and that they could do so independently and additively within the host. Several studies have found that
435 fish skin microbiomes are highly variable and may not reflect the host's genetics but drift due to stochasticity (Jones
436 et al., 2022; Sadeghi et al., 2023; Wang et al., 2023), in agreement with our results. Nevertheless, while host genetics
437 within our artificial selection lines do not appear to influence the skin microbiome, that many studies including ours
438 have shown a strong correlation between bacterial communities and infection severity nevertheless points to
439 important interactions between the skin microbiome and parasite (Le Sage et al., 2021; Scheifler et al., 2022; Speer,
440 2022; Xavier et al., 2019). While within our study the skin microbiome was not altered by artificial selection, a
441 recent study that artificially selected for body size in fish significantly altered the host-associated microbiome and
442 found that the microbiome in the fast-growing line had the ability to resist parasite colonization (Piazzon et al.,
443 2020). Piazzon's study indicates that selection on other traits, i.e., size or color could have important effects on the
444 microbiome that could alter their susceptibility to parasites. Indeed, Bartuseviciute *et al.* that found life history and
445 size selection experiments have been shown to have an indirect effect on guppies resistance to *G. turnbulli*
446 (Bartuseviciute et al., 2022), potentially mediated through the microbiome.

447 The microbiome's role in determining resistance has been seen in other systems, where pre-infection
448 microbiome composition, alpha diversity, or microbiome function is a powerful tool to predict individuals that are
449 most susceptible to parasites within a population (Bates et al., 2022; Lee et al., 2019). Our results further emphasize
450 the potential of the microbiome in predicting infectious disease dynamics and lend further credence to the
451 microbiome having an active role on host-parasite interactions and not passively reflecting host's genetics. Further
452 investigation of our bacterial community findings were found to be similar to a recent study of skin microbial
453 metabolism function in amphibian chytridiomycosis (Bates et al., 2022): we found five of the eight ASVs reported
454 to be indicator taxa of epizootic dynamics; *Burkholderiales, Pseudomonadales, Sphingomonadales, Rhizobiales,*

455 *Flavobacteriales* were also important in our selected bacterial community types (see ***Figure S2*** for all contributing
456 species). These results taken together indicate a complex and dynamic relationship between skin microbiome,
457 ectoparasite infection, and host genetics. (Casadei et al., 2023; Douglas et al., 2021).

458 Our results show intricate patterns of evolution between the sexes in the multiple defenses against their
459 specialist parasite *G. turnbulli*. We found significant differences between the sexes in the extent to which artificial
460 selection line predicted infection severity This finding is similar to those found in Dargent et al., where male guppies
461 did not show significant evolution of resistance to *Gyrodactylus*, compared to females (Dargent et al., 2016). A
462 potential explanation for this finding is that male guppies in the ancestral population already had greater resistance
463 than did females due to sexual selection, and thus perhaps the evolution of further resistance among males is
464 constrained. Indeed, females in many populations prefer males with larger orange ornaments (Endler et al., 2001;
465 Houde, 1988, 1997), with implications for disease dynamics (Rovenolt et al., 2024). Larger orange ornaments
466 indicate male resistance to this parasite (Stephenson et al., 2020). We also see that males tend to develop lower
467 infection loads in general in the lab (Johnson et al., 2011), are less likely, and less heavily infected in surveys of
468 natural populations (Clark et al., 2023; Gotanda et al., 2013; Stephenson, van Oosterhout, Mohammed, et al., 2015).
469 Furthermore, our experimental design allowed female preference for such males to continue in our experiment,
470 potentially resulting in the overall high and invariable resistance we observe in males.

471 We saw differences in the evolution of susceptibility between males and females in our lines. Natural
472 selection on female guppies for larger body sizes and longevity has likely resulted in the evolution of increased
473 infection tolerance compared to males (Stephenson et al., 2016).That we saw the tolerance-resistant tradeoff only
474 within our female guppies could have fundamental links to how male and female guppies respond to parasite
475 selection pressure. However, that we saw males within the susceptible lines were significantly different to control
476 lines in infection severity, but not tolerance, might suggest that the sexes face different tradeoffs. This possibility
477 will hopefully inspire exciting and novel exploration of the evolutionary dynamics of resistance and tolerance
478 between the sexes.

We did not find an interaction between bacterial community types and sex within our main models, and males and females were even spread across the two largest community types A and B. Though, we did find that sex was a significant indicator of both alpha and beta diversity metrics. Sex has been seen to have influence on skin microbiome in other studies (Jung et al., 2023; Rodríguez-Barreto et al., 2023; Sadeghi et al., 2023). The observed sex-specific differences in both artificial selection and microbiome suggest potential co-evolutionary patterns between host sex and microbiome in parasite resistance that need to be disentangled with methodical experimental designs.

This study is the first to artificially select guppies for resistance and susceptibility to *G. turnbulli*, revealing novel insights into the evolution of host-parasite dynamics. By incorporating both resistant and susceptible lines, we uncovered evidence for a tolerance-resistance tradeoff, particularly in females, where resistant fish showed lower tolerance to infection. Surprisingly, while the microbiome did not differ across selection lines, bacterial community types were significantly associated with infection outcomes, suggesting that the microbiome plays an active role in shaping disease dynamics. This study is the first to highlight the role of the microbiome in the well-known system between Trinidadian guppies and *G. turnbulli* parasites. These findings have broader implications for understanding how host genetics and microbiome interact to influence disease susceptibility and tolerance, with potential applications in disease management, conservation, and evolutionary biology. The study also highlights sex-specific evolutionary trajectories in response to parasite pressure, encouraging further exploration into how sex differences shape resistance and tolerance in host populations.

References

Bakke, T. A., Cable, J., & Harris, P. D. (2007). The biology of gyrodactylid monogeneans: the "Russian-doll killers." *Advances in Parasitology*, *64*, 161–376.

Bates, K. A., Sommer, U., Hopkins, K. P., Shelton, J. M. G., Wierzbicki, C., Sergeant, C., Tapley, B., Michaels, C. J., Schmeller, D. S., Loyau, A., Bosch, J., Viant, M. R., Harrison, X. A., Garner, T. W. J., & Fisher, M. C. (2022). Microbiome function predicts amphibian chytridiomycosis disease dynamics. *Microbiome*, *10*(1), 44.

505 Benson, A., Pifer, R., Behrendt, C. L., Hooper, L. V., & Yarovinsky, F. (2009). Gut commensal bacteria direct a
506 protective immune response against Toxoplasma gondii. *Cell Host & Microbe*, *6*(2), 187–196.
507 Bernardo-Cravo, A. P., Schmeller, D. S., Chatzinotas, A., Vredenburg, V. T., & Loyau, A. (2020). Environmental
508 Factors and Host Microbiomes Shape Host-Pathogen Dynamics. *Trends in Parasitology*, *36*(7), 616–633.
509 Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts
510 and Hologenomes. *PLoS Biology*, *13*(8), e1002226.
511 Brucker, R. M., Harris, R. N., Schwantes, C. R., Gallaher, T. N., Flaherty, D. C., Lam, B. A., & Minbiole, K. P. C.
512 (2008). Amphibian chemical defense: antifungal metabolites of the microsymbiont Janthinobacterium
513 lividum on the salamander Plethodon cinereus. *Journal of Chemical Ecology*, *34*(11), 1422–1429.
514 de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut*,
515 *71*(5), 1020–1032.
516 Haehling, M. B., Toscano, J. H. B., Giraldelo, L. A., Santos, I. B., Esteves, S. N., Benavides, M. V., Tholon, P., &
517 Chagas, A. C. S. (2021). Is selection for resistance and resilience to Haemonchus contortus possible in
518 Morada Nova sheep? *Small Ruminant Research: The Journal of the International Goat Association*, *201*,
519 106447.
520 Hanage, W. P. (2014, August 20). *Microbiology: Microbiome science needs a healthy dose of scepticism*. Nature
521 Publishing Group UK. https://doi.org/10.1038/512247a
522 Holmes, I., Harris, K., & Quince, C. (2012). Dirichlet multinomial mixtures: generative models for microbial
523 metagenomics. *PloS One*, *7*(2), e30126.
524 Hopkins, S. R., Wojdak, J. M., & Belden, L. K. (2017). Defensive Symbionts Mediate Host-Parasite Interactions at
525 Multiple Scales. *Trends in Parasitology*, *33*(1), 53–64.
526 Le Sage, E. H., LaBumbard, B. C., Reinert, L. K., Miller, B. T., Richards-Zawacki, C. L., Woodhams, D. C., &
527 Rollins-Smith, L. A. (2021). Preparatory immunity: Seasonality of mucosal skin defences and
528 Batrachochytrium infections in Southern leopard frogs. *The Journal of Animal Ecology*, *90*(2), 542–554.
529 Lee, K. H., Gordon, A., Shedden, K., Kuan, G., Ng, S., Balmaseda, A., & Foxman, B. (2019). The respiratory
530 microbiome and susceptibility to influenza virus infection. *PloS One*, *14*(1), e0207898.

531 Li, F., Li, C., Chen, Y., Liu, J., Zhang, C., Irving, B., Fitzsimmons, C., Plastow, G., & Guan, L. L. (2019). Host
532 genetics influence the rumen microbiota and heritable rumen microbial features associate with feed
533 efficiency in cattle. *Microbiome*, *7*(1), 92.
534 Libertucci, J., & Young, V. B. (2019). The role of the microbiota in infectious diseases. *Nature Microbiology*, *4*(1),
535 35–45.
536 Lorgen-Ritchie, M., Clarkson, M., Chalmers, L., Taylor, J. F., Migaud, H., & Martin, S. A. M. (2022). Temporal
537 changes in skin and gill microbiomes of Atlantic salmon in a recirculating aquaculture system – Why do
538 they matter? *Aquaculture* , *558*, 738352.
539 Mazel, F., Davis, K. M., Loudon, A., Kwong, W. K., Groussin, M., & Parfrey, L. W. (2018). Is Host Filtering the
540 Main Driver of Phylosymbiosis across the Tree of Life? *MSystems*, *3*(5).
541 https://doi.org/10.1128/mSystems.00097-18
542 Minniti, G., Rød Sandve, S., Padra, J. T., Heldal Hagen, L., Lindén, S., Pope, P. B., Ø Arntzen, M., & Vaaje-
543 Kolstad, G. (2019). The Farmed Atlantic Salmon (Salmo salar) Skin-Mucus Proteome and Its Nutrient
544 Potential for the Resident Bacterial Community. *Genes*, *10*(7). https://doi.org/10.3390/genes10070515
545 Mueller, U. G., Juenger, T. E., Kardish, M. R., Carlson, A. L., Burns, K. M., Edwards, J. A., Smith, C. C., Fang,
546 C.-C., & Des Marais, D. L. (2021). Artificial Selection on Microbiomes To Breed Microbiomes That Confer
547 Salt Tolerance to Plants. *MSystems*, *6*(6), e0112521.
548 Nelson, P., & May, G. (2020). Defensive Symbiosis and the Evolution of Virulence. *The American Naturalist*,
549 *196*(3), 333–343.
550 Parks, D. H., & Beiko, R. G. (2013). Measures of phylogenetic differentiation provide robust and complementary
551 insights into microbial communities. *The ISME Journal*, *7*(1), 173–183.
552 Piazzon, M. C., Naya-Català, F., Perera, E., Palenzuela, O., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2020). Genetic
553 selection for growth drives differences in intestinal microbiota composition and parasite disease resistance
554 in gilthead sea bream. *Microbiome*, *8*(1), 168.
555 Reverter, M., Sasal, P., Suzuki, M. T., Raviglione, D., Inguimbert, N., Pare, A., Banaigs, B., Voisin, S. N., Bulet,
556 P., & Tapissier-Bontemps, N. (2020). Insights into the Natural Defenses of a Coral Reef Fish Against Gill

557 Ectoparasites: Integrated Metabolome and Microbiome Approach. *Metabolites*, *10*(6).
558 https://doi.org/10.3390/metabo10060227
559 Shanahan, F. (2012). The microbiota in inflammatory bowel disease: friend, bystander, and sometime-villain.
560 *Nutrition Reviews*, *70 Suppl 1*, S31-7.
561 Smallbone, W., Ellison, A., Poulton, S., van Oosterhout, C., & Cable, J. (2021). Depletion of MHC supertype during
562 domestication can compromise immunocompetence. *Molecular Ecology*, *30*(3), 736–746.
563 Smith, M. I., Yatsunenko, T., Manary, M. J., Trehan, I., Mkakosya, R., Cheng, J., Kau, A. L., Rich, S. S.,
564 Concannon, P., Mychaleckyj, J. C., Liu, J., Houpt, E., Li, J. V., Holmes, E., Nicholson, J., Knights, D.,
565 Ursell, L. K., Knight, R., & Gordon, J. I. (2013). Gut microbiomes of Malawian twin pairs discordant for
566 kwashiorkor. *Science*, *339*(6119), 548–554
567 Abt, M. C., Osborne, L. C., Monticelli, L. A., Doering, T. A., Alenghat, T., Sonnenberg, G. F., Paley, M. A.,
568 Antenus, M., Williams, K. L., Erikson, J., Wherry, E. J., & Artis, D. (2012). Commensal bacteria calibrate
569 the activation threshold of innate antiviral immunity. *Immunity*, *37*(1), 158–170.
570 Alberto, F. (2013). MsatAllele: Visualizes the scoring and binning of microsatellite fragment sizes. *R Package*
571 *Version*.
572 Amenyogbe, N., Kollmann, T. R., & Ben-Othman, R. (2017). Early-Life Host-Microbiome Interphase: The Key
573 Frontier for Immune Development. *Frontiers in Pediatrics*, *5*, 111.
574 Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1),
575 32–46.
576 Bartuseviciute, V., Diaz Pauli, B., Salvanes, A. G. V., & Heino, M. (2022). Size-selective harvesting affects the
577 immunocompetence of guppies exposed to the parasite Gyrodactylus. *Proceedings. Biological Sciences /*
578 *The Royal Society*, *289*(1981), 20220534.
579 Bates, K. A., Sommer, U., Hopkins, K. P., Shelton, J. M. G., Wierzbicki, C., Sergeant, C., Tapley, B., Michaels, C.
580 J., Schmeller, D. S., Loyau, A., Bosch, J., Viant, M. R., Harrison, X. A., Garner, T. W. J., & Fisher, M. C.
581 (2022). Microbiome function predicts amphibian chytridiomycosis disease dynamics. *Microbiome*, *10*(1),
582 44.

583 Becher, S. A., Russell, S. T., & Magurran, A. E. (2002). Isolation and characterization of polymorphic
584 microsatellites in the Trinidadian guppy (Poecilia reticulata). *Molecular Ecology Notes*, *2*(4), 456–458.
585 Becker, M. H., Brucker, R. M., Schwantes, C. R., Harris, R. N., & Minbiole, K. P. C. (2009). The bacterially
586 produced metabolite violacein is associated with survival of amphibians infected with a lethal fungus.
587 *Applied and Environmental Microbiology*, *75*(21), 6635–6638.
588 Benson, A., Pifer, R., Behrendt, C. L., Hooper, L. V., & Yarovinsky, F. (2009). Gut commensal bacteria direct a
589 protective immune response against Toxoplasma gondii. *Cell Host & Microbe*, *6*(2), 187–196.
590 Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E.
591 J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown,
592 C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible,
593 interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8),
594 852–857.
595 Bordenstein, S. R., & Theis, K. R. (2015). Host Biology in Light of the Microbiome: Ten Principles of Holobionts
596 and Hologenomes. *PLoS Biology*, *13*(8), e1002226.
597 Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler,
598 M., & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated
599 Generalized Linear Mixed Modeling. In *The R Journal* (Vol. 9, Issue 2, pp. 378–400).
600 https://doi.org/10.32614/RJ-2017-066
601 Brucker, R. M., Harris, R. N., Schwantes, C. R., Gallaher, T. N., Flaherty, D. C., Lam, B. A., & Minbiole, K. P. C.
602 (2008). Amphibian chemical defense: antifungal metabolites of the microsymbiont Janthinobacterium
603 lividum on the salamander Plethodon cinereus. *Journal of Chemical Ecology*, *34*(11), 1422–1429.
604 Cable, J., & Van Oosterhout, C. (2007). The role of innate and acquired resistance in two natural populations of
605 guppies (Poecilia reticulata) infected with the ectoparasite Gyrodactylus turnbulli. *Biological Journal of*
606 *the Linnean Society. Linnean Society of London*, *90*(4), 647–655.

Casadei, E., Mani, A., Cisco, M., Vågnes, Ø., Salinas, I., & Patel, S. (2023). Sex-dependent effects of mechanical delousing on the skin microbiome of broodstock Atlantic salmon (Salmo salar L.). *Scientific Reports*, *13*(1), 10824.

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *358*(1434), 1051–1070.

Chen, J. Z., Kwong, Z., Gerardo, N. M., & Vega, N. M. (2024). Ecological drift during colonization drives within-host and between-host heterogeneity in an animal-associated symbiont. *PLoS Biology*, *22*(4), e3002304.

Chen, X., Wu, R., Li, L., Zeng, Y., Chen, J., Wei, M., Feng, Y., Chen, G., Wang, Y., Lin, L., Luo, H., Chen, A., Zeng, Z., He, F., Bai, Y., Zhang, S., Han, Y., Wang, Z., Zhao, X., … Gong, S. (2023). Pregnancy-induced changes to the gut microbiota drive macrophage pyroptosis and exacerbate septic inflammation. *Immunity*, *56*(2), 336-352.e9.

Clark, D. R., Young, K. A., Kitzes, J., Moore, P. J., Evans, A. J., & Stephenson, J. F. (2023). Aggregation of symbionts on hosts depends on interaction type and host traits. *Ecography*. https://doi.org/10.1111/ecog.06858

Cockerham, C. C., & Weir, B. S. (1973). Descent measures for two loci with some applications. *Theoretical Population Biology*, *4*(3), 300–330.

Cortazar-Chinarro, M., Richter-Boix, A., Rödin-Mörch, P., Halvarsson, P., Logue, J. B., Laurila, A., & Höglund, J. (2024). Association between the skin microbiome and MHC class II diversity in an amphibian. *Molecular Ecology*, *33*(1), e17198.

Dargent, F., Rolshausen, G., Hendry, A. P., Scott, M. E., & Fussmann, G. F. (2016). Parting ways: parasite release in nature leads to sex-specific evolution of defence. *Journal of Evolutionary Biology*, *29*(1), 23–34.

Dargent, F., Scott, M. E., Hendry, A. P., & Fussmann, G. F. (2013). Experimental elimination of parasites in nature leads to the evolution of increased resistance in hosts. *Proceedings. Biological Sciences / The Royal Society*, *280*(1773), 20132371.

David, P., Pujol, B., Viard, F., Castella, V., & Goudet, J. (2007). Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*, *16*(12), 2474–2487.

633 De Meeûs, T. (2018). Revisiting FIS, FST, Wahlund Effects, and Null Alleles. *The Journal of Heredity*, *109*(4),
634 446–456.
635 de Vos, W. M., Tilg, H., Van Hul, M., & Cani, P. D. (2022). Gut microbiome and health: mechanistic insights. *Gut*,
636 *71*(5), 1020–1032.
637 Debray, R., Herbert, R. A., Jaffe, A. L., Crits-Christoph, A., Power, M. E., & Koskella, B. (2022). Priority effects
638 in microbiome assembly. *Nature Reviews. Microbiology*, *20*(2), 109–121.
639 Dheilly, N. M., Martínez Martínez, J., Rosario, K., Brindley, P. J., Fichorova, R. N., Kaye, J. Z., Kohl, K. D., Knoll,
640 L. J., Lukeš, J., Perkins, S. L., Poulin, R., Schriml, L., & Thompson, L. R. (2019). Parasite microbiome
641 project: Grand challenges. *PLoS Pathogens*, *15*(10), e1008028.
642 Douglas, A. J., Hug, L. A., & Katzenback, B. A. (2021). Composition of the North American Wood Frog (Rana
643 sylvatica) Bacterial Skin Microbiome and Seasonal Variation in Community Structure. *Microbial Ecology*,
644 *81*(1), 78–92.
645 Endler, J. A., Basolo, A., Glowacki, S., & Zerr, J. (2001). Variation in response to artificial selection for light
646 sensitivity in guppies (Poecilia reticulata). *The American Naturalist*, *158*(1), 36–48.
647 Fox, J., & Weisberg, S. (2019). Companion to Applied Regression [R package car version 3.1-2]. *Sage*, *3rd*.
648 https://cran.r-project.org/web/packages/car/index.html
649 Fraser, B. A., & Neff, B. D. (2009). MHC class IIB additive and non-additive effects on fitness measures in the
650 guppy *Poecilia reticulata*. *Journal of Fish Biology*, *75*(9), 2299–2312.
651 Fraser, B. A., Ramnarine, I. W., & Neff, B. D. (2010). TEMPORAL VARIATION AT THE MHC CLASS IIB IN
652 WILD POPULATIONS OF THE GUPPY (*POECILIA RETICULATA*). *Evolution; International Journal*
653 *of Organic Evolution*, *64*(7), 2086–2096.
654 Garrigues, Q., Apper, E., Chastant, S., & Mila, H. (2022). Gut microbiota development in the growing dog: A
655 dynamic process influenced by maternal, environmental and host factors. *Frontiers in Veterinary Science*,
656 *9*, 964649.
657 Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine*, *27*(15),
658 2865–2873.

659 Gorczyca, K., Obuchowska, A., Kimber-Trojnar, Ż., Wierzchowska-Opoka, M., & Leszczyńska-Gorzelak, B.
660 (2022). Changes in the Gut Microbiome and Pathologies in Pregnancy. *International Journal of*
661 *Environmental Research and Public Health*, *19*(16). https://doi.org/10.3390/ijerph19169961
662 Gotanda, K. M., Delaire, L. C., Raeymaekers, J. A. M., Pérez-Jvostov, F., Dargent, F., Bentzen, P., Scott, M. E.,
663 Fussmann, G. F., & Hendry, A. P. (2013). Adding parasites to the guppy-predation story: insights from
664 field surveys. *Oecologia*, *172*(1), 155–166.
665 Goudet, J. (01 2001). FSTAT, Version. *1. 2: A Computer Program to Calculate F-Statistics. Heredity*, *86*, 485–
666 486.
667 Hanage, W. P. (2014, August 20). *Microbiology: Microbiome science needs a healthy dose of scepticism*. Nature
668 Publishing Group UK. https://doi.org/10.1038/512247a
669 Hand, D., Wallis, C., Colyer, A., & Penn, C. W. (2013). Pyrosequencing the canine faecal microbiota: breadth and
670 depth of biodiversity. *PloS One*, *8*(1), e53115.
671 Hardy, B. M., Muths, E., Funk, W. C., & Bailey, L. L. (2024). Quantifying intraspecific variation in host resistance
672 and tolerance to a lethal pathogen. *The Journal of Animal Ecology*. https://doi.org/10.1111/1365-
673 2656.14106
674 Hartig, F. (2022). *DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R*
675 *package version 0.4. 6*.
676 Hawley, D. M., Etienne, R. S., Ezenwa, V. O., & Jolles, A. E. (2011). Does animal behavior underlie covariation
677 between hosts' exposure to infectious agents and susceptibility to infection? Implications for disease
678 dynamics. *Integrative and Comparative Biology*, *51*(4), 528–539.
679 Holmes, I., Harris, K., & Quince, C. (2012). Dirichlet multinomial mixtures: generative models for microbial
680 metagenomics. *PloS One*, *7*(2), e30126.
681 Hopkins, S. R., Wojdak, J. M., & Belden, L. K. (2017). Defensive Symbionts Mediate Host-Parasite Interactions at
682 Multiple Scales. *Trends in Parasitology*, *33*(1), 53–64.

683 Hosseini, M., Kolangi Miandare, H., Shabani, A., Hoseinifar, S. H., & Yarahmadi, P. (2016). Dietary Lactobacillus
684 acidophilus modulated skin mucus protein profile, immune and appetite genes expression in gold fish
685 (Carassius auratus gibelio). *Fish & Shellfish Immunology*, *59*, 149–154.
686 Houde, A. E. (1988). Genetic difference in female choice between two guppy populations. *Animal Behaviour*, *36*(2),
687 510–516.
688 Houde, A. E. (1997). Effect of artificial selection on male colour patterns on mating preference of female guppies.
689 *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *256*(1346), 125–130.
690 Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota
691 regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National*
692 *Academy of Sciences of the United States of America*, *108*(13), 5354–5359.
693 Janecka, M. J., Rovenolt, F., & Stephenson, J. F. (2021). How does host social behavior drive parasite non-selective
694 evolution from the within-host to the landscape-scale? *Behavioral Ecology and Sociobiology*, *75*(11), 150.
695 Jog, M. G., Sackett, M. E., Kisty, S. D., Hansen, J. A., & Stephenson, J. F. (2022). The behaviour of infected guppies
696 depends on social context, parasite tolerance and host sex. *Animal Behaviour*, *187*, 97–104.
697 Johnson, M. B., Lafferty, K. D., van Oosterhout, C., & Cable, J. (2011). Parasite transmission in social interacting
698 hosts: monogenean epidemics in guppies. *PloS One*, *6*(8), e22634.
699 Jones, E. W., Carlson, J. M., Sivak, D. A., & Ludington, W. B. (2022). Stochastic microbiome assembly depends
700 on context. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(7),
701 e2115877119.
702 Jung, Y., Cui, H. S., Joo, S. Y., Lee, E. K., Seo, C. H., & Cho, Y. S. (2023). Sex differences in the skin microbiome
703 of burn scars. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and]*
704 *the European Tissue Repair Society*, *31*(4), 547–558.
705 Kohl, K. D. (2020). Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-
706 associated microbial communities. *Philosophical Transactions of the Royal Society of London. Series B,*
707 *Biological Sciences*, *375*(1798), 20190251.

Kramp, R. D., Kohl, K. D., & Stephenson, J. F. (2022). Skin bacterial microbiome diversity predicts lower activity levels in female, but not male, guppies, Poecilia reticulata. *Biology Letters*, *18*(8), 20220167.

Le Sage, E. H., LaBumbard, B. C., Reinert, L. K., Miller, B. T., Richards-Zawacki, C. L., Woodhams, D. C., & Rollins-Smith, L. A. (2021). Preparatory immunity: Seasonality of mucosal skin defences and Batrachochytrium infections in Southern leopard frogs. *The Journal of Animal Ecology*, *90*(2), 542–554.

Lee, K. H., Gordon, A., Shedden, K., Kuan, G., Ng, S., Balmaseda, A., & Foxman, B. (2019). The respiratory microbiome and susceptibility to influenza virus infection. *PloS One*, *14*(1), e0207898.

Lim, S. J., & Bordenstein, S. R. (2020). An introduction to phylosymbiosis. *Proceedings. Biological Sciences / The Royal Society*, *287*(1922), 20192900.

Linehan, J. L., Harrison, O. J., Han, S.-J., Byrd, A. L., Vujkovic-Cvijin, I., Villarino, A. V., Sen, S. K., Shaik, J., Smelkinson, M., Tamoutounour, S., Collins, N., Bouladoux, N., Dzutsev, A., Rosshart, S. P., Arbuckle, J. H., Wang, C.-R., Kristie, T. M., Rehermann, B., Trinchieri, G., … Belkaid, Y. (2018). Non-classical immunity controls Microbiota impact on skin immunity and tissue repair. *Cell*, *172*(4), 784-796.e18.

Lozupone, C., & Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235.

Lush, J. L. (1937). *Animal Breeding Plans - Scholar's Choice Edition*. Bibliolife DBA of Bibilio Bazaar II LLC.

Luzzi, G. A., Merry, A. H., Newbold, C. I., Marsh, K., & Pasvol, G. (1991). Protection by alpha-thalassaemia against Plasmodium falciparum malaria: modified surface antigen expression rather than impaired growth or cytoadherence. *Immunology Letters*, *30*(2), 233–240.

Madhavi, R., & Anderson, R. M. (1985). Variability in the susceptibility of the fish host, Poecilia reticulata, to infection with Gyrodactylus bullatarudis (Monogenea). *Parasitology*, *91*(3), 531–544.

Mazel, F., Davis, K. M., Loudon, A., Kwong, W. K., Groussin, M., & Parfrey, L. W. (2018). Is Host Filtering the Main Driver of Phylosymbiosis across the Tree of Life? *MSystems*, *3*(5). https://doi.org/10.1128/mSystems.00097-18

Medzhitov, R., Schneider, D. S., & Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science*, *335*(6071), 936–941.

Mikaberidze, A., & McDonald, B. A. (2020). A tradeoff between tolerance and resistance to a major fungal pathogen in elite wheat cultivars. *The New Phytologist*, *226*(3), 879–890.

Miller, M. R., White, A., & Boots, M. (2006). The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. *Evolution; International Journal of Organic Evolution*, *60*(5), 945–956.

Morgan, M. (2023). *DirichletMultinomial: Dirichlet-Multinomial Mixture Model Machine Learning for Microbiome Data*. https://doi.org/10.18129/B9.bioc.DirichletMultinomial

Morris, J., Darolti, I., van der Bijl, W., & Mank, J. E. (2020). High-resolution characterization of male ornamentation and re-evaluation of sex linkage in guppies. *Proceedings. Biological Sciences / The Royal Society*, *287*(1937), 20201677.

Nater, A., Krützen, M., & Lindholm, A. K. (2008). Development of polymorphic microsatellite markers for the livebearing fish Poecilia parae. *Molecular Ecology Resources*, *8*(4), 857–860.

Nei, M., Maruyama, T., & Chakraborty, R. (1975). The Bottleneck Effect and Genetic Variability in Populations. *Evolution; International Journal of Organic Evolution*, *29*(1), 1–10.

Nelson, P., & May, G. (2020). Defensive Symbiosis and the Evolution of Virulence. *The American Naturalist*, *196*(3), 333–343.

Ni, J., Wu, G. D., Albenberg, L., & Tomov, V. T. (2017). Gut microbiota and IBD: causation or correlation? *Nature Reviews. Gastroenterology & Hepatology*, *14*(10), 573–584.

Ost, K. S., & Round, J. L. (2017). [Review of *A Few Good Commensals: Gut Microbes Use IFN-γ to Fight Salmonella*]. *Immunity*, *46*(6), 977–979.

Pagán, I., & García-Arenal, F. (2018). Tolerance to plant pathogens: Theory and experimental evidence. *International Journal of Molecular Sciences*, *19*(3). https://doi.org/10.3390/ijms19030810

Paterson, I. G., Crispo, E., Kinnison, M. T., Hendry, A. P., & Bentzen, P. (2005). Characterization of tetranucleotide microsatellite markers in guppy (Poecilia reticulata). *Molecular Ecology Notes*, *5*(2), 269–271.

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* , *118*(12), 1883–1891.

760 Phillips, K. P., Cable, J., Mohammed, R. S., Herdegen-Radwan, M., Raubic, J., Przesmycka, K. J., van Oosterhout,
761 C., & Radwan, J. (2018). Immunogenetic novelty confers a selective advantage in host-pathogen
762 coevolution. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(7),
763 1552–1557.
764 Piazzon, M. C., Naya-Català, F., Perera, E., Palenzuela, O., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2020). Genetic
765 selection for growth drives differences in intestinal microbiota composition and parasite disease resistance
766 in gilthead sea bream. *Microbiome*, *8*(1), 168.
767 R Core Team. (2020). *R: A language and environment for statistical computing* (Version v.4.1.0) [R Foundation
768 for Statistical Computing, Vienna, Austria.].
769 Råberg, L., Sim, D., & Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to infectious
770 diseases in animals. *Science*, *318*(5851), 812–814.
771 Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population Genetics Software for Exact Tests and
772 Ecumenicism. *The Journal of Heredity*, *86*(3), 248–249.
773 Reverter, M., Sasal, P., Suzuki, M. T., Raviglione, D., Inguimbert, N., Pare, A., Banaigs, B., Voisin, S. N., Bulet,
774 P., & Tapissier-Bontemps, N. (2020). Insights into the Natural Defenses of a Coral Reef Fish Against Gill
775 Ectoparasites: Integrated Metabolome and Microbiome Approach. *Metabolites*, *10*(6).
776 https://doi.org/10.3390/metabo10060227
777 Robertson, S. J., Lemire, P., Maughan, H., Goethel, A., Turpin, W., Bedrani, L., Guttman, D. S., Croitoru, K.,
778 Girardin, S. E., & Philpott, D. J. (2019). Comparison of Co-housing and Littermate Methods for Microbiota
779 Standardization in Mouse Models. *Cell Reports*, *27*(6), 1910-1919.e2.
780 Rodríguez-Barreto, D., Sanz-González, J. C., Martín, M. V., Arrieta, J. M., & Almansa, E. (2023). Sex-specific
781 bacterial microbiome variation in octopus vulgaris skin. *Frontiers in Microbiology*, *14*, 1233661.
782 Rovenolt, F., Walsman, J., Martin, C., Gotanda, K., & Stephenson, J. (2024). Parasite prevalence depends on female
783 preference: Integrating parasite-mediated sexual selection and infectious disease dynamics. In *Authorea*
784 *Inc.* https://doi.org/10.22541/au.172447480.06236699/v1

785 Sadeghi, J., Chaganti, S. R., Johnson, T. B., & Heath, D. D. (2023). Host species and habitat shape fish-associated
786 bacterial communities: phylosymbiosis between fish and their microbiome. *Microbiome*, *11*(1), 258.
787 Santos-Marcos, J. A., Mora-Ortiz, M., Tena-Sempere, M., Lopez-Miranda, J., & Camargo, A. (2023). Interaction
788 between gut microbiota and sex hormones and their relation to sexual dimorphism in metabolic diseases.
789 *Biology of Sex Differences*, *14*(1), 4.
790 Scheifler, M., Sanchez-Brosseau, S., Magnanou, E., & Desdevises, Y. (2022). Diversity and structure of sparids
791 external microbiota (Teleostei) and its link with monogenean ectoparasites. *Animal Microbiome*, *4*(1), 27.
792 Shanahan, F. (2012). The microbiota in inflammatory bowel disease: friend, bystander, and sometime-villain.
793 *Nutrition Reviews*, *70 Suppl 1*, S31-7.
794 Smallbone, W., Van Oosterhout, C., & Cable, J. (2016). The effects of inbreeding on disease susceptibility:
795 *Gyrodactylus turnbulli* infection of guppies, *Poecilia reticulata*. *Experimental Parasitology*, *167*, 32–37.
796 Speer, K. A. (2022). [Review of *Microbiomes mediate host-parasite interactions*]. *Molecular Ecology*, *31*(7), 1925–
797 1927.
798 Stephenson, J. F., & Adelman, J. S. (2022). The behavior of infected hosts: Behavioral tolerance, behavioral
799 resilience, and their implications for behavioral competence. *Animal Behavior and Parasitism*.
800 https://books.google.ca/books?hl=en&lr=&id=xSZ_EAAAQBAJ&oi=fnd&pg=PA287&ots=xW36pEEkt
801 3&sig=asg6-91ifwE5LAwCypVYASxqwO4
802 Stephenson, J. F., Kinsella, C., & Cable, J. (2016). A further cost for the sicker sex? Evidence for male-biased
803 parasite-induced vulnerability to predation. *Ecology*.
804 https://onlinelibrary.wiley.com/doi/abs/10.1002/ece3.2049
805 Stephenson, J. F., Stevens, M., Troscianko, J., & Jokela, J. (2020). The size, symmetry, and color saturation of a
806 male guppy's ornaments forecast his resistance to parasites. *The American Naturalist*, *196*(5), 597–608.
807 Stephenson, J. F., van Oosterhout, C., & Cable, J. (2015). Pace of life, predators and parasites: predator-induced
808 life-history evolution in Trinidadian guppies predicts decrease in parasite tolerance. *Biology Letters*, *11*(11),
809 20150806.

810 Stephenson, J. F., van Oosterhout, C., Mohammed, R. S., & Cable, J. (2015). Parasites of Trinidadian guppies:
811 evidence for sex- and age-specific trait-mediated indirect effects of predators. *Ecology*, *96*(2), 489–498.
812 Stoffel, M. A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., & Hoffman, J. I. (2016). inbreedR: an R
813 package for the analysis of inbreeding based on genetic markers. *Methods in Ecology and Evolution*, *7*(11),
814 1331–1339.
815 Sylvia, K. E., Jewell, C. P., Rendon, N. M., St John, E. A., & Demas, G. E. (2017). Sex-specific modulation of the
816 gut microbiome and behavior in Siberian hamsters. *Brain, Behavior, and Immunity*, *60*, 51–62.
817 Trevelline, B. K., Stephenson, J. F., & Kohl, K. D. (2020). [Review of *Two's company, three's a crowd: Exploring*
818 *how host-parasite-microbiota interactions may influence disease susceptibility and conservation of*
819 *wildlife*]. *Molecular Ecology*, *29*(8), 1402–1405.
820 Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., & Warman, M. L. (2000). Preparation of PCR-
821 quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques*, *29*(1), 52,
822 54.
823 van der Bijl, W., Shu, J., Goberdhan, V. S., Sherin, L., Cortazar-Chinarro, M., Corral-Lopez, A., & Mank, J. E.
824 (2023). Deep learning reveals the role of copy number variation in the genetic architecture of a highly
825 polymorphic sexual trait. In *bioRxiv* (p. 2023.09.29.560175). https://doi.org/10.1101/2023.09.29.560175
826 van Oosterhout, C., Harris, P. D., & Cable, J. (2003). Marked variation in parasite resistance between two wild
827 populations of the Trinidadian guppy, Poecilia reticulata (Pisces: Poeciliidae). *Biological Journal of the*
828 *Linnean Society. Linnean Society of London*, *79*(4), 645–651.
829 van Oosterhout, C., Joyce, D. A., & Cummings, S. M. (2006). Evolution of MHC class IIB in the genome of wild
830 and ornamental guppies, Poecilia reticulata. *Heredity*, *97*(2), 111–118.
831 Walsman, J. C., Duffy, M. A., Cáceres, C. E., & Hall, S. R. (2023). "Resistance Is Futile": Weaker Selection for
832 Resistance by Abundant Parasites Increases Prevalence and Depresses Host Density. *The American*
833 *Naturalist*, *201*(6), 864–879.

834 Walsman, J. C., Janecka, M. J., Clark, D. R., Kramp, R. D., Rovenolt, F., Patrick, R., Mohammed, R. S., Konczal,
835 M., Cressler, C. E., & Stephenson, J. F. (2022). Shoaling guppies evade predation but have deadlier
836 parasites. *Nature Ecology & Evolution*, *6*(7), 945–954.
837 Walsman, J. C., Lambe, M., & Stephenson, J. F. (2024). Associating with kin selects for disease resistance and
838 against tolerance. *Proceedings. Biological Sciences*, *291*(2023), 20240356.
839 Wang, L.-C., Chen, L.-H., Chiu, Y.-C., Liou, C.-Y., Chen, H.-C., Lu, C.-Y., & Chen, J.-L. (2023). Teleost skin
840 microbiome: An intimate interplay between the environment and the host immunity. *Fish & Shellfish*
841 *Immunology*, *139*, 108869.
842 Watanabe, T., Yoshida, M., Nakajima, M., & Taniguchi, N. (2005). Linkage mapping of AFLP and microsatellite
843 DNA markers with the body color- and sex-determining loci in the guppy (Poecilia reticulata). *Zoological*
844 *Science*, *22*(8), 883–889.
845 Weiler, I. L. G., Kramp, R. D., Rovenolt, F., & Stephenson, J. F. (2024). Sex-dependent effects of infection on
846 guppy reproductive fitness and offspring parasite resistance. In *bioRxiv* (p. 2023.10.29.564622).
847 https://doi.org/10.1101/2023.10.29.564622
848 Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer.
849 Wilde, J., Slack, E., & Foster, K. R. (2024). Host control of the microbiome: Mechanisms, evolution, and disease.
850 *Science (New York, N.Y.)*, *385*(6706), eadi3338.
851 Xavier, R., Mazzei, R., Pérez-Losada, M., Rosado, D., Santos, J. L., Veríssimo, A., & Soares, M. C. (2019). A
852 Risky Business? Habitat and Social Behavior Impact Skin and Gut Microbiomes in Caribbean Cleaning
853 Gobies. *Frontiers in Microbiology*, *10*, 716.
854 Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease.
855 *Cell Research*, *30*(6), 492–506.
856 Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed Effects Models and Extensions*
857 *in Ecology with R*. Springer New York.

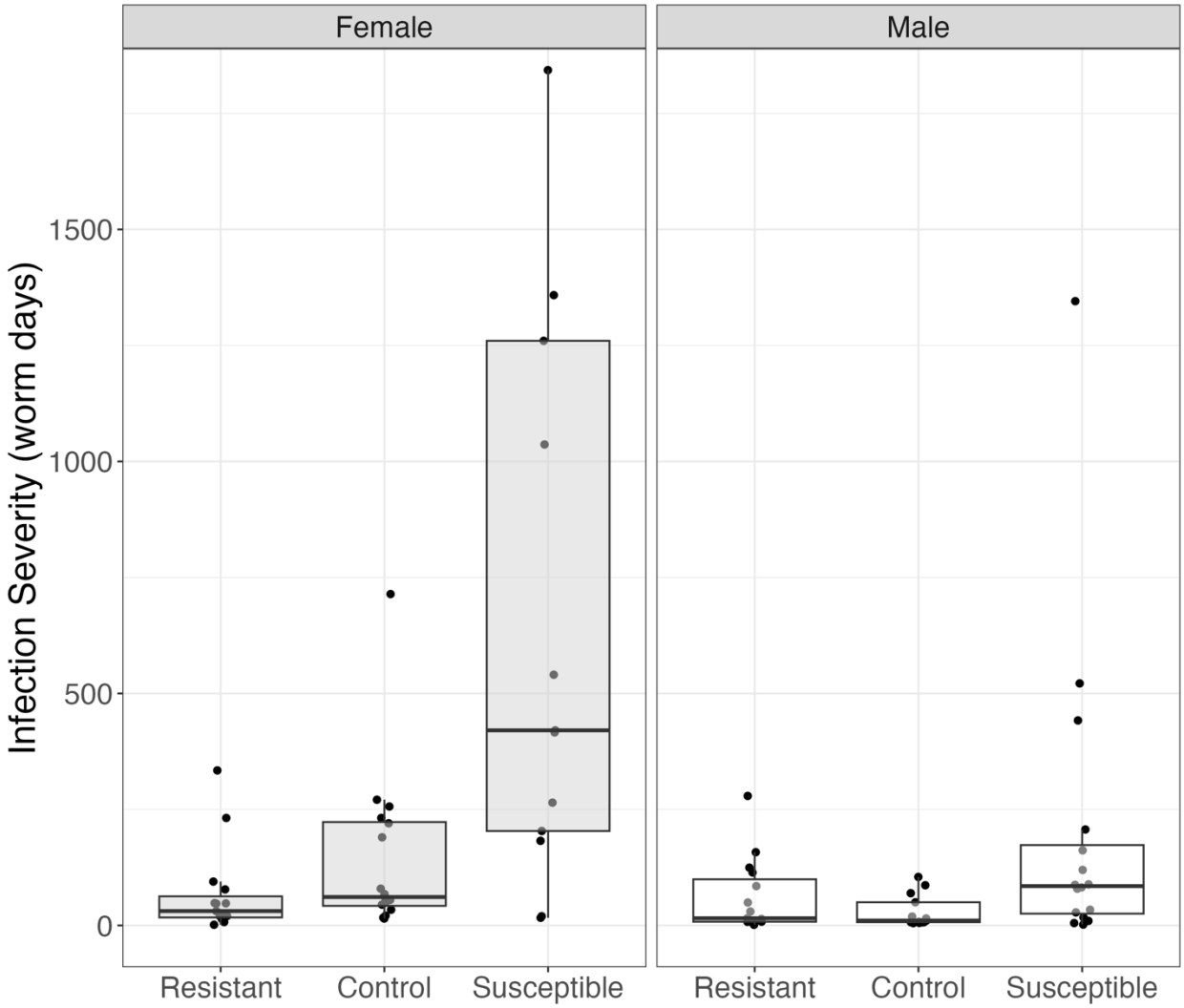

Figure 1. Trinidadian guppy artificial selection lines significantly predicted G. turnbulli infection severity *(χ^2=37.74, P =6.4e-09). Infection severity (worm days)* is the area under the curve of infection load over the course of an individual's infection. *Female guppies (n=44) susceptible and resistant artificial selection lines differed significantly from the control line (Susceptible-Control, z=3.49, P=0.001; Resistant-Control, z =-2.74, P=0.016). Male guppies (n=43) susceptible, but not resistant, artificial selection line differed significantly from control line (Susceptible-control, z=4.19, P<0.001; Resistant-Control, z =1.86, P=0.15). Box plots give the median (dark line), interquartile range (box) and values within 1.5× the interquartile range (whiskers) of the infection severity.*

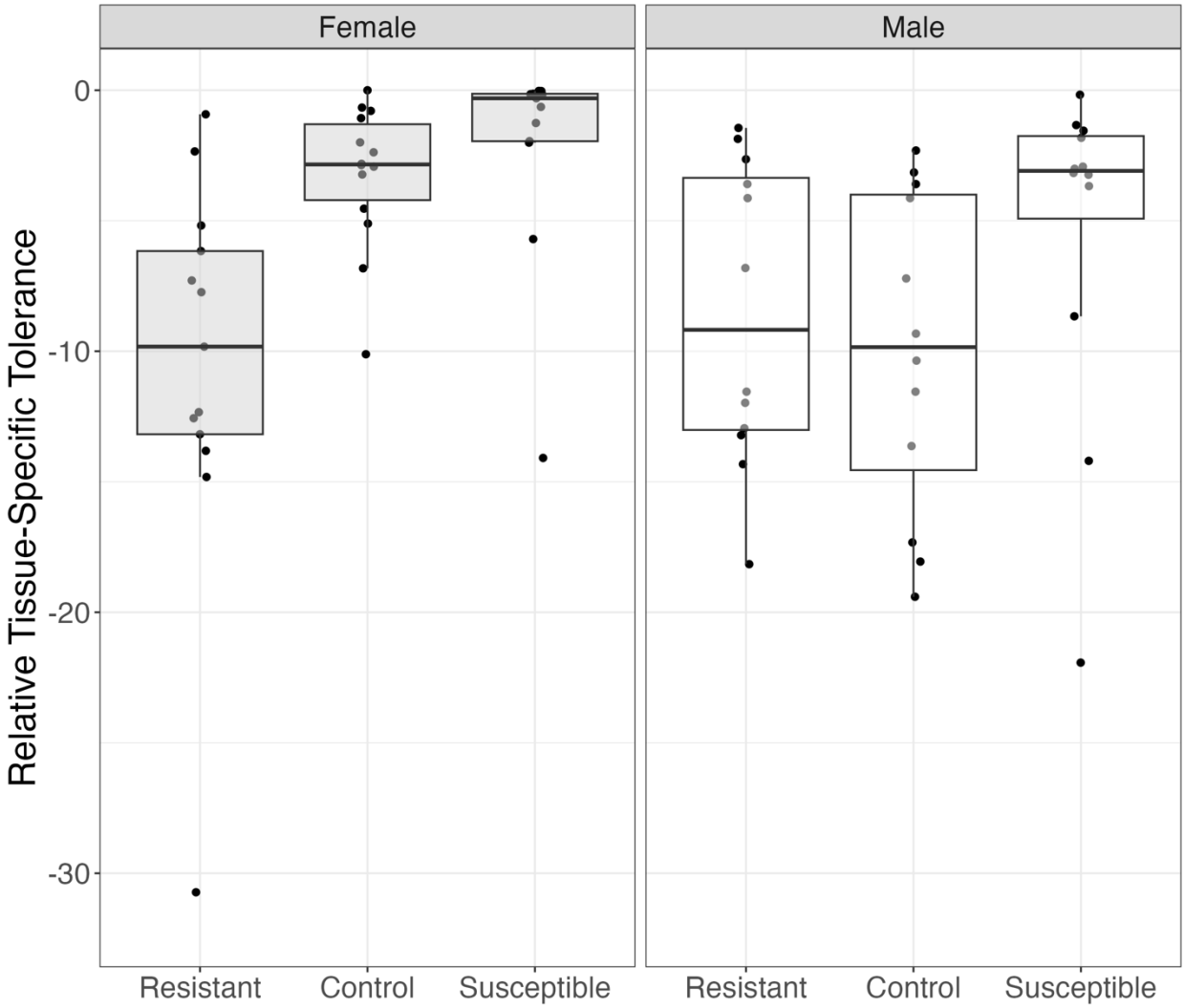

Figure 2. Trinidadian guppy artificial selection lines significantly predicted Relative Tissue-Specific Tolerance *(χ^2=14.56, P =0.00069).* We quantified relative tissue-specific tolerance as the per-parasite percentage mass change across infection minus max tolerance value, greater values indicate greater tolerance. *Female guppies (n=44) resistant artificial selection lines differed significantly from the control and susceptible lines (Resistant-Control, z=-3.92, P=0.0003; Resistant-Susceptible, z =4.05, P=0.0001). Male guppies (n=43) artificial selection lines did not predict relative tissue specific tolerance (χ^2=2.08, P =0.35). Box plots give the median (dark line), interquartile range (box) and values within 1.5× the interquartile range (whiskers) of the infection severity.*

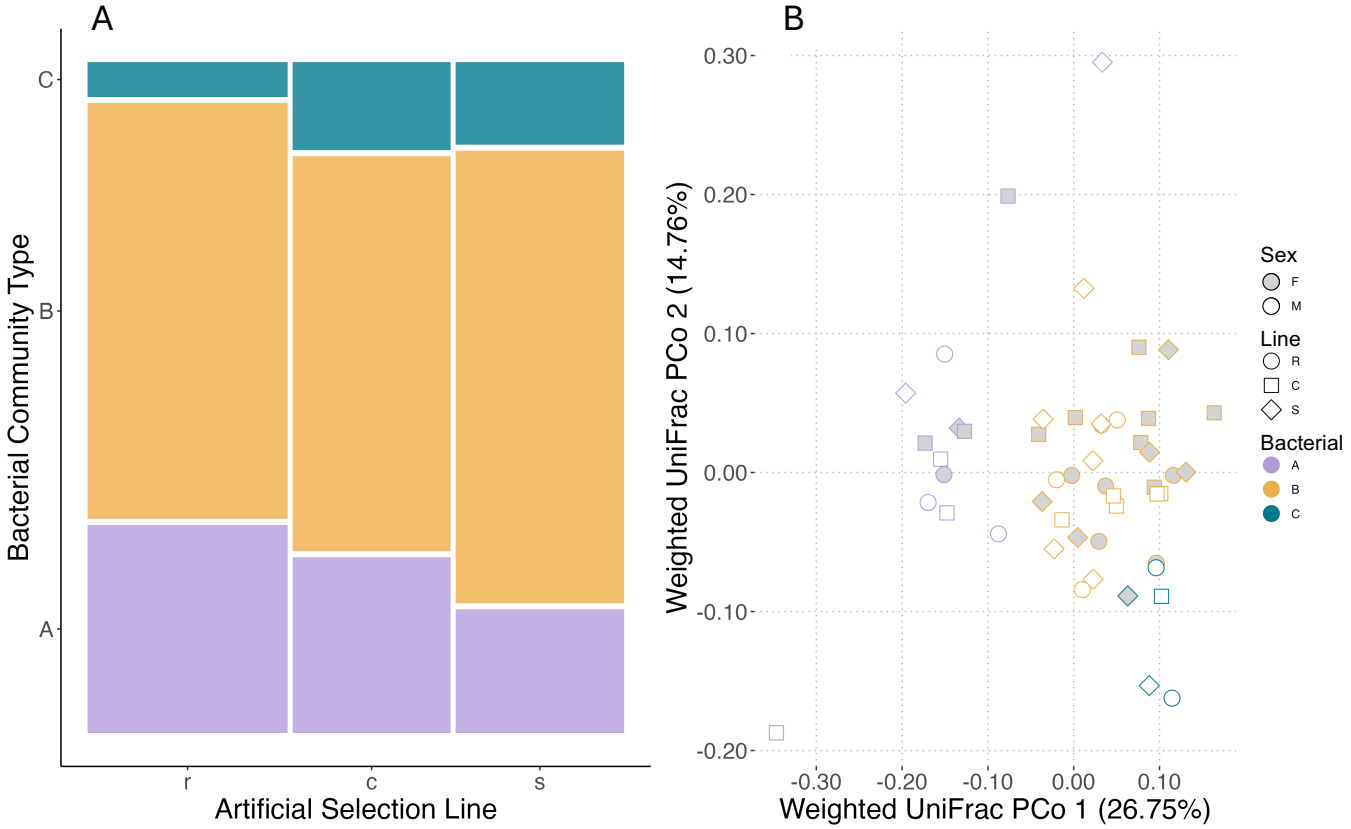

Figure 3. Uniform distribution of skin bacterial community types across guppy artificial selection lines suggests no association with selection mechanism. A*. Mosaic plot presenting proportion of Bacterial community types (A, B, C) across, Control (c), Resistant (r), and Susceptible lines (s). Bacterial communities do not show a significantly uneven distribution across lines (Fisher's exact test, p = 0.8).* ***B****. A Principal Coordinate (PCoA) analysis plot based on Weighted UniFrac similarity of fish skin microbial communities (n=52) across sex, bacterial community type, and artificial selection line. Percentages represent the proportion of variation explained by each axis. Bacterial community type was significant in explaining variation* (PERMANOVA: F=7.37, *P=0.0003*). *No significant pattern or clustering is was detected for artificial selection lines (PERMANOVA: F=0.68, P=0.89*).

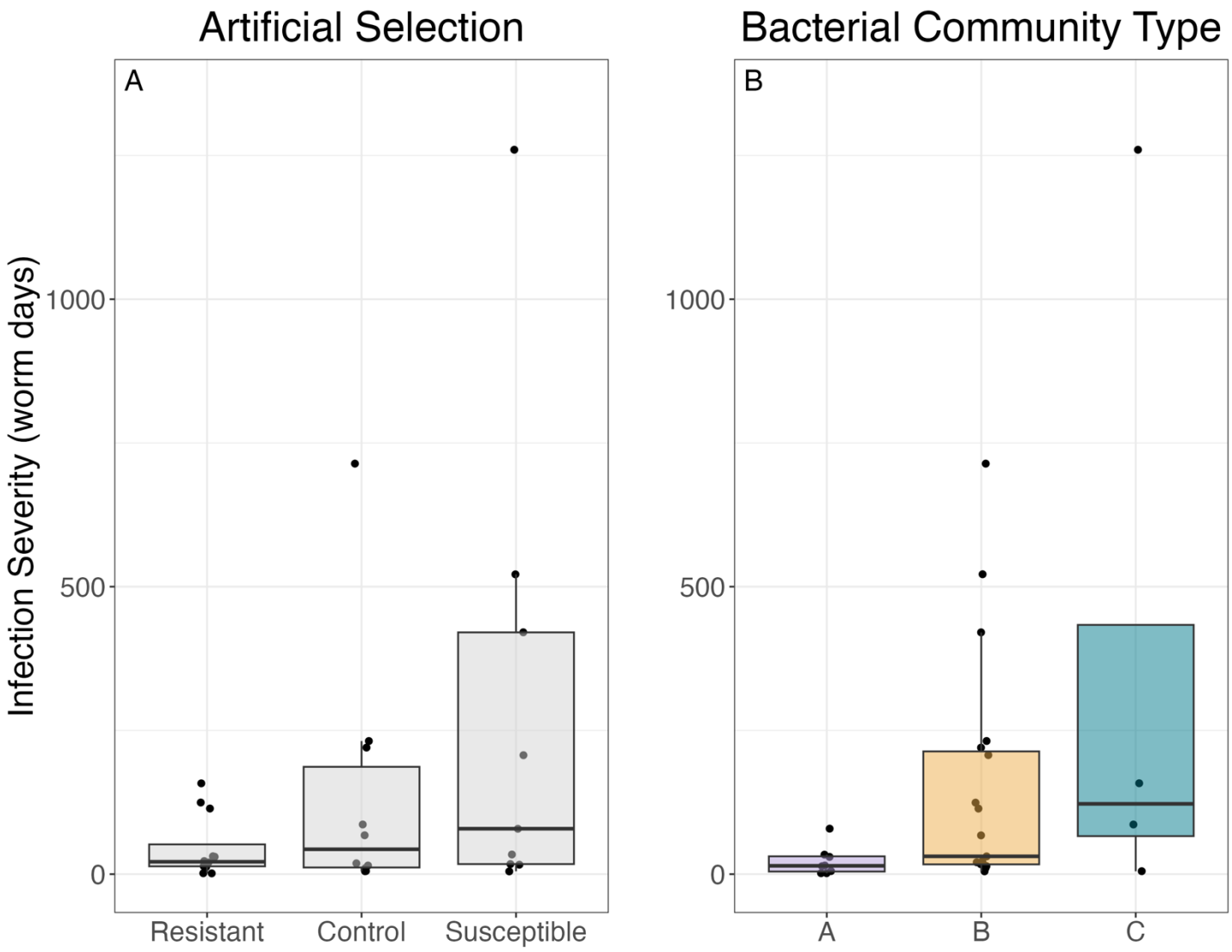

Figure 4. Trinidadian guppy artificial selection lines and bacterial community types predict G. turnbulli infection severity. **A.** The artificial selection lines still significantly predicted infection severity (Anova; χ^2*=9.39, P=0.009)* within the swabbed subset of fish (n=31). **B.** Bacterial community types differed in their infection severity (n=31). Bacterial community type A had significantly lower infection severity compared to bacterial community type B & C (A-B*, z=2.59, P=0.0034; A-C, z =4.92, P<0.001*). No interactions between bacterial community and artificial selection line (Anova; χ^2*=7.4, P=0.116).* Post-hoc tests showed that a model including both line and bacterial community types were significantly better at explaining infection severity than either effect alone (χ^2*=13.40, P=0.001).*