

1 Title: Host sex and genotype modify the gut microbiome response to helminth infection

2
3 Fei Ling^{1,2*}, Natalie Steinel^{2,3,4}, Jesse Weber^{2,5,6}, Lei Ma^{2,7}, Chris Smith^{2,8}, Decio Correa²,
4 Bin Zhu¹, Daniel Bolnick^{2,9*}, Gaoxue Wang¹

5
6 ¹ College of Animal Science and Technology, Northwest A&F University, Yangling, 712100, PR
7 China

8 ² Department of Integrative Biology, University of Texas at Austin, Austin TX 78712 USA

9 ³ Dell Medical School, University of Texas at Austin, Austin TX 78712 USA

10 ⁴ Department of Biological Sciences, University of Massachusetts Lowell, Lowell MA 01854
11 USA

12 ⁵ Division of Biological Sciences, University of Montana, Missoula, MT 59812 USA

13 ⁶ Department of Biological Sciences, University of Alaska, Anchorage AK 99508 USA

14 ⁷ Department of Civil and Environmental Engineering, Massachusetts Institute of Technology,
15 Cambridge MA 02139 USA

16 ⁸ Department of Ecology and Evolutionary Biology, University of Colorado, Boulder CO 80309
17 USA

18 ⁹ Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs CT 06268
19 USA

20 * Correspondence and requests for materials should be addressed to F.L. (feiling@nwsuaf.edu.cn)
21 and D.I.B. (email: daniel.bolnick@uconn.edu)

22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 **Abstract**

51 The microbial community can be altered by direct/indirect interactions with parasites infecting
52 host. Direct interactions can arise from physical/chemical contact with the parasite. Indirect
53 interactions can involve parasite-induced changes in host immunity. If so, this would represent a
54 case of genetic polymorphism in one species controlling an ecological interaction between other
55 species. Here, we report a test of this expectation: we experimentally exposed *Gasterosteus*
56 *aculeatus* to their naturally co-evolved parasite, *Schistocephalus solidus*. The host microbiome
57 differed in response to parasite exposure, and between infected and uninfected fish. The
58 microbial response to infection differed between host sexes, and also varied between variants at
59 autosomal quantitative trait loci (QTL). These results indicate that host genotype regulates the
60 indirect effect of infection on a vertebrate gut microbiome. Our results also raise the possibility
61 that this sex-bias may be related to sex-specific microbial responses to the presence (or, absence)
62 of helminthes. Therefore, helminth-based therapeutics as possible treatments for inflammatory
63 bowel diseases might need to take account of these interactions, potentially requiring therapies
64 tailored to host sex or genotype.

65 **Keywords:** gut microbiome, helminth infection, sex, genotype, *Gasterosteus aculeatus*

66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84

85 **Introduction**

86 Helminth infection is often associated with changes in the host's gut microbiome [1, 2], resulting
87 in a complex multi-way ecological interaction between host, parasite, and diverse microbiota.
88 For example, *Hymenolepis diminuta* infection reduces the abundance of Bacilli species in the gut
89 of mammalian hosts, in both the lab and wild [3, 4]. However, other studies report no effects of
90 infection on the microbiome: *Trichuris trichiura* and *Necator americanus* infections in humans
91 do not alter the fecal microbiota [5, 6]. Sometimes the gut microbiota protects its host against
92 helminth infection, such as the maternally transmitted bacterium *Spiroplasma* that protects
93 *Drosophila neotestacea* against nematode parasitism [7], or *Bifidobacterium animalis* that
94 protects mice against *Strongyloides venezuelensis* infection [8]. A recent study demonstrated that
95 *T.muris* infection altered the gut microbiota in mice, and inhibited subsequent rounds of infection
96 [9]. Conversely, *Lactobacillus* facilitates *H. polygyrus* and *T. muris* infections in mice [10, 11].
97 These opposing findings illustrate the inconsistent nature of helminth-microbiome interactions
98 within their shared hosts. What biological variables explain such heterogeneous results? If we
99 can identify the causes of these variable helminth-microbe associations, then we may be better
100 able to treat dysbiosis and/or macroparasite infections [12-14].

101 The mechanisms underlying helminth-microbiome interactions are still being elucidated.
102 Macroparasites can interact directly with the microbiome, for instance by secreting antibacterial
103 peptides (e.g., the gastrointestinal nematode *Heligmosomoides polygyrus* secretes at least eight
104 products with antibacterial effects [15]). Or, parasites can indirectly alter the gut microbiota via
105 changes in host physiology, especially immune state. *H. polygyrus bakeri* infection induces
106 colonic regulatory T cells in mice, which are widely regarded to arise in response to gut
107 microbiota colonization [16], and protect mice from colitis [17]. Also, *H. polygyrus* can
108 negatively regulate host intestinal mucosal IL-22 and IL-17, and decrease the host's expression
109 of epithelial antimicrobial peptides [18]. But non-immune mechanisms also occur: helminth
110 damage to the host gut epithelium can cause malnutrition that changes the microbiome [19].

111 Indirect interactions between the parasite and microbiota, acting through host traits, should
112 presumably be contingent on host traits. Hosts often vary in their immune response to helminth
113 infection, due to immunogenetic polymorphism [20, 21], energetic reserves [22], stress [23], and
114 prior parasite exposure [24]. Males and females within a species also can respond differently to
115 infections [25], because sex hormones modulate immune traits [26]. But, we lack evidence that

116 variation within host species changes the microbial response to parasitic infection. Here, we
117 show that host sex and autosomal genotype alter the gut microbiota response to cestode
118 infection. We experimentally exposed laboratory-bred threespine stickleback (*Gasterosteus*
119 *aculeatus*) fish to their native cestode parasite (*Schistocephalus solidus*). We then assayed the gut
120 microbial response to infection and host genotype, and non-additive interactions between
121 infection and genotype. This host-parasite system is especially valuable for such studies because
122 the microbiota have little opportunity for direct interaction with this cestode parasite. After the
123 fish ingests infected copepods, the parasite exits the copepod and rapidly penetrates the intestinal
124 wall (within hours) to establish a long-term infection in the peritoneal cavity, physically
125 separated from the gut microbiota. So, cestode effects on the microbiota are likely to be indirect,
126 mediated via host traits such as immune responses which are often polymorphic within, and
127 divergent between, host species. In an initial exposure experiment (experiment 1), we show that
128 cestode exposure suffices to alter the gut microbiota, even when the infection subsequently fail,
129 but this effect varied among host full-sibling families. In a follow-up experiment (experiment 2)
130 we show that infection presence/absence (controlling for exposure) does also alter the gut
131 microbiota, but this effect varies between host sexes (which are genetically determined) and
132 autosomal genotypes. This result demonstrates that genetic variation in a host species modifies
133 the ecological interaction between helminth and microbiome communities.

134

135 **Results**

136 **Effects of cestode exposure versus infection**

137 Experiment 1 was designed to test if exposure alone, or infection presence, change microbial
138 communities. This experimental design is illustrated in Additional file: Figure S1a and sample
139 sizes of fish used in this experiment was listed Additional file: Table S1. The results show that
140 cestode-exposed fish (infected or uninfected) had different microbial communities than the
141 sham-exposed control fish (different unweighted PCoA1 axis scores), though this trend varied
142 among host full-sib families (Fig. 1a and Additional file: Table S2; fish family $P < 0.0001$,
143 exposure $P = 0.2589$, exposure*family $P = 0.0363$). The significant exposure by family interaction
144 occurs because one full-sib family (GG12) showed an atypical microbial response to exposure.
145 Omitting this one family, the remaining families all showed a consistent microbiome response to
146 parasite exposure (exposure $P = 0.0378$, family $P = 0.0579$, exposure*family $P = 0.1675$). In

147 contrast, we observed no difference between infected versus exposed-but-uninfected fish (Fig. 1b
148 and Additional file: Table S2). However, this initial experiment was had modest statistical power,
149 so in Experiment 2 we focused on evaluating only the effect of cestode presence/absence, among
150 fish who were all exposed to the cestode.

151

152 **Gut microbial community composition**

153 In the Experiment 2, we exposed 711 lab-raised adult F2 hybrid sticklebacks to *S.solidus*,
154 resulting in 256 successful infections (the experimental design is illustrated in Additional file:
155 Figure S1b and sample sizes of this experiment was listed in Additional file: Table S3). We
156 obtained 16S sequences 693 of these 711 fish (a few individuals' intestines were not retained),
157 yielding 11,586 Operational Taxonomic Units (OTUs). We retained an average of 11,066
158 sequence reads per fish (s.d.=13,394; median=7,575; range from 48 to 126,943). We excluded all
159 individual stickleback with fewer than 500 sequence reads ($N=43$ removed, 650 retained,
160 Additional file: Table S4). A summary of the gut microbial community composition is provided
161 in Dataset 1. The five most abundant Phyla in the sticklebacks' intestines were Firmicutes
162 (61.07% of reads on average), Proteobacteria (28.68%), Actinobacteria (5.13%), Bacteroidetes
163 (0.93%) and Planctomycetes (0.67%). The microbial Orders Bacillales (0.53% ~ 98.34%),
164 Burkholderiales (0.095% ~ 91.79%) and Actinomycetales (0.027% ~ 98.46%), and Family
165 Alcaligenaceae (0.025% ~ 98.27%) were detected in all individual stickleback. As previously
166 noted [27, 28], the stickleback gut microbiota differed dramatically among individuals
167 (Additional file: Figure S2), despite their similar ages and being reared on the same foods in the
168 same research facility (split between two adjacent rooms).

169

170 **Host sex, cross, and infection jointly affect the gut microbiome**

171 General linear model analyses of the 181 most common microbial Families (those present in at
172 least 20 fish) suggest that cestode infection and host genotype jointly affect the stickleback gut
173 microbiome. All models included room effect as a covariate to account for the large effect of
174 rearing room (Additional file: Figure S3). Host sex, cross, and mass each had significant main
175 effects on nearly a third of microbial Families (32.6%, 29.8%, and 27.1% respectively;
176 Additional file: Figure S4 and Dataset 2). Main effects of cestode infection were relatively

177 uncommon, however, affecting only 11.0% of microbial Families (still significantly more than
178 expected from our 5% type I error rate; $\chi^2=12.7$, $P=0.0003$).

179 Although relatively few microbial Families were associated with cestode presence/absence
180 (consistent with the lower-powered Experiment 1), substantially more Families (50 of 181, or
181 27.6%) exhibit interactions between infection and other host traits. Specifically, 19.9% of
182 microbial Families exhibited significant infection*cross interactions (contrasting ROB
183 backcross, F2 intercross, or GOS backcross), and 17.1% of Families depend on infection*sex
184 interactions. Fish mass had little effect on the cestode-microbe interaction: only 8.8% of
185 microbial Families exhibited a mass*infection interaction, slightly more common than the 5%
186 false positive rate ($\chi^2=4.8$, $P=0.027$). Thus, the statistical main effect of infection (our focus in
187 Experiment 1) underestimated the impacts of cestode presence, the majority of which were
188 contingent on genetic characteristics of the host (sex, cross type). Comparable trends were
189 observed if we examined other taxonomic ranks (e.g., 84 common microbial Orders, Fig. 2).

190

191 **Examples of interactions between helminth infection and host genotype**

192 A few illustrative examples of such interaction effects are plotted in Fig. 3 and Additional file:
193 Figure S5. We observed a strong main effect of infection for some microbe Orders, such as
194 Fusobacteriales (Fig. 3a and Additional file: Figure S5a, infection effect $t=-2.65$, $P=0.0083$),
195 which were less abundant in infected sticklebacks' gut regardless of sex or genotype. As an
196 example of a main effect of host sex, Lactobacillales were more abundant in male than in female
197 stickleback regardless of infection status (Fig. 3b and Additional file: Figure S5b, sex effect $t=$
198 3.37 , $P<0.001$).

199 Clostridiales illustrate the contingent effect of parasite infection (Fig. 3c and Additional file:
200 Figure S5c), with a sex main effect ($t=5.56$, $P<0.0001$) and a sex*infection interaction ($t=-7.46$,
201 $P<0.0001$). Clostridiales abundance was insensitive to infection in female hosts, but *S.solidus*
202 infection strongly reduced Clostridiales abundance in male fish. Equivalently, one could say that
203 Clostridiales abundance differed strongly between uninfected males and females, but did not
204 differ between infected males and females. Rhodobacterales also show a sex*infection
205 interaction Fig. 3d and Additional file: Figure S5d, increasing with infection in males but
206 decreasing with infection in females. As a result, among uninfected fish Rhodobacterales were
207 most abundant in females, whereas among infected fish this taxon was most abundant in males.

208 Host autosomal genotype also influenced the gut microbiota, as indicated by widespread
209 differences between backcross versus F2 hybrids. Caulobacterales were significantly more
210 common in F2 hybrids (Fig. 3e and Additional file: Figure S5e, cross main effect $F_{2,629}=71.6$,
211 $P<0.0001$) than in either backcross. Other Orders were more common in one particular cross, or
212 exhibited an additive trend (F2s intercrosses being intermediate between backcrosses). This
213 autosomal effect altered the impact of cestode infection. Rhodobacterales were more abundant
214 in hosts with greater ancestry from Roberts Lake, but only in the absence of the cestode (Fig. 3f
215 and Additional file: Figure S5f). In contrast, in cestode-infected fish Rhodobacterales were
216 uniformly common in all genotypes.

217

218 **Discriminant function analyses of host cross and sex effects on microbiota**

219 We next used multivariate analyses to evaluate the response of the overall gut microbiome
220 community to infection and host genotype (summarized in Additional file: Table S5). LDA
221 separating the four combinations of sex and infection confirmed that helminth infections alter the
222 gut microbiome but sex modifies the effects of helminthiasis (Fig. 4a). The two leading LDA
223 axes, respectively, exhibited a significant effect of sex ($F_{1,466}=90.8$, $P<0.0001$), and an effect of
224 infection ($F_{1,466}=4.64$, $P=0.0317$). For LDA axis 1 (LDA1), these variables also interacted
225 ($F_{1,466}=9.6$, $P=0.0021$). Overall, cestode infection changed female gut microbiota composition
226 more strongly than it changed male microbiota.

227 A separate discriminant function analysis of fish cross type and infection status (6
228 combinations) revealed several insights. The first LDA axis separated F2 hybrid from both
229 reciprocal backcross populations (Fig. 4b), suggesting that there may be transgressive genetic
230 effects on the microbiota (e.g., Fig. 3e). F2 hybrids were intermediate between the backcrosses
231 along LDA2, consistent with additive genetic control of other aspects of the microbiota
232 (Additional file: Figure S6). The third axis separated infected and uninfected fish, but in a highly
233 genotype-dependent manner (Fig. 4b and Additional file: Figure S6). In F2 cross fish, LDA3
234 scores were insensitive to infection, whereas in both backcross populations the LDA3 scores
235 changed strongly in response to infection. All three crosses had similar LDA3 scores when
236 uninfected, but Gosling and Roberts backcross fishes' microbiomes diverged in response to
237 infection.

238

239 **QTL mapping of gut microbiota composition**

240 Based on the effects of cross type, described above, we expected to be able to locate host loci
241 that (i) explain variation in gut microbiota composition, and (ii) do so differently for infected
242 versus uninfected fish. We used quantitative trait locus (QTL) mapping to test this expectation,
243 and to identify chromosomal regions for future detailed mapping, and possible candidate gene
244 identification and validation.

245 ddRADseq identified 234 SNPs with fixed differences between the Roberts and Gosling
246 populations and sufficiently deep coverage within and among the F2 hybrid individuals, yielding
247 approximately 10 markers per linkage group. Another paper will describe QTL mapping of
248 infection outcomes and host immune traits (Weber et al, in preparation); here we focus only on
249 mapping the gut microbiota. We located no significant QTL for any of the top 10 weighted
250 PCoA axes, and no significant QTL for microbial diversity (at a rarefaction of 2000 or 4000
251 reads per fish). But, we did detect weak-effect QTL for an unweighted PCoA axis. Unweighted
252 PCoA axis 5 exhibited three QTLs that narrowly exceeded our stringent threshold for
253 significance (Additional file: Figure S7a-c). We had a stronger signal when we fused the many
254 PCoA axes into a single metric using linear discriminant analysis (trained to distinguish the two
255 backcrosses then applied to all samples). Using this first LDA axis we detected a single well-
256 supported QTL on Chr9 (Additional file: Figure S7d-e). Note that because QTL mapping was
257 done within each cross, using LDA to define an axis that distinguishes between crosses is not
258 tautological. Chromosome 9 does not have any noteworthy effect on cestode infection success
259 (infection QTL described in Weber et al, in preparation).

260 The lack of strong QTL for microbial ordination metrics led us to hypothesize that host
261 control of the whole microbial community is highly polygenic. If host genetic variation acts on
262 particular microbe taxa, it might act only weakly on PCoA scores and be correspondingly hard to
263 map. So we next mapped microbial Orders separately, revealing numerous taxon-specific QTL.
264 To illustrate, Fusobacteriales exhibit two strong autosomal QTL, plus an association with the X
265 chromosome (ChrX) indicating a sex effect (Fig. 5). Other examples are plotted in
266 supplementary figures (Additional file: Figure S8), and their overlapping distributions in the
267 genome are presented in Fig. 6. This summary reveals genomic ‘hotspots’ for QTL affecting the
268 microbiota, on Chr1, Chr2, and Chr3. Many of these microbial Orders also mapped to the sex

269 chromosome (Chr19), consistent with the common main effect of sex. Similar results were
270 obtained for Family level QTL mapping.

271 We next tested whether these QTL are contingent on cestode presence or absence, as
272 expected from the interactive effect of infection and cross type, described above. We mapped
273 QTL separately for infected fish and for uninfected fish, and then looked for loci with QTL in
274 only one of these groups. We found numerous host loci that are only associated with microbial
275 variation in uninfected fish, whereas the same locus is unrelated to the microbiota among
276 infected fish (e.g., Additional file: Figure S9a-f). In fewer instances, we identified host QTL for
277 microbiota in infected fish only (Additional file: Figure S9g-h). In several cases stratifying by
278 cestode infection revealed QTL that would not have been detected otherwise. For example,
279 Solirubrobacterales (Additional file: Figure S9e-f) have a QTL on Chr3 that is observed in all
280 fish, but which in fact only acts on the uninfected fish (a larger sample size). When we ignored
281 infection status, the same microbial taxon had a non-significant QTL on stickleback Chr16. The
282 lack of Solirubrobacterales in infected fish was masking the effect of host genetic variation at
283 that site.

284

285 **Discussion**

286 The interaction between hosts, parasites, and gut microbes represents a rich opportunity for
287 experimental studies of multi-way ecological interactions. A growing literature suggests that
288 helminth infection can alter the vertebrate gut microbiota [1]. Our experiment adds an important
289 twist to this literature: the magnitude and direction of helminth-induced microbial changes
290 depend on both the sex of the host, and the host's autosomal genotype. By examining F2 hybrids
291 between two recently-diverged host populations, we were able to identify autosomal loci (QTL)
292 that contribute to variation in microbiota composition, as well as variation in microbiota response
293 to infection. The implication is that genetic variation within one species (the host) alters the
294 ecological effect of another species (the cestode parasite) on a third party (the microbial
295 community).

296 The inconvenient implication of this finding is that we may not readily generalize helminth-
297 microbe effects beyond the genotypes (and sex) we study. This unwelcome news is tempered by
298 the opportunity it presents: host genetic variation can therefore help us identify the mechanisms
299 by which helminths alter the gut microbiota (or, vice versa). Doing so may allow us to develop a

300 more general predictive model that can account for such heterogeneity among study subjects, and
301 ultimately explain why individual hosts differ in their microbial response to infection.

302 To date, many studies have evaluated effects of helminth infection on the gut microbiota of
303 animals [1, 13]. Yet, we remain largely ignorant as to whether these helminth effects depend on
304 other biological variables, whether environmental effects or host genotype. Here, we have
305 provided evidence of such interactions: host genotype (sex and autosomal) and helminth
306 infection synergistically alter the microbiota. One third of the common microbial Orders
307 exhibited significant interactions between infection and host genotype (Fig. 2), whereas infection
308 by itself affected only 7% of the Orders. For example, the abundances of some microbial Orders
309 in female hosts were significantly different with those males when uninfected, but no difference
310 between them when infected. Discriminant analysis suggested that females' microbiota were
311 generally more sensitive to infection than males' were. This sex-dependent effect of infection is
312 consistent with other recent papers on stickleback suggesting that sex modifies the gut
313 microbiome's response to diet [27], and MHC genotype [29].

314 Host autosomal genotype also affected gut microbial composition, and modified microbes'
315 responses to helminth infection. This inference is consistent with many other studies of
316 vertebrates that have yielded many examples of host genetic control of microbiome composition
317 [30, 31]. What makes the above results novel is that this host genetic variation also alters the
318 microbiome's response to a third (parasitic) species. We coarsely localize these autosomal
319 effects to a modest number of chromosomal loci (QTL). These QTL do not contain MHCIIb,
320 which has previously been associated with natural variation in stickleback gut microbiota [29].
321 At present we lack the resolution fine-map down to specific candidate genes, but some intriguing
322 possibilities exist. The QTL on Chr2, which affect multiple microbial Orders, contains
323 transcription factor c-MAF, which activates the expression of IL-4 in Th2 cells and attenuates
324 Th1 differentiation [32]. Interestingly, recently Xu et al. reported c-MAF-dependent regulatory
325 T cells mediate immunological tolerance to intestinal microbiota [33]. In addition, the QTL on
326 this chromosome contains reticulocalbin and synaptotagmin IX, which are associated with
327 calcium ion binding; dietary calcium can affect the intestinal microbiota composition [34-36].
328 The QTL on Chr3 contains cadherins, which play a key role in intestinal homeostasis and barrier
329 function [37]. Substantial future work is needed to fine-map candidate genes and experimentally
330 validate their suspected effects on the gut microbiota, and on the cestode-microbiome interaction.

331 Interactions between host genotype and infection likely arise from host genetic variation in
332 immune response to cestodes. The presence of helminths can interfere with TLR or downstream
333 signaling pathways [38], which may have ancillary effects on the microbiota. Adaptive immune
334 responses may be involved as well. Helminths can trigger a Th2 response that increases mucus
335 production and epithelial cell turnover, altering the mucosal microbiota [39]. Any polymorphism
336 in immune genes (e.g. TLRs) involved in these pathways may lead to an exaggerated (or,
337 reduced) immune response to helminth infection, and thereby modify the effect of helminths on
338 gut microbes. This logic applies equally to immune differences between males and females [40,
339 41].

340 In the specific case examined here, helminth effects on the microbiota are especially likely
341 to involve indirect effects via host immunity, rather than a direct microbe-helminth interaction.
342 This is because *S.solidus* is not an intestinal parasite. After being ingested, it quickly transits into
343 the body cavity where it persists for months but cannot directly affect the microbiota. Prior
344 studies confirm that stickleback vary in their ability to detect *S.solidus*, mount an effective
345 immune response, and susceptibility to cestode immune suppression [42-44]. Polymorphism in
346 these immune responses may cascade throughout the host body, imposing collateral effects on
347 the microbiota.

348 The above results specifically apply to variation among cestode-exposed stickleback. But,
349 our Experiment 1 suggests that stickleback gut microbiota depended most strongly on parasite
350 exposure (regardless of outcome), rather than infection itself. Apparently there is a lasting effect
351 of the transient presence of a tapeworm in the stickleback gut lumen, which outweighs the effect
352 of tapeworm presence or absence in the peritoneum. If indeed brief parasite exposure sends
353 lasting ripples through the microbial community, then the study of the wild microbiome will be
354 still more difficult. We rarely if ever know our study animals' history of unsuccessful infections,
355 so these ripples might generate substantial and untraceable variation among wild individuals. Yet
356 infection success also alters the gut microbiome, as we then revealed in the follow-up
357 Experiment 2.

358 At present we do not know the fitness consequences of the altered microbiota that we
359 document here. Some studies have reported detrimental helminth effects on the microbiota.
360 Experimental *T. muris* infection in mice alters the microbiota composition leading to reduced
361 availability of microbial metabolomics products needed by the host (vitamin D2/D3 derivatives,

362 many fatty acids, and amino acid synthesis intermediates) [45]. Conversely, helminth infection
363 can be beneficial, such as reducing the risk of *H. pylori*-induced gastric lesions [46]. Our results
364 suggest that these positive or negative effects of helminth-microbiota interactions will be
365 contingent on the specific sex and genotype of the hosts considered.

366 The absence of helminths in wealthy counties has been postulated to contribute to the
367 increasing prevalence of allergic and autoimmune diseases [47], which might be attributed, at
368 least in part, to alterations to the intestinal microbiota [13]. Some of these auto-immune diseases
369 are sex-biased in humans [48, 49]. Our results raise the possibility that this sex-bias may be
370 related to sex-specific microbial responses to the presence (or, absence) of helminthes. Therefore,
371 we propose that sex-specific environmental effects on the gut microbiota might contribute to
372 understanding sex-specific inflammatory diseases and sex differences in dysbiosis. Recently,
373 helminth-based therapeutics, e.g. infection with *T. suis*, have been tested as possible treatments
374 for inflammatory bowel diseases [47]. If our results hold beyond stickleback, we predict that
375 clinical therapeutic efficacy may differ between sexes, or between host genotypes. Consequently,
376 such treatments might need to take account of these interactions, potentially requiring therapies
377 tailored to host sex or genotype.

378

379 **Materials and Methods**

380 **Stickleback breeding**

381 Gosling Lake and Roberts Lake on Vancouver Island, British Columbia contain stickleback
382 populations that differ in immune phenotypes, immune response to infection, parasite growth in
383 the lab, and cestode prevalence in the wild (>70% and 0% respectively) [50, 51]. We generated
384 pure Roberts (ROB), pure Gosling (GOS), and reciprocal F1 hybrid families (RG or GR), and
385 raised these to maturity in the lab, at which time we interbred them to generate second generation
386 (F2) hybrids. These F2 hybrids included intercross families (F1*F1 matings) and reciprocal
387 backcross families (ROB*F1, F1*ROB, GOS*F1, F1*GOS). All fish were reared in freshwater
388 conditions and housed with full-siblings (9-39 animals per family) in either 40-L or 10-L tanks.

389

390 **Parasite Collection and Experimental Exposures**

391 These fish crosses and infections were designed to obtain a quantitative trait locus (QTL) map of
392 the genetic basis of host control of cestode growth and viability; those results will be reported

393 elsewhere (Weber et al, in preparation). Here, we focus on testing the hypothesis that host
394 genetic variation alters the impact of helminth infection on the vertebrate gut microbiota. We
395 conducted two sets of experimental infections (Additional file: Figure S1).

396 (Experiment 1) As a pilot study, we evaluated the effects of cestode exposure, and
397 successful infection, on the host gut microbiome. We experimentally exposed adult pure-bred
398 fish from six full-sib families from Gosling Lake, to *S.solidus*. Within each family, five
399 individuals were controls fed uninfected copepods (sham exposure), while the rest received
400 infected copepods, only some of whom were ultimately infected. This experimental design
401 yielded three categories of fish within each family: unexposed controls, exposed-but-uninfected
402 controls, and infected fish ($N=30$, Additional file: Figure S1a and Table S1). For these exposures
403 we followed a standard procedure described by Weber et al. [50]. Briefly, we dissected mature
404 *S.solidus* out of infected wild-caught fish from Gosling, and bred the tapeworms in culture media
405 in dark waterbath (mimicking the gut of piscivorous birds), to collect eggs. (Roberts Lake lacks
406 *S.solidus*, so this cannot be used as a source population.) We hatched the tapeworm eggs and fed
407 them to copepods (*Macrocyclus albidus*), then visually isolated infected copepods to feed in
408 controlled doses to the lab-raised stickleback. Forty-three days after parasite exposure, we
409 euthanized fish with MS-222 to check for infection success. We froze the entire intestine in
410 ethanol for subsequent microbiome analysis.

411 (Experiment 2) To evaluate the effect of host genotype on cestode-microbiome
412 interactions, we experimentally exposed adult F2 hybrid fish (intercross and backcross, $N=711$,
413 Additional file: Figure S1b and Table S3) to *S.solidus*, then assayed infection outcomes and
414 sequenced the gut microbiome. All fish from this experiment were exposed to *S.solidus*, but not
415 all were infected successfully, providing a contrast between fish with versus without the parasite,
416 controlling for initial exposure history. By excluding the unexposed (sham) treatment, we are
417 able to devote more statistical power to evaluating host genotype interactions with cestode
418 presence versus absence, without the substantial additional confounding variation associated
419 with parasite exposure (see Experiment 1 results).

420

421 **Amplification, sequencing and analysis of 16S rRNA amplicons**

422 We extracted DNA from the entire intestine (both content and mucosa) of stickleback ($N=30$
423 pure-bred GOS fish in Experiment 1; $N=693$ F2 hybrids in Experiment 2) using the MoBio

424 Powersoil DNA Isolation Kits, as described by Bolnick et al. [27, 28]. For the pure-bred GOS
425 fish, we used the V4-V5 primers [52]. For the F2 fish, 16S rRNA amplicons were generated for
426 the V4 hypervariable [27]. Sample-specific barcodes were used as described in Bolnick et al.
427 [27]. PCR amplification was performed in triplicate for each sample in a reaction volume of 25
428 μ L containing 1 \times Q5 High-Fidelity Master Mix, 5 pmol forward and reverse primers and 1 μ L
429 template DNA. The PCR products for each sample were pooled and quantified with Picogreen
430 double-stranded DNA reagent to facilitate pooling equimolar amounts of amplicons for
431 sequencing. To test for contamination, negative controls (without samples) were set up for both
432 the DNA extraction and 16S PCR amplification stages. The results indicated there was no
433 detectable contamination because the PCRs yielded negligible DNA concentrations during
434 Picogreen quantitation. Amplicon pools were paired-end sequenced on an IlluminaMiseq
435 platform at GSAF (Genomic Sequencing and Analysis Facility) at the University of Texas at
436 Austin.

437 The raw paired-end reads were demultiplexed, and subsequent sequence processing was
438 performed using the mothur software package (v.1.39.1), following standard operating
439 procedures (SOP) [53, 54] (https://www.mothur.org/wiki/MiSeq_SOP). Briefly, the sequences
440 were trimmed for 16S rRNA gene primer sequences, and then assembled into contigs and
441 aligned with 16S rRNA gene sequences from the ARB Silva v128 reference database [55].
442 Chimeric sequences were detected using VSEARCH within mothur in each sample and removed.
443 The remaining sequences were classified by using Bayesian classifier with a training set (version
444 16) from the Ribosomal Database Project (<http://rdp.cme.msu.edu>) [56]. Operational Taxonomic
445 Units (OTUs) were identified using the UCLUST algorithm based on 97% similarity. We
446 rarefied the data to 4000 sequences per sample to calculate unweighted and weighted UniFrac
447 distance and PCoA scores by phyloseq library in R [57].

448

449 **Analyses of pure GOS fish experimentally exposed to *S.solidus* or a negative control**

450 We sequenced the gut microbiota of GOS fish (Experiment 1) to obtain counts of microbe
451 abundance. We used mixed model linear models to analyze effects of family and infection status
452 on unweighted or weighted PCoA axis 1. Family was a random effect, with a random
453 family*infection interaction. First, we contrasted unexposed (sham infections) versus exposed

454 fish (regardless of the outcome of exposure). Second, we compared uninfected fish (sham or
455 failed infections) versus infected fish.

456

457 **Analyses of F2 hybrid fish experimentally exposed to *S.solidus***

458 **General Linear Model Analyses**

459 Evaluating the results of Experiment 2, we used quasibinomial general linear models (GLMs),
460 implemented in R, to examine the effects of infection and host genotype on microbial
461 composition (the relative abundance of each commonly observed Order/Family, found in at least
462 20 fish [$N = 84$ Orders/181 Families]). For each taxon, we estimated a GLM in which the
463 fraction of reads attributed to that taxon (out of all reads) was related to *S.solidus*
464 presence/absence, and effects of host cross (Gosling backcross, F2 intercross, or Roberts
465 backcross), host sex, and host mass. We included interactions of particular interest to us here:
466 infection*cross, infection*sex, and infection*mass. As fish were reared in two rooms, we used a
467 room effect as a covariate. We used a sequential Bonferroni correction to P-values when
468 evaluating effects of particular microbial Orders/Families.

469

470 **Discriminant Function Analyses**

471 We next examined the whole-microbiome effects of infection, sex, and cross direction. We
472 applied linear discriminant analysis (LDA) to the top 50 weighted/unweighted microbial PCoA
473 axes (which cumulatively account for 99.99% of variance in microbial alpha diversity using
474 phylogenetically weighted or unweighted presence-absence data). We first used LDA to
475 distinguish four groups (combinations of sex and infection status). Second, we used LDA to
476 distinguish six groups (factorial combinations of three host cross types and infection status). We
477 used ANOVAs to test for effects of sex, cross, and infection status on each LDA axis. We also
478 confirmed these statistical effects using a MANOVA applied directly to the top 50 PCoA axes to
479 test for effects of sex, cross, infection, host mass, and interactions among these variables.

480

481 **QTL mapping: ddRAD genotyping**

482 We used a Promega Wizard SV 96-well plate kit to extract DNA from alcohol-preserved fin
483 clips from all F2 hybrid fish (intercross and backcrosses), as well as all grandparents used to
484 generate the crosses. We then genotyped the fish to obtain SNPs for quantitative trait locus (QTL)

485 mapping, using the ddRADseq protocol described in Peterson et al. [58], with bioinformatics
486 steps to identify SNPs conducted as described in Stuart et al. [59]. We retained only SNPs
487 exhibiting fixed differences between the Roberts and Gosling Lake grandparents (e.g., fully
488 informative for QTL mapping). This conservative approach yielded 236 genetic markers for
489 mapping, on average slightly more than 10 markers per linkage group.

490

491 **QTL mapping: analyses**

492 We mapped quantitative trait loci (QTL) for several microbiome measures: alpha diversity (using
493 2000 or 4000 read depth normalization), the top 10 weighted PCoA axes (or unweighted axes),
494 and the relative abundance of the common microbial Orders (as described above for GLMs). We
495 built linkage maps for each cross separately in R/qtl [60], and used the *scanone* function with
496 ‘hk’ interval mapping (using rank-based nonparametric tests for microbial Order relative
497 abundance). To account for the complex cross design (with backcrosses and F2 intercrosses), we
498 built separate QTL maps within each of the three cross types, then summed their LOD scores.
499 This yields one summary statistic measuring a locus’ association with the focal trait, while
500 accounting for between-cross differences in QTL effect size or marker linkage. We compared
501 this summed LOD against null expectations obtained by 1,000 permutations of the focal
502 phenotype across fish within each cross, each time redoing each cross’ QTL map and summing
503 cross null LOD scores. Conservatively, we consider an observed QTL significant when its
504 summed LOD exceeded the 99.99% quantile from that marker’s null values at that same marker.
505 We double-checked each significant QTL with a GLM (as described above) testing for fish
506 genotype effect (at the nearest genetic marker) on the focal microbiome variable.

507

508 **Acknowledgements**

509 We thank the Genome Sequencing and Analysis Facility (GSAF) at the University of Texas at Austin for sequencing
510 support, and the Texas Advanced Computing Center (TACC) and High Performance Computing platform of
511 Northwest A&F University for computational resources. This research was supported by funding from NSFC (Grant
512 31672680) to Fei Ling, and the Howard Hughes Medical Institute to DIB, NIH (Grant 1R01AI123659-01A1) to DIB.

513

514 **Authors’ contributions**

515 D.I.B., J.W., and N.C.S. designed the study; F.L., N.C.S., and L.M. performed the study; D.I.B., F.L., C.S., D.C.,
516 B.Z., and G.X.W. analyzed the data; D.I.B., and F.L. wrote the paper.

517

518 **Availability of data materials**

519 Sequence data have been deposited in the Sequence Read Database (SRA) under project IDs SRP115642
520 (BioProject PRJNA398629) and SRP115678 (BioProject PRJNA398630) for experimental 1 and 2, respectively. All

521 other relevant data are available in this article and its Supplementary Information files, or from the corresponding
522 authors upon request.

523

524 **Competing interests**

525 The authors declare no conflict of interest.

526

527 **References**

- 528 1. Reynolds LA, Finlay BB, Maizels RM. Cohabitation in the intestine: Interactions among
529 helminth parasites, bacterial microbiota, and host immunity. *J Immunol.* 2015;195:4059-66.
- 530 2. Peachey LE, Jenkins TP, Cantacessi C. This gut ain't big enough for both of us. Or is it?
531 Helminth-microbiota interactions in veterinary species. *Trends Parasitol.* 2017;3:619-32.
- 532 3. McKenney EA, Williamson L, Yoder AD, Rawls JF, Bilbo SD, Parker W. Alteration of the
533 rat cecal microbiome during colonization with the helminth *Hymenolepis diminuta*. *Gut*
534 *Microbes.* 2015;6:182-93.
- 535 4. Aivelo T, Norberg A. Parasite-microbiota interactions potentially affect intestinal
536 communities in wild mammals. *J Anim Ecol.* 2018;87:438-47.
- 537 5. Cooper P, Walker AW, Reyes J, Chico M, Salter SJ, Vaca M, Parkhill J. Patent human
538 infections with the whipworm, *Trichuris trichiura*, are not associated with alterations in the
539 faecal microbiota. *PLoS One.* 2013;8:e76573.
- 540 6. Cantacessi C, Giacomin P, Croese J, Zakrzewski M, Sotillo J, McCann L, Nolan MJ,
541 Mitreva M, Krause L, Loukas A. Impact of experimental hookworm infection on the human
542 gut microbiota. *J Infect Dis.* 2014;210:1431-4.
- 543 7. Jaenike J, Unckless R, Cockburn SN, Boelio LM, Perlman SJ. Adaptation via symbiosis:
544 recent spread of a *Drosophila* defensive symbiont. *Science.* 2010;329:212-5.
- 545 8. Oliveira-Sequeira TC, David ÉB, Ribeiro C, Guimarães S, Masseno AP, Katagiri S,
546 Sequeira JL. Effect of *Bifidobacterium animalis* on mice infected with *Strongyloides*
547 *venezuelensis*. *Rev Inst Med Trop Sao Paulo.* 2014;56:105-9.
- 548 9. White EC, Houlden A, Bancroft AJ, Hayes KS, Goldrick M, Grecis RK, Roberts IS.
549 Manipulation of host and parasite microbiotas: Survival strategies during chronic nematode
550 infection. *SciAdv.* 2018;4:eaap7399.
- 551 10. Dea-Ayuela MA, Rama-Iniguez S, Bolas-Fernandez F. Enhanced susceptibility to *Trichuris*
552 *muris* infection of B10Br mice treated with the probiotic *Lactobacillus casei*. *Int*
553 *Immunopharmacol.* 2008;8:28-35.
- 554 11. Reynolds LA, Smith KA, Filbey KJ, Harcus Y, Hewitson JP, Redpath SA, Valdez Y, Yebra
555 MJ, Finlay BB, Maizels RM. Commensal-pathogen interactions in the intestinal tract:
556 Lactobacilli promote infection with, and are promoted by, helminth parasites. *Gut Microbes.*
557 2014;5:522-32.
- 558 12. Loke P, Lim YA. Helminths and the microbiota: parts of the hygiene hypothesis. *Parasite*
559 *Immunol.* 2015;37:314-23.
- 560 13. Zaiss MM, Harris NL. Interactions between the intestinal microbiome and helminth
561 parasites. *Parasite Immunol.* 2016;38:5-11.
- 562 14. Rapin A, Harris NL. Helminth-bacterial interactions: Cause and consequence. *Trends*
563 *Immunol.* 2018;39:724-33.
- 564 15. Hewitson JP, Harcus Y, Murray J, van Agtmaal M, Filbey KJ, Grainger JR, Bridgett S,
565 Blaxter ML, Ashton PD, Ashford DA, Curwen RS, Wilson RA, Dowle AA, Maizels RM.
566 Proteomic analysis of secretory products from the model gastrointestinal nematode

- 567 *Heligmosomoides polygyrus* reveals dominance of venom allergen-like (VAL) proteins. J
568 Proteomics. 2011;74: 1573-94.
- 569 16. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD,
570 Macpherson AJ. Intestinal bacterial colonization induces mutualistic regulatory T cell
571 responses. Immunity 2011;34:794-806.
- 572 17. Hang L, Blum AM, Setiawan T, Urban JP Jr, Stoyanoff KM, Weinstock JV.
573 *Heligmosomoides polygyrus bakeri* infection activates colonic Foxp³⁺ T cells enhancing
574 their capacity to prevent colitis. J Immunol. 2013;191:1927-34.
- 575 18. Su L, Su CW, Qi Y, Yang G, Zhang M, Cherayil BJ, Zhang X, Shi HN. Coinfection with an
576 intestinal helminth impairs host innate immunity against *Salmonella enterica* serovar
577 Typhimurium and exacerbates intestinal inflammation in mice. Infect Immun.
578 2014;82:3855-66.
- 579 19. Glendinning L, Nausch N, Free A, Taylor DW, Mutapi F. The microbiota and helminths:
580 sharing the same niche in the human host. Parasitology. 2014;141:1255-71.
- 581 20. Grant AV, Araujo MI, Ponte EV, Oliveira RR, Cruz AA, Barnes KC, Beaty TH.
582 Polymorphisms in IL10 are associated with total Immunoglobulin E levels and *Schistosoma*
583 *mansoni* infection intensity in a Brazilian population. Genes Immun. 2011;12:46-50.
- 584 21. Costa RD, Figueiredo CA, Barreto ML, Alcantara-Neves NM, Rodrigues LC, Cruz AA,
585 Vergara C, Rafaels N, Foster C, Potee J, Campbell M, Mathias RA, Barnes KC. Effect of
586 polymorphisms on TGFB1 on allergic asthma and helminth infection in an African admixed
587 population. Ann Allergy Asthma Immunol. 2017;118:483-8. e1.
- 588 22. Janet K, Tommy LFL. Flying with diverse passengers: greater richness of parasitic
589 nematodes in migratory birds. Oikos. 2015;124:399-405.
- 590 23. Guivier E, Bellenger J, Sorci G, Faivre B. Helminth interaction with the host immune
591 system: Short-term benefits and costs in relation to the infectious environment. Am Nat.
592 2016;188:253-63.
- 593 24. Bourke CD, Maizels RM, Mutapi F. Acquired immune heterogeneity and its sources in
594 human helminth infection. Parasitology. 2011;138:139-59.
- 595 25. Fischer J, Jung N, Robinson N, Lehmann C. Sex differences in immune responses to
596 infectious diseases. Infection. 2015;43:399-403.
- 597 26. Klein SL. The effects of hormones on sex differences in infection: from genes to behavior.
598 Neurosci. Biobehav. Rev. 2000;24:627-38.
- 599 27. Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusia AJ, Knight R,
600 Caporaso JG, Svanbäck R. Individual diet has sex-dependent effects on vertebrate gut
601 microbiota. Nat Commun. 2014;5: 4500.
- 602 28. Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Knight R, Caporaso JG, Svanbäck R.
603 Individuals' diet diversity influences gut microbial diversity in two freshwater fish
604 (threespine stickleback and Eurasian perch). Ecol Lett. 2014;17:979-87.
- 605 29. Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, Stutz WE. Major
606 Histocompatibility Complex class IIb polymorphism influences gut microbiota composition
607 and diversity. Mol Ecol. 2014;23:4831-45.
- 608 30. Benson AK, Kelly SA, Legge R, Ma F, Low SJ, Kim J, Zhang M, Oh PL, Nehrenberg D,
609 Hua K, Kachman SD, Moriyama EN, Walter J, Peterson DA, Pomp D. Individuality in gut
610 microbiota composition is a complex polygenic trait shaped by multiple environmental and
611 host genetic factors. Proc Natl Acad Sci USA. 2010;07: 18933-8.

- 612 31. Goodrich JK, Davenport ER, Waters JL, Clark AG, Ley RE. Cross-species comparisons of
613 host genetic associations with the microbiome. *Science*. 2016;52:532-5.
- 614 32. Ho IC, Lo D, Glimcher LH. c-maf promotes T helper cell type 2 (Th2) and attenuates Th1
615 differentiation by both interleukin 4-dependent and -independent mechanisms. *J Exp Med*.
616 1998;188:1859-66.
- 617 33. Xu M, Pokrovskii M, Ding Y, Yi R, Au C, Harrison OJ, Galan C, Belkaid Y, Bonneau R,
618 Littman DR. c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut
619 pathobiont. *Nature*. 2018;554:373-7.
- 620 34. Mai V, McCrary QM, Sinha R, Gleis M. Associations between dietary habits and body mass
621 index with gut microbiota composition and fecal water genotoxicity: an observational study
622 in African American and Caucasian American volunteers. *Nutr J*. 2009;8:49.
- 623 35. Mann E, Schmitz-Esser S, Zebeli Q, Wagner M, Ritzmann M, Metzler-Zebeli BU. Mucosa-
624 associated bacterial microbiome of the gastrointestinal tract of weaned pigs and dynamics
625 linked to dietary calcium-phosphorus. *PLoS One*. 2014;9: e86950.
- 626 36. Gomes JM, Costa JA, Alfenas RC. Could the beneficial effects of dietary calcium on
627 obesity and diabetes control be mediated by changes in intestinal microbiota and integrity?
628 *Br J Nutr*. 2015;14:1756-65.
- 629 37. Zhu Y, Michelle LT, Jobin C, Young HA. Gut microbiota and probiotics in colon
630 tumorigenesis. *Cancer Lett*. 2011;309:119-27.
- 631 38. Venugopal PG, Nutman TB, Semnani RT. Activation and regulation of toll-like receptors
632 (TLRs) by helminth parasites. *Immunol Res*. 2009;43:252-63.
- 633 39. Broadhurst MJ, Ardeshir A, Kanwar B, Mirpuri J, Gundra UM, Leung JM, Wiens KE,
634 Vujkovic-Cvijin I, Kim CC, Yarovinsky F, Lerche NW, McCune JM, Loke P. Therapeutic
635 helminth infection of macaques with idiopathic chronic diarrhea alters the inflammatory
636 signature and mucosal microbiota of the colon. *PLoS Pathog*. 2012;8:e1003000.
- 637 40. Scotland RS, Stables MJ, Madalli S, Watson P, Gilroy DW. Sex differences in resident
638 immune cell phenotype underlie more efficient acute inflammatory responses in female
639 mice. *Blood*. 2011;118:5918-27.
- 640 41. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*.
641 2016;16:626-38.
- 642 42. Scharsack JP, Koch K, Hammerschmidt K. Who is in control of the stickleback immune
643 system: interactions between *Schistocephalus solidus* and its specific vertebrate host. *Proc*
644 *Biol Sci*. 2007;274:3151-8.
- 645 43. Barber I, Scharsack JP. The three-spined stickleback-*Schistocephalus solidus* system: an
646 experimental model for investigating host-parasite interactions in fish. *Parasitology*.
647 2010;137: 411-24.
- 648 44. Hendry AP, Peichel CL, Matthews B, Boughman JW, Nosil P. Stickleback research: the
649 now and the next. *Evol Ecol Res*. 2013;15:111-41.
- 650 45. Houlden A, Hayes KS, Bancroft AJ, Worthington JJ, Wang P, Grecis RK, Roberts IS.
651 Chronic *Trichuris muris* Infection in C57BL/6 mice causes significant changes in host
652 microbiota and metabolome: Effects reversed by pathogen clearance. *PLoS One*.
653 2015;10:e0125945.
- 654 46. Whary MT, Muthupalani S, Ge Z, Feng Y, Lofgren J, Shi HN, Taylor NS, Correa P,
655 Versalovic J, Wang TC, Fox JG. Helminth co-infection in *Helicobacter pylori* infected INS-
656 GAS mice attenuates gastric premalignant lesions of epithelial dysplasia and glandular

- 657 atrophy and preserves colonization resistance of the stomach to lower bowel microbiota.
658 *Microbes Infect.* 2014;16:345-55.
- 659 47. Khan AR, Fallon PG. Helminth therapies: translating the unknown unknowns to known
660 knowns. *Int J Parasitol.* 2013;43:293-9.
- 661 48. Whitacre, C.C. Sex differences in autoimmune disease. *Nat Immunol.* 2011;9:777-80.
- 662 49. Chiaroni-Clarke RC, Munro JE, Ellis JA. Sex bias in paediatric autoimmune disease - Not
663 just about sex hormones? *J Autoimmun.* 2016;69:12-23.
- 664 50. Weber JN, Steinel NC, Shim KC, Bolnick DI. Recent evolution of extreme cestode growth
665 suppression by a vertebrate host. *Proc Natl Acad Sci USA.* 2017;114:6575-80.
- 666 51. Lohman BK, Steinel NC, Weber JN, Bolnick DI. Gene expression contributes to the recent
667 evolution of host resistance in a model host parasite system. *Front Immunol.* 2017;8:1071.
- 668 52. Smith CC, Snowberg LK, Gregory CJ, Knight R, Bolnick DI. Dietary input of microbes and
669 host genetic variation shape among-population differences in stickleback gut microbiota.
670 *ISME J.* 2015;9:2515-26.
- 671 53. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA,
672 Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ,
673 Weber CF. Introducing mothur: open-source, platform-independent, community-supported
674 software for describing and comparing microbial communities. *Appl Environ Microbiol.*
675 2009;5:7537-41.
- 676 54. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-
677 index sequencing strategy and curation pipeline for analyzing amplicon sequence data on
678 the MiSeq Illumina sequencing platform. *Appl Environ Microbiol.* 2013;79:5112-20.
- 679 55. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The
680 SILVA ribosomal RNA gene database project: improved data processing and web-based
681 tools. *Nucleic Acids Res.* 2013;41:D590-6.
- 682 56. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A,
683 Kuske CR, Tiedje JM. Ribosomal Database Project: data and tools for high throughput
684 rRNA analysis. *Nucleic Acids Res.* 2014;42:D633-42.
- 685 57. McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and
686 graphics of microbiome census data. *PLoS One.* 2013;8:e61217.
- 687 58. Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. Double digest RADseq: an
688 inexpensive method for de novo SNP discovery and genotyping in model and non-model
689 species. *PLoS One.* 2012;7:e37135.
- 690 59. Stuart YE, Veen T, Weber JN, Hanson D, Ravinet M, Lohman BK, Thompson CJ, Tasneem
691 T, Doggett A, Izen R, Ahmed N, Barrett RDH, Hendry AP, Peichel CL, Bolnick DI.
692 Contrasting effects of environment and genetics generate a continuum of parallel evolution.
693 *Nat Ecol Evol.* 2017;1:158.
- 694 60. Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses.
695 *Bioinformatics.* 2003;19:889-90.

696
697 **Figure legends**

698 **Fig. 1 Microbiome community structure of stickleback differs between cestode exposure versus infection.** For
699 the purpose of plotting, microbiome community is measured here by scoring fish along the first linear discriminant
700 axis trained on three groups of fish (control, exposed, and infected). The six families are identified by separate point
701 and line colors, underscoring one family with an atypical response to exposure that generated a significant family by
702 exposure interaction; without this family there is a significant exposure main effect. (a) Microbiome community
703 structure differs between stickleback that were experimentally exposed to *S.solidus* ('exposed') versus control fish

704 that were sham infected. (b) No significant effect of cestode infection success on experimentally exposed
705 sticklebacks' gut microbiota.

706
707
708 **Fig. 2 Many microbial Orders ($N=84$, found in a minimum of 20 stickleback) exhibit significant effects of**
709 **infection or fish characteristics.** Here, we identify which microbial Orders exhibit various main and interaction
710 effects. For each Order, a cell is colored if the relevant effect is significant at $P<0.05$. Note that there is a strong
711 excess of significant results at this threshold above the null expectation of a 5% false positive rate (χ^2 tests $P<0.0001$
712 for all but the mass*infection interaction). Generalized linear model results for all common Orders (found in at least
713 20 fish) are summarized in File S2. Color denotes an effect direction. For cross, red denotes higher abundance with
714 more Roberts Lake alleles (ROB backcross), blue denotes higher abundance in Gosling Lake alleles (GOS
715 backcross). For mass, red denotes higher abundance in larger fish, blue in smaller fish. For sex, red denotes higher
716 abundance in males, blue higher in females. For infection, red denotes higher abundance in infected than in
717 uninfected fish, blue is the reverse. For the cross*infection interaction, red indicates instances where infection
718 increases microbe abundance in ROB backcrosses while blue indicates infection increases abundance in GOS. For
719 mass*infection, red indicates that infection increases microbe abundance more strongly in larger fish. For
720 sex*infection, red denotes cases where infection increases microbe abundance most strongly in males, whereas blue
721 implies infection increases microbe abundance mostly in females.

722
723
724 **Fig. 3 Examples of how the gut microbiome composition depends on host sex, genotype (cross), and infection**
725 **status, focusing on the relative abundance of microbial Orders.** To visualize these effects we first adjusted for
726 variation due to rearing location (room), by calculating residuals from a quasibinomial general linear model of the
727 focal Order's read counts (out of the total reads per fish) regressed on rearing room. Here we plot the mean (and ± 1
728 s.e. confidence intervals) for this residual abundance, for various groups of fish to illustrate infection and host
729 effects. (a) Fusobacteriales exhibit a significant decrease in cestode-infected fish, both males (blue) and females
730 (red). (b) Lactobacillales are more abundant in males than in females regardless of infection status. (c) Clostridiales
731 abundance is reduced in infected males, but not infected females. (d) Rhodobacterales are more common in females
732 for uninfected fish, but more common in males for infected fish. (e) Caulobacterales are more abundant in F2
733 hybrids than in either backcross, regardless of infection status (black = uninfected, red = infected). (f)
734 Rhodobacterales are more abundant in fish with a greater fraction of Roberts lake ancestry, but only among
735 uninfected fish; infection leads to a higher Rhodobacterales abundance that is similar across fish genotypes. Fig. S5
736 shows the same plots, but with all data points included to show the small effect size relative to high among-
737 individual variation.

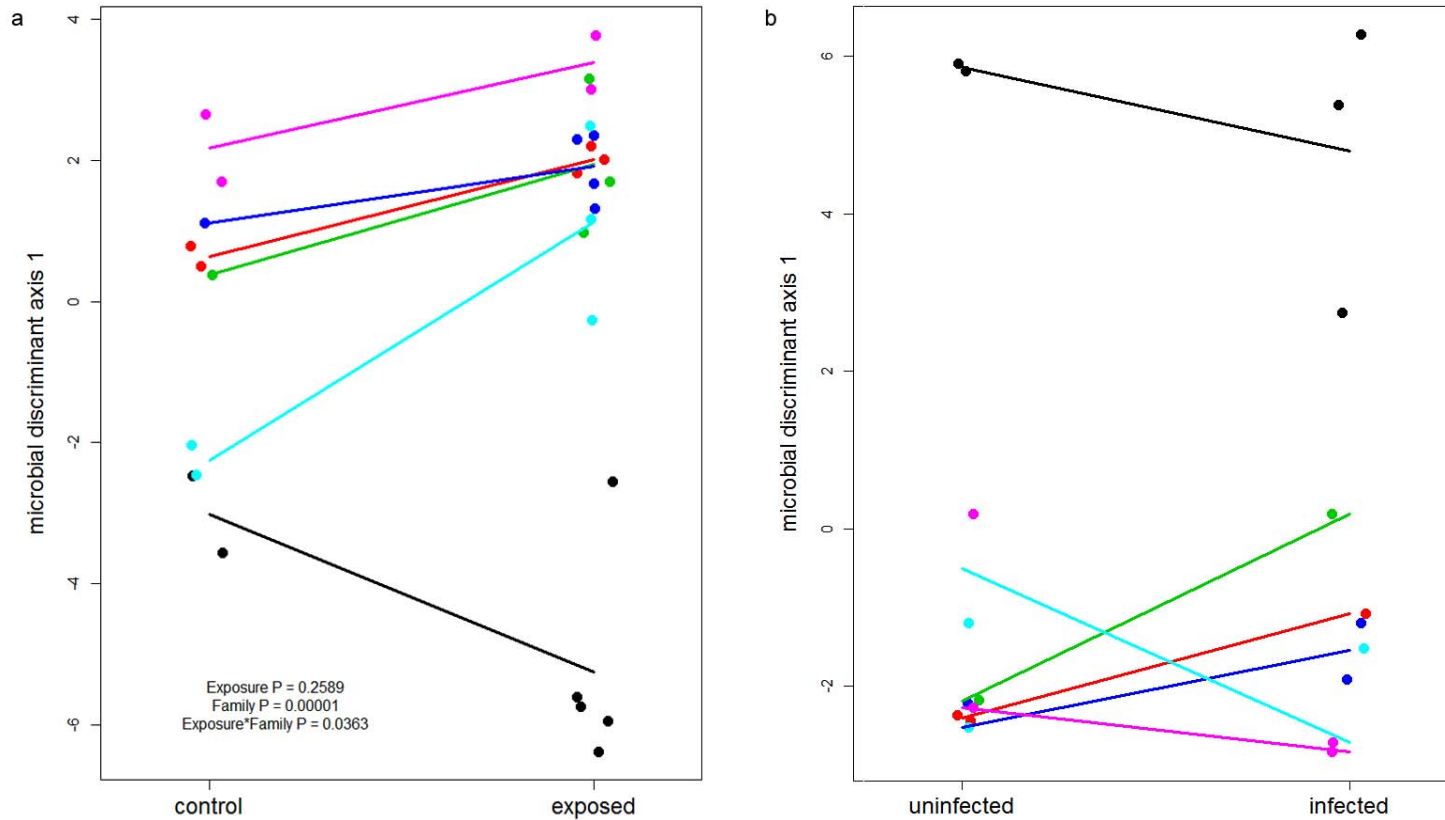
738
739
740 **Fig. 4 Linear discriminant analysis of the top 50 unweighted PCoA axes of microbial composition reveals**
741 **effects of host sex, host cross type, and infection status.** (a) Results of LDA to separate all combinations of sex
742 and infection status. LDA axis 1 (LDA1) separates infected (filled points) from uninfected (open points; $P<0.0001$).
743 LDA2 separates males and females (blue and red points; $P<0.0001$), and exhibits a significant interaction effect
744 ($P=0.002$). (b) Results of LDA to separate combinations of cross (blue = Gosling backcross, purple = F2, red =
745 Roberts backcross) and infection status (open circles = uninfected, filled circles = infected). LDA1 (68% of
746 variation) separates F2 hybrids from both backcrosses. LDA2 (16% of variation; not shown here) separates Roberts
747 from Gosling backcross fish, with F2 hybrids being intermediate. LDA3 explains 7% of the microbial variation and
748 is most strongly associated with infection status, but in a manner that depends on host cross: the three host crosses
749 are on average almost identical along LDA3 when uninfected (open circles), but diverge when infected with F2s
750 intermediate as expected from additive genetic control. Raw points are shown in faded colors, overlain by larger
751 darker circles representing bivariate averages with ± 1 s.e. error bars.

752
753
754 **Fig. 5 QTL map of Fusobacteriales relative abundance (with non-parametric statistical tests) reveals two**
755 **autosomal QTL plus an association with the X chromosome.** Analyses were run separately for each cross, with
756 rearing room as an additive covariate, then the LOD scores from the three maps were summed. The observed
757 summed LOD scores are plotted in black for each linkage group, measuring statistical association between the focal
758 trait and the chromosomal region. Marker locations are indicated as tick marks along the horizontal axis. Thin blue
759 lines represent null summed LOD scores from within-cross permutations of traits. The horizontal dashed line

760 indicates the upper 99.99% quantile for the null LOD scores. Three QTL exceed this threshold, on chromosomes 4, 9,
761 and X. Two of these are plotted in the lower panels (left, locus X109 on Chr 4, genotype $P=0.0013$; right, locus
762 X156 on Chr 9, genotype $P=0.0060$). The y axis in these effect plots are the residuals from a regression of
763 unweighted PCoA5 on rearing room. We plot the means microbial abundance with ± 1 standard error bars, for each
764 of the three genotypes at each locus.

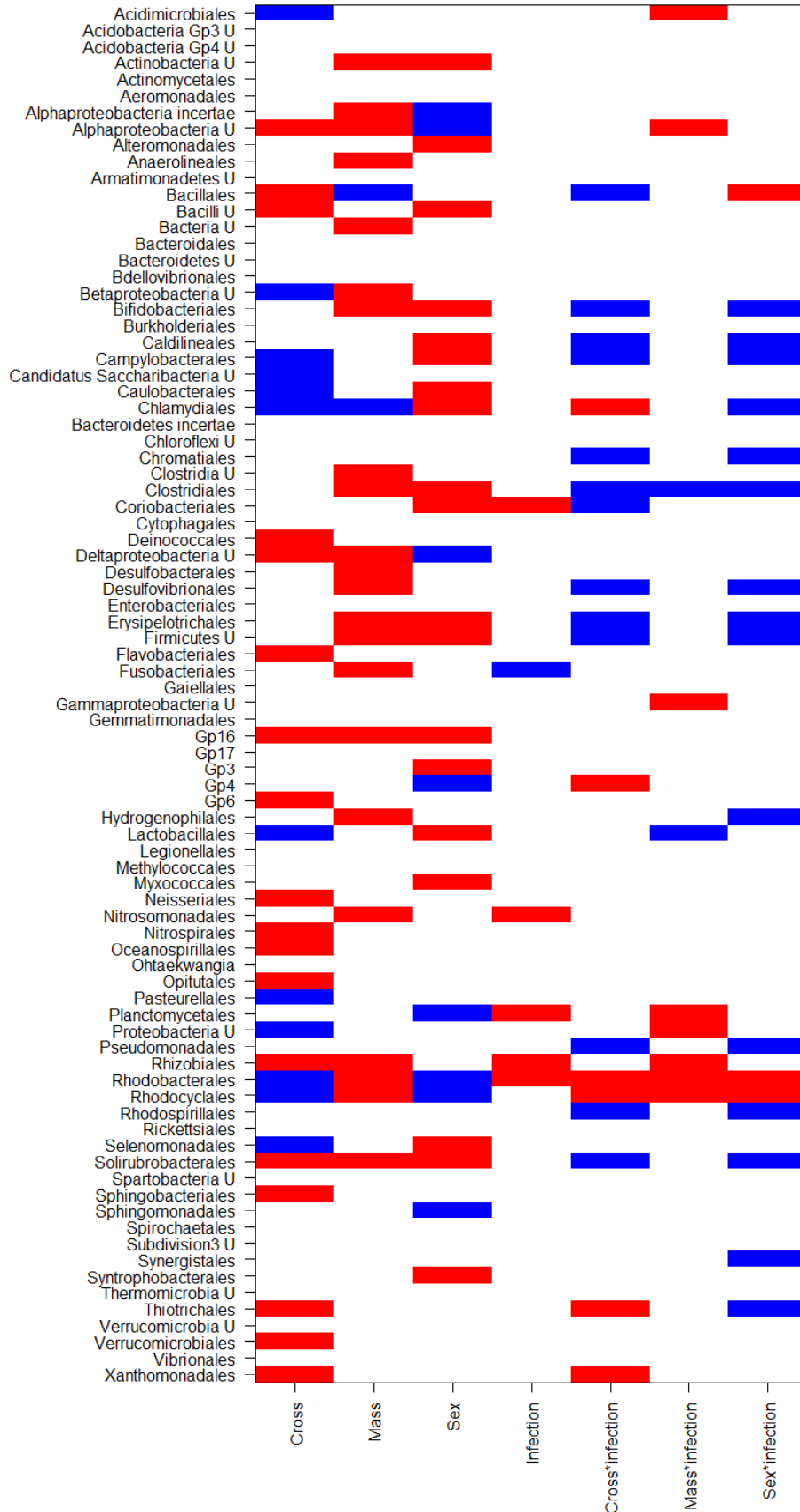
765
766 **Fig. 6 A summary of the locations of QTL affecting the relative abundance of individual microbial Order.**
767 Each chromosome is plotted as a vertical grey bar, and to its right we plot microbial QTL located on that
768 chromosome. A dot symbol indicates the location of maximum LOD score for each QTL, and thin vertical lines
769 indicate the inferred width of the QTL. For sex-linked microbes, their QTL span the entire X chromosome, so we
770 omit that linkage group. The key to the right lists the locations of the significant QTL for each focal taxon.

771
772
773
774
775
776
777
778
779
780

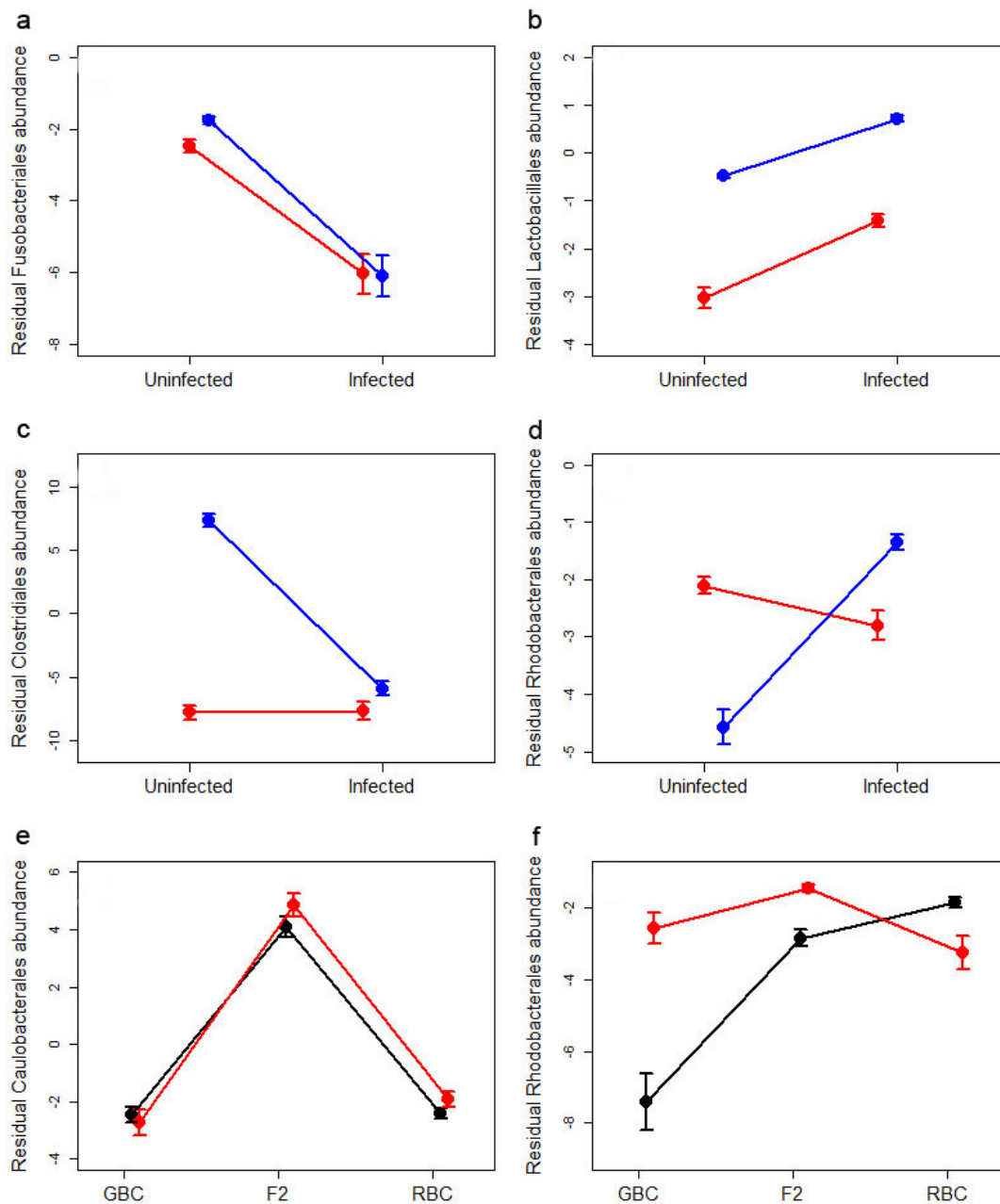


781
782
783
784
785
786
787

Fig. 1 Microbiome community structure of stickleback differs between cestode exposure versus infection. For the purpose of plotting, microbiome community is measured here by scoring fish along the first linear discriminant axis trained on three groups of fish (control, exposed, and infected). The six families are identified by separate point and line colors, underscoring one family with an atypical response to exposure that generated a significant family by exposure interaction; without this family there is a significant exposure main effect. (a) Microbiome community structure differs between stickleback that were experimentally exposed to *S.solidus* ('exposed') versus control fish that were sham infected. (b) No significant effect of cestode infection success on experimentally exposed sticklebacks' gut microbiota



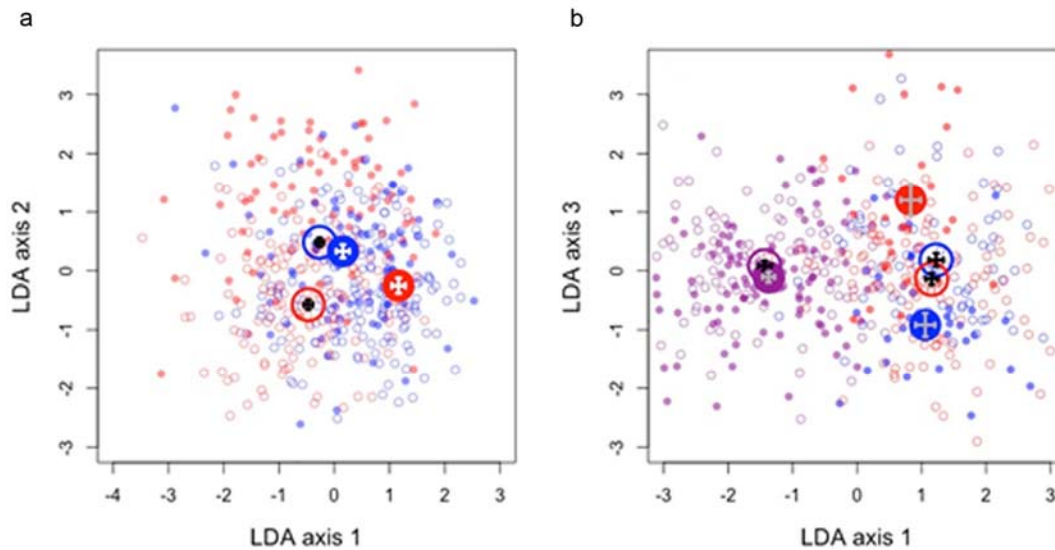
789 **Fig. 2 Many microbial Orders ($N=84$, found in a minimum of 20 stickleback) exhibit significant effects of**
790 **infection or fish characteristics.** Here, we identify which microbial Orders exhibit various main and interaction
791 effects. For each Order, a cell is colored if the relevant effect is significant at $P<0.05$. Note that there is a strong
792 excess of significant results at this threshold above the null expectation of a 5% false positive rate (χ^2 tests $P<0.0001$
793 for all but the mass*infection interaction). Generalized linear model results for all common Orders (found in at least
794 20 fish) are summarized in File S2. Color denotes an effect direction. For cross, red denotes higher abundance with
795 more Roberts Lake alleles (ROB backcross), blue denotes higher abundance in Gosling Lake alleles (GOS
796 backcross). For mass, red denotes higher abundance in larger fish, blue in smaller fish. For sex, red denotes higher
797 abundance in males, blue higher in females. For infection, red denotes higher abundance in infected than in
798 uninfected fish, blue is the reverse. For the cross*infection interaction, red indicates instances where infection
799 increases microbe abundance in ROB backcrosses while blue indicates infection increases abundance in GOS. For
800 mass*infection, red indicates that infection increases microbe abundance more strongly in larger fish. For
801 sex*infection, red denotes cases where infection increases microbe abundance most strongly in males, whereas blue
802 implies infection increases microbe abundance mostly in females.



803
 804 **Fig. 3 Examples of how the gut microbiome composition depends on host sex, genotype (cross), and infection**
 805 **status, focusing on the relative abundance of microbial Orders.** To visualize these effects we first adjusted for
 806 variation due to rearing location (room), by calculating residuals from a quasibinomial general linear model of the
 807 focal Order's read counts (out of the total reads per fish) regressed on rearing room. Here we plot the mean (and ± 1
 808 s.e. confidence intervals) for this residual abundance, for various groups of fish to illustrate infection and host
 809 effects. (a) Fusobacteriales exhibit a significant decrease in cestode-infected fish, both males (blue) and females
 810 (red). (b) Lactobacillales are more abundant in males than in females regardless of infection status. (c) Clostridiales
 811 abundance is reduced in infected males, but not infected females. (d) Rhodobacterales are more common in females
 812 for uninfected fish, but more common in males for infected fish. (e) Caulobacterales are more abundant in F2
 813 hybrids than in either backcross, regardless of infection status (black = uninfected, red = infected). (f)
 814 Rhodobacterales are more abundant in fish with a greater fraction of Roberts lake ancestry, but only among
 815 uninfected fish; infection leads to a higher Rhodobacterales abundance that is similar across fish genotypes. Fig. S5

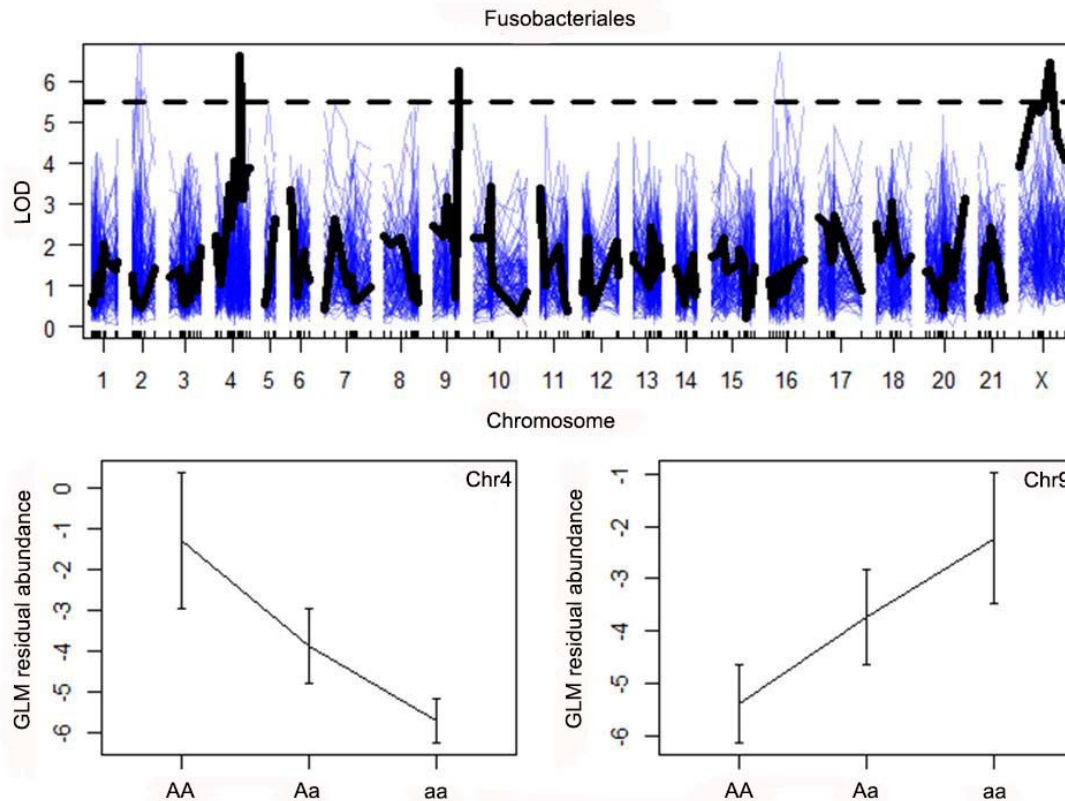
816 shows the same plots, but with all data points included to show the small effect size relative to high among-
817 individual variation.

818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833



834
835 **Fig. 4 Linear discriminant analysis of the top 50 unweighted PCoA axes of microbial composition reveals**
836 **effects of host sex, host cross type, and infection status.** (a) Results of LDA to separate all combinations of sex
837 and infection status. LDA axis 1 (LDA1) separates infected (filled points) from uninfected (open points; $P < 0.0001$).
838 LDA2 separates males and females (blue and red points; $P < 0.0001$), and exhibits a significant interaction effect
839 ($P = 0.002$). (b) Results of LDA to separate combinations of cross (blue = Gosling backcross, purple = F2, red =
840 Roberts backcross) and infection status (open circles = uninfected, filled circles = infected). LDA1 (68% of
841 variation) separates F2 hybrids from both backcrosses. LDA2 (16% of variation; not shown here) separates Roberts
842 from Gosling backcross fish, with F2 hybrids being intermediate. LDA3 explains 7% of the microbial variation and
843 is most strongly associated with infection status, but in a manner that depends on host cross: the three host crosses
844 are on average almost identical along LDA3 when uninfected (open circles), but diverge when infected with F2s
845 intermediate as expected from additive genetic control. Raw points are shown in faded colors, overlain by larger
846 darker circles representing bivariate averages with ± 1 s.e. error bars.

847
848
849
850
851
852
853
854



855
856 **Fig. 5 QTL map of Fusobacteriales relative abundance (with non-parametric statistical tests) reveals two**
857 **autosomal QTL plus an association with the X chromosome.** Analyses were run separately for each cross, with
858 rearing room as an additive covariate, then the LOD scores from the three maps were summed. The observed
859 summed LOD scores are plotted in black for each linkage group, measuring statistical association between the focal
860 trait and the chromosomal region. Marker locations are indicated as tick marks along the horizontal axis. Thin blue
861 lines represent null summed LOD scores from within-cross permutations of traits. The horizontal dashed line
862 indicates the upper 99.99% quantile for the null LOD scores. Three QTL exceed this threshold, on chromosomes 4, 9,
863 and X. Two of these are plotted in the lower panels (left, locus X109 on Chr 4, genotype $P=0.0013$; right, locus
864 X156 on Chr 9, genotype $P=0.0060$). The y axis in these effect plots are the residuals from a regression of
865 unweighted PCoA5 on rearing room. We plot the means microbial abundance with ± 1 standard error bars, for each
866 of the three genotypes at each locus.

867
868
869
870
871
872
873
874
875
876
877
878
879
880

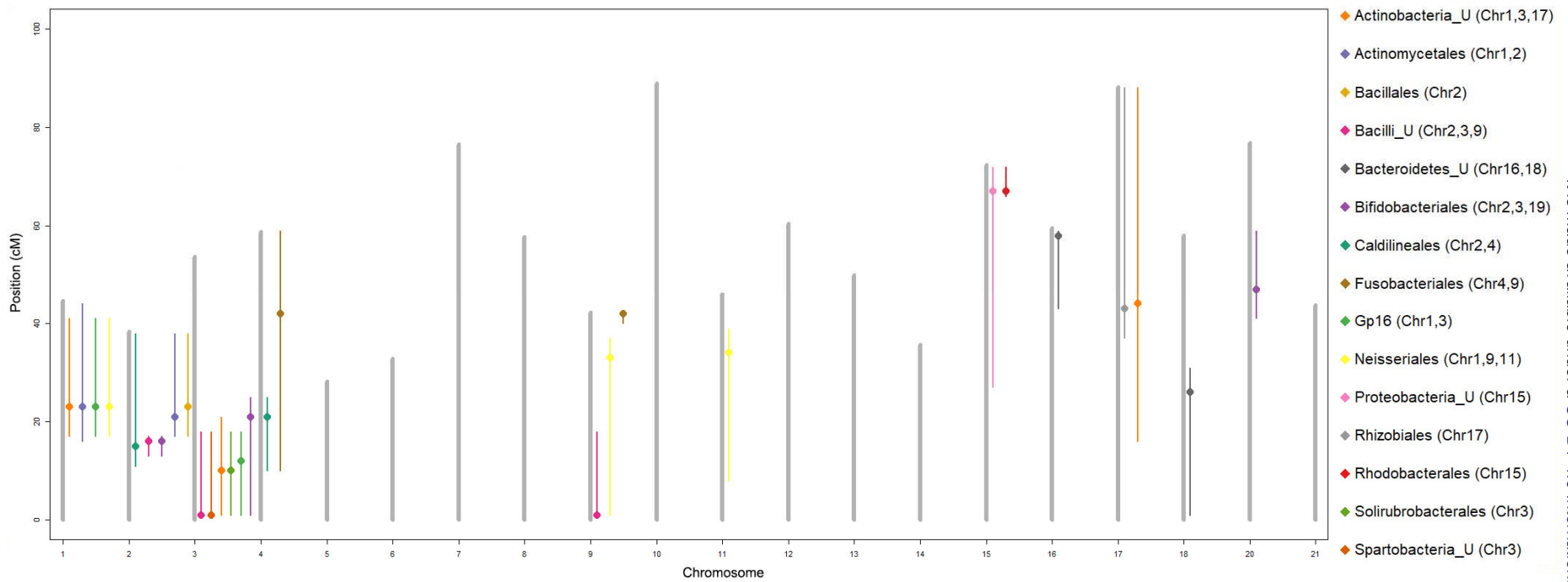


Fig. 6 A summary of the locations of QTL affecting the relative abundance of individual microbial Order. Each chromosome is plotted as a vertical grey bar, and to its right we plot microbial QTL located on that chromosome. A dot symbol indicates the location of maximum LOD score for each QTL, and thin vertical lines indicate the inferred width of the QTL. For sex-linked microbes, their QTL span the entire X chromosome, so we omit that linkage group. The key to the right lists the locations of the significant QTL for each focal taxon.