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1 **Title:** The evolution of parasite host range in genetically diverse host populations

2 **Running Title:** Host diversity and generalist parasites

3 **Authors:**

4 Amanda K Gibson[1,2*], Helena S Baffoe-Bonnie[1], McKenna J Penley, Julie Lin[1], Raythe Owens[1],
5 Arooj Khalid[1], and Levi T. Morran[1]

6 **Affiliations:**

7 [1]Department of Biology, Emory University, Atlanta, GA 30322

8 [2]Department of Biology, University of Virginia, Virginia 22902, USA

9 **Corresponding author**: Amanda K Gibson, Department of Biology, University of Virginia,
10 Virginia 22902, USA; akg5nq@virginia.edu

11 **Author contributions:** AKG conceived and directed the study, performed experimental
12 evolution and assays, collected data, analyzed data, and wrote the manuscript. HSBB assisted in
13 experimental evolution, performed assays, collected and analyzed data, and contributed to drafts
14 of the manuscript. MJP, JL, RO and AK assisted in experimental evolution and assays. LTM
15 conceived the study, provided guidance, collected data, and revised the manuscript.

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26 **Abstract:** Parasites vary enormously in their host range. Why are some parasites specialists and
27 others generalists? We tested the hypothesis that genetically diverse host populations select for
28 parasites with broader host ranges than genetically homogeneous host populations. To do so, we
29 selected for increased killing ability of the bacterial parasite *Serratia marcescens* in populations
30 of the host *Caenorhabditis elegans* that were either diverse (50% mix of genotypes N2 and
31 LTM1) or homogenous (100% N2 or LTM1). We found mixed support for the hypothesis.
32 After 20 generations of selection, parasites selected in diverse host populations maintained a
33 broad host range, as shown by the retention of high killing ability of a novel host genotype.
34 Parasites selected in diverse populations killed N2 hosts equally well as parasites selected in
35 homogenous populations of N2. However, N2-selected parasites lost killing ability against the
36 novel host, consistent with the evolution of narrow host ranges in homogenous environments. In
37 contrast, parasites selected in homogenous LTM1 populations did not specialize: they did not
38 increase in their killing ability on LTM1 and did not lose killing ability against the novel host.
39 Our results argue that the evolution of host range depends upon both the identity and diversity of
40 hosts that a parasite encounters.

41

42 **Keywords:** Host range, spatial variation, generalist, specialist, experimental evolution,
43 *Caenorhabditis elegans*

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48 **Introduction**

49 Closely-related parasites may vary in the number of host genotypes (Carius et al. 2001; Thrall et
50 al. 2001; Poullain et al. 2008), species (Desdevises et al. 2002; Poulin and Mouillot 2003;
51 Krasnov et al. 2004), or even families (Ross et al. 2013) they can attack. Why does host range
52 vary so much? Here, we use experimental evolution to test the hypothesis that the variance of
53 the host environment drives the evolution of parasite host range, such that genetically diverse
54 host populations favor generalist parasites and genetically homogeneous host populations favor
55 specialist parasites.

56 This hypothesis derives from theory on the evolution of niche width. This body of theory
57 argues that heterogeneous environments select for large niche widths (i.e. generalists), while
58 homogeneous environments select for narrow niche widths (i.e. specialists) (Levins 1962; Pianka
59 1966; Via and Lande 1985; Lynch and Gabriel 1987; Futuyma and Moreno 1988).
60 Specialization in a homogeneous environment is thought to arise from either antagonistic
61 pleiotropy, where mutations that increase performance in the focal environment reduce
62 performance in alternate environments (Rausher 1984; Jaenike 1990; Via 1990), or mutation
63 accumulation, where populations accumulate mutations that are neutral in the focal environment
64 and deleterious in alternate environments (Fry 1996; Whitlock 1996). The probability of fixation
65 of such mutations declines if individuals have a high probability of encountering multiple
66 environments, due to either temporal or fine-scale spatial heterogeneity. Experimental evolution
67 studies of free-living systems support the maintenance of generalists under abiotic heterogeneity,
68 notably under temporal heterogeneity (Bennett et al. 1992; Reboud and Bell 1997) (rev. in
69 Kassen 2002).

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70 This body of theory has been extended to the evolution of host range in parasites.
71 Substantial evidence now exists for the evolution of host specialization under temporal
72 homogeneity: during serial passage, many parasites adapt to infect individual host species or
73 genotypes and simultaneously decline in their ability to infect alternate hosts (Cunfer 1984; Fry
74 1990; Ebert 1998). In contrast, tests of the associated prediction, that diverse host environments
75 select for generalist parasites, are few in number and limited to viral systems. A common
76 approach involves creating temporal host heterogeneity by alternating a viral lineage between
77 cell lines derived from different host species (Novella et al. 1999; Weaver et al. 1999; Coffey
78 and Vignuzzi 2011) (though see Bedhomme et al. 2011). Turner et al. (2010) adopted this
79 approach, finding mixed support for the idea that diverse host communities select for parasites
80 that can infect a broad range of host species: after alternation between human and canine cells,
81 populations of vesicular stomatitis virus populations showed higher performance on cell lines of
82 novel host species relative to viral populations serially passaged on human cells. In contrast,
83 viral populations serially passaged on canine cells showed no average reduction in performance
84 relative to viral populations selected under temporal alternation. Studies have not explicitly
85 tested if spatial variation in hosts favor parasites with broad host ranges.

86 We build on these studies by directly testing the hypothesis that genetically diverse host
87 populations select for parasites with a broader range of host genotypes than genetically
88 homogeneous host populations. To test this hypothesis, we used experimental evolution to select
89 on populations of the bacterial parasite *Serratia marcescens* for increased performance in
90 populations of the nematode host *Caenorhabditis elegans* that varied in their diversity. Some
91 host populations were genetically diverse (an even mix of two genotypes), creating fine-scale
92 spatial variation in the host environment. Others were genetically homogeneous (one of two

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93 possible genotypes), creating spatial homogeneity. We then compared the breadth of host ranges
94 across treatments by evaluating the performance of evolved parasite lineages on a novel host
95 genotype. We predicted that 1) parasites selected in diverse host populations would maintain or
96 increase in their ability to kill a novel host genotype, consistent with selection for a broad host
97 range, and 2) parasites selected in homogeneous populations would show reduced killing ability
98 of the novel host, consistent with specialization. We found mixed support for these predictions.

99 Materials and Methods

100 *Host and parasite genotypes*

101 For experimental evolution, we used two genotypes of the nematode *Caenorhabditis*
102 *elegans*: N2 and LTM1. Slowinksi et al. (2016) described the origins of the LTM1 line, which is
103 a single genotype derived from ethylmethane sulfonate mutagenesis of the CB4856 genotype.
104 We selected these two host genotypes for experimental evolution because 1) N2 and CB4856 are
105 among the most genetically divergent genotypes within *C. elegans* (Barrière and Félix 2005),
106 and 2) preliminary assays demonstrated that the parasite *Serratia marcescens* is equally virulent
107 to N2 and LTM1.

108 For assays of parasite virulence, we also included the host genotype JU1395. JU1395 is
109 roughly equally genetically divergent from N2 and LTM1(Andersen et al. 2012). Hence we
110 limited the potential that genetic proximity alone would generate differences between parasites
111 adapted to N2 vs. LTM1 in their virulence against JU1395. Assays of parasite performance
112 against JU1395 allowed us to compare the host range of evolved parasite lineages. We
113 subsequently refer to JU1395 as a novel host genotype, because parasite lineages never
114 encountered this host genotype during experimental evolution. We refer to N2 and LTM1 as

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115 sympatric host genotypes, because parasite lineages encountered one or both of these host
116 genotypes during experimental evolution.

117 We initiated replicate parasite lineages from Sm2170, a genotype of the bacterial parasite
118 *Serratia marcescens*. Sm2170 is known to be highly virulent towards *C. elegans* hosts
119 (Schulenburg and Ewbank 2004). The interaction of *C. elegans* and Sm2170 is a novel host-
120 parasite interaction constructed in the lab: there is no evidence that *C. elegans* encounters this
121 particular strain of *S. marcescens* in the wild, and Sm2170 had not previously been
122 experimentally evolved with *C. elegans*. Hosts acquire infection while feeding.

123 *Parasite selection treatments*

124 We established four treatments, each with six replicate parasite lineages (Fig. 1). In three
125 of these treatments, we subjected replicate parasite lineages to direct selection for increased
126 virulence against host populations that differed in their level of genetic diversity. In the first two
127 treatments, parasites were selected to kill hosts in homogeneous host populations. These host
128 populations comprised either 100% N2 hosts or 100% LTM1 hosts. In the third treatment,
129 parasites were selected to kill hosts in populations that were genetically diverse. These
130 populations were 50% N2 and 50% LTM1 hosts. We assumed that parasites have no ability for
131 host choice, such that parasites passaged with genetically diverse host populations had an equal
132 probability of encountering an N2 or LTM1 host each generation of selection.

133 We did not allow for host evolution during experimental evolution. Hence, each passage,
134 parasites were re-exposed to host populations of the same make-up as the prior generation. We
135 limited host evolution by maintaining stock populations of N2 and LTM1 at 15°C. Every few
136 weeks, we refreshed these stocks by thawing hosts archived at -80°C. Our experimental

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137 treatments therefore limited temporal host heterogeneity in order to contrast spatial host
138 heterogeneity with homogeneity.

139 The fourth treatment was the control treatment, where we did not directly select for
140 increased virulence. We designed this treatment to serve as the baseline against which to
141 measure evolutionary change in the prior three treatments. In this treatment, we passaged
142 bacteria without hosts. In doing so, we subjected bacterial populations to genetic drift and to the
143 non-focal selection pressures of the experiment in the absence of selection for increased
144 virulence.

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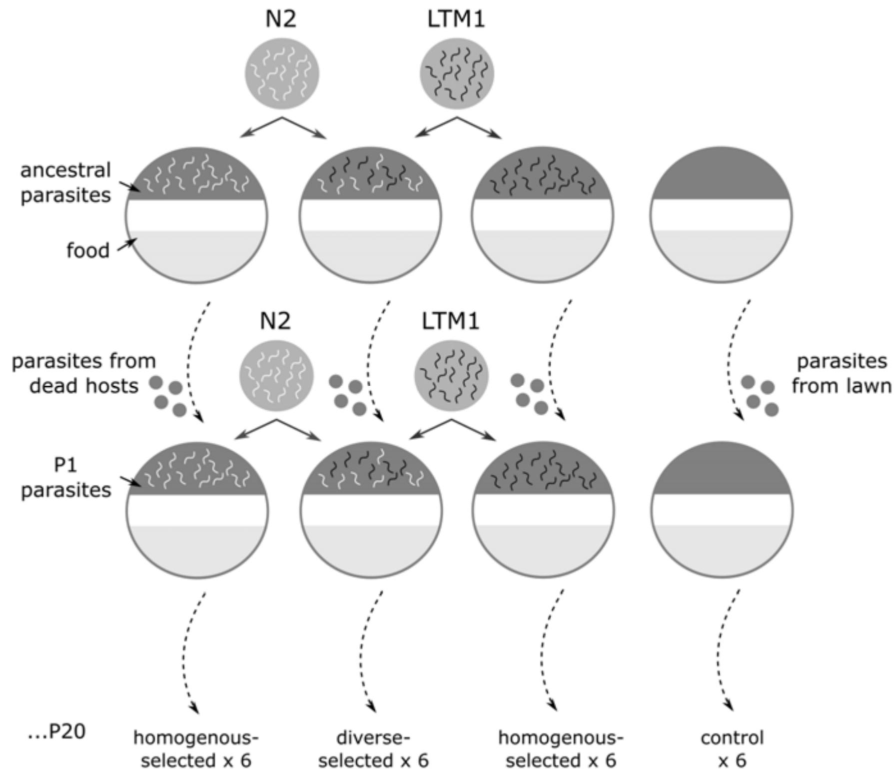

Figure 1: Experimental evolution scheme. We initiated experimental evolution by adding 500 *C. elegans* hosts to *Serratia* selection plates seeded with a lawn of Sm2170, the ancestral parasite genotype (dark lawn on upper portion of plates). For homogeneous selection, we added 100% N2 (left, white) or 100% LTM1 (right, black) hosts. For diverse selection, we added 50% N2 and 50% LTM1 hosts. We then selected for virulent parasites by extracting parasite colonies from hosts that died rapidly, within 24 hours. We used this passage of parasites (shown here as P1, second row) to seed lawns on *Serratia* selection plates, to which the same genotype(s) of hosts were added to commence the second round of selection. For the control treatment, we did not add any hosts and passaged parasite colonies directly from the lawn. We continued these selection regimes for a total of 20 passages. Each of the four treatments was replicated six times, for a total of 24 independent parasite lineages.

149

150 *Experimental evolution design*

151 Selection was performed using *Serratia* selection plates, as in Morran et al. (2009) (Fig.
152 1). Under this design, we seeded 100 mm petri dishes of Nematode Growth Media (NGM-lite,
153 United States Biological) with 35 uL of bacterial parasites (*Serratia marcescens*) on one side of
154 the plate and 35 uL of food (*Escherichia coli*,strain OP50) on the other. Adding nematodes to the

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155 *Serratia* lawn forced interaction between hosts and parasites. Hosts could then migrate towards
156 the lawn of food. We used this particular design in order to maintain the conditions of prior
157 evolution experiments (Morran et al. 2009; Morran et al. 2011; Slowinski et al. 2016) and
158 thereby facilitate comparison with their results.

159 To initiate experimental evolution, we harvested large numbers of L4 larvae of N2 and
160 LTM1 hosts. We established host populations that were 100% N2, 100% LTM1, or 50%
161 N2:50% LTM1 hosts by mixing the appropriate volumes of larvae of each host genotype. All
162 initial *Serratia* selection plates were seeded with the same culture of Sm2170. In order to
163 establish six replicate parasite lineages per treatment, we deposited ~500 L4 larvae of the
164 appropriate host population onto the Sm2170 lawns of six different *Serratia* selection plates. For
165 the control treatment, we did not add any larvae to the Sm2170 lawns. This resulted in a total of
166 24 plates representing 24 independent parasite lineages, six per each of four treatments.

167 We maintained these plates for 24 hours at 20°C. We then selected the most virulent
168 parasites by isolating and transferring those that killed hosts rapidly, within 24 hours. To
169 accomplish this, we picked 20-30 dead hosts from the Sm2170 lawn of each plate. We removed
170 external bacteria from these hosts by repeated rinsing, then crushed the hosts to extract the
171 internal bacteria that had killed them (Morran et al. 2011). We grew these bacteria on NGM-lite
172 plates at room temperature (~22°C) for 48 hours, then maintained them at 4°C for 48 hours. We
173 then randomly selected 40 colonies from these plates and grew them at 28°C overnight in 5 mL
174 of LB media. These liquid cultures were used to produce the next round of *Serratia* selection
175 plates, to which we added the same host population encountered by the parasite lineage in the
176 prior passage.

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177 For the control treatment, we collected ~30 samples of free-living bacteria directly from
178 the lawn of *Serratia* in order to mimic the sample sizes obtained in the other treatments. We
179 otherwise treated these populations in the same manner as the host-associated lineages. We
180 repeated this passaging scheme for a total of 20 passages, at which point we froze liquid cultures
181 of parasite lineages at -80°C.

182 *Survival assays of parasite virulence*

183 We measured parasite virulence as the mortality rate of a host genotype after 48 hours of
184 exposure to a parasite lineage. In setting up the assays, we replicated the experimental passaging
185 scheme. For each host genotype tested, we added a fixed volume of L4 larvae (100% focal host
186 genotype) to multiple replicate *Serratia* selection plates of all 24 parasite lineages. We
187 determined the mean number of L4 larvae added to *Serratia* selection plates by adding this same
188 volume to 10 standard plates seeded with OP50 and counting the number of hosts after 24 hours.
189 We maintained *Serratia* selection plates at 20°C for 48 hours, then counted the number of live
190 worms that had migrated out of the *Serratia* lawn. The mortality rate was obtained from the
191 survival rate, which we calculated as the number of live hosts divided by the mean number
192 added.

193 For the N2 genotype, we added 494 ± 26 hosts (mean ± standard error of the mean) per
194 *Serratia* selection plates. Each parasite lineage was replicated four times, for a total of 24
195 experimental replicates per selection treatment. For the LTM1 genotype, we added 498 ± 25
196 hosts. Each parasite lineage was also replicated four times. For our novel genotype, JU1395, we
197 added 270 ± 12 hosts. Each parasite lineage was replicated eight times, for a total of 48
198 experimental replicates per selection treatment.

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199 *Statistical Analyses*

200 All statistical analyses were performed in R (ver. 3.5.3; R Core Team 2013). We
201 conducted three separate analyses, one for each host genotype tested in the survival assays, in
202 order to compare the virulence of parasite lineages from different experimental treatments
203 towards a given host genotype. Statistical analyses with the N2 and LTM1 genotypes served to
204 evaluate adaptation of parasite lineages to their sympatric host genotypes. The statistical analysis
205 with the JU1395 genotype served to evaluate the host range of selected parasite lineages, by
206 testing their ability to kill a novel host genotype.

207 We began with survival assay data for N2, one of the sympatric host genotypes. We fit a
208 Poisson regression with experimental evolution treatment (control, homogeneous N2,
209 homogeneous LTM1, diverse) as a predictor of the number of live worms in an experimental
210 replicate. We included parasite lineage (1-6) as a random effect. We found evidence of
211 significant overdispersion (variance inflation factor, $\hat{c}$=19.98)(Venables and Ripley 2002), so we
212 re-fit the model as a negative binomial regression with the glmer.nb function in the lme4 package
213 (Bates et al. 2015). A likelihood ratio test indicated a substantially better fit with the negative
214 binomial regression relative to the Poisson regression (Likelihood-ratio test: χ^2=1319.2, df=1,
215 $p<0.001$). We applied this same modeling approach for the LTM1 and JU1395 genotypes. In
216 both cases, we found evidence of overdispersion (LTM1, $\hat{c}$=21.65; JU1395, $\hat{c}$=11.63) and a
217 better fit to our data with a negative binomial regression (LTM1, χ^2=1504.5, df=1, $p<0.001$;
218 JU1395, χ^2=1448.1, df=1, $p<0.001$).

219 We then evaluated treatment as a predictor of variation in the number of surviving hosts
220 by using likelihood ratio tests to compare models with and without the treatment factor. For
221 models in which treatment was a significant predictor of variation in survival, we examined

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222 model coefficients to compare between treatments. In analysis of sympatric host genotypes, we
223 tested the prediction that parasite lineages evolved increased virulence against hosts with which
224 they were passaged during experimental evolution. In analysis of the novel host genotype, we
225 tested the prediction that parasite lineages selected in diverse host populations would have higher
226 virulence against a novel host than parasite lineages selected in homogeneous host populations,
227 consistent with a larger host range for parasites selected in diverse host populations.

228 Lastly, we conducted a post-hoc analysis, based on observation of the data, to test if
229 parasite lineages selected in diverse host populations varied less in their virulence against a novel
230 host than parasite lineages selected in homogeneous host populations. To test this prediction, we
231 calculated the coefficient of variation in virulence (both number of surviving hosts and mortality
232 rate) against JU1395 across the six independent parasite lineages per treatment. We calculated
233 95% confidence intervals for the coefficient of variation by bootstrapping the JU1395 data set
234 10,000 times. Specifically, we re-sampled the experimental replicates per parasite lineage eight
235 times with replacement and re-calculated the coefficient of variation for each treatment.

236 Results

237 *Adaptation to sympatric host genotypes*

238 We first evaluated the virulence of experimentally evolved parasites when paired with
239 their sympatric hosts, N2 and LTM1. We predicted an increase in virulence when parasite
240 lineages were paired with the host genotypes on which they were selected.

241 The mortality rate of N2 was 82.3% when paired with the ancestral parasite genotype.
242 This closely matched the mortality rate of N2 when paired with control parasite lineages after 20
243 experimental passages (82.6 ± 0.8% SEM) (Table S1). When paired with experimentally evolved

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244 parasites, the mortality rate of N2 varied with parasite selection treatment (Table 1A, Fig. 2A).
245 Consistent with our prediction, parasite lineages selected to kill hosts in populations that were
246 diverse or homogeneous for N2 showed increased ability to kill N2 hosts relative to control
247 parasites (Generalized linear mixed model, number of surviving worms relative to control: 50%:
248 coefficient = -0.315, z = -2.287, p = 0.022; 100% N2: coefficient = -0.514, z = -3.677, $p < 0.001$)
249 and parasites selected to kill homogeneous LTM1 populations (50%: coefficient = 0.383, z =
250 2.755, p = 0.006; 100% N2: coefficient = 0.582, z = 4.232, $p < 0.001$). Specifically, the
251 mortality rate of N2 was 87.3±0.7% and 88.4±1.4% for parasites selected in diverse or
252 homogeneous N2 populations, respectively. Therefore, in relation to control parasites, survival
253 of N2 hosts declined by approximately a third when paired with parasites selected to kill N2
254 (homogeneous N2: 33% decline; diverse: 27% decline). Parasites selected in diverse or
255 homogeneous N2 populations did not differ from one another in their killing ability (coefficient
256 = 0.198, z = 1.417, p = 0.157). Parasite lineages selected to kill hosts in populations that were
257 homogeneous for LTM1 showed equivalent ability to kill N2 as control parasites (79.7 ± 2.1%,
258 coefficient = 0.068, z = 0.494, p = 0.621).

259 When paired with the ancestral parasite genotype, the mortality rate of LTM1 was 82.2%,
260 identical to that of N2 hosts. This mortality rate was slightly lower than the mortality rate of
261 LTM1 when paired with control parasite lineages after 20 experimental passages (83.8 ± 0.5%
262 SEM) (Table S2). Counter to our prediction, and in contrast to the results obtained for the N2
263 host genotype, the mortality rate of LTM1 did not vary across selection treatments for
264 experimentally evolved parasite lineages (Table 1B, Fig. 2B). The changes in virulence
265 qualitatively matched those observed with N2: relative to control parasites, the mortality rate of
266 LTM1 was slightly higher when paired with parasites selected in populations that were diverse or

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267 homogeneous for LTM1 (85.8 ± 0.6%, 86.5 ± 0.8%). We likewise observed no increase from
268 control parasites in the mortality rate of LTM1 when paired with parasite lineages selected to kill
269 hosts in populations that were homogeneous N2 (82.3 ± 1.5%).

Table 1: Experimental evolution treatment as a predictor of variation in parasite virulence on sympatric and novel host genotypes

	df	*D*	*p*
On sympatric hosts			
A. N2	3	22.93	<0.001
B. LTM1	3	3.73	0.292
On novel host			
C. JU1395	3	30.01	<0.001

Results of three separate generalized linear mixed models in which we fit parasite experimental evolution treatment (homogeneous N2, homogeneous LTM1, diverse, or control) as a predictor of the number of host individuals that survived parasite exposure. The three models correspond to three separate killing assays, one for each host genotype tested (A - N2, B - LTM1, and C - JU1395). We included parasite lineage (six independent lineages per experimental evolution treatment) as a random effect. We show the results of likelihood ratio (*D*) tests of models with and without treatment as a predictor.

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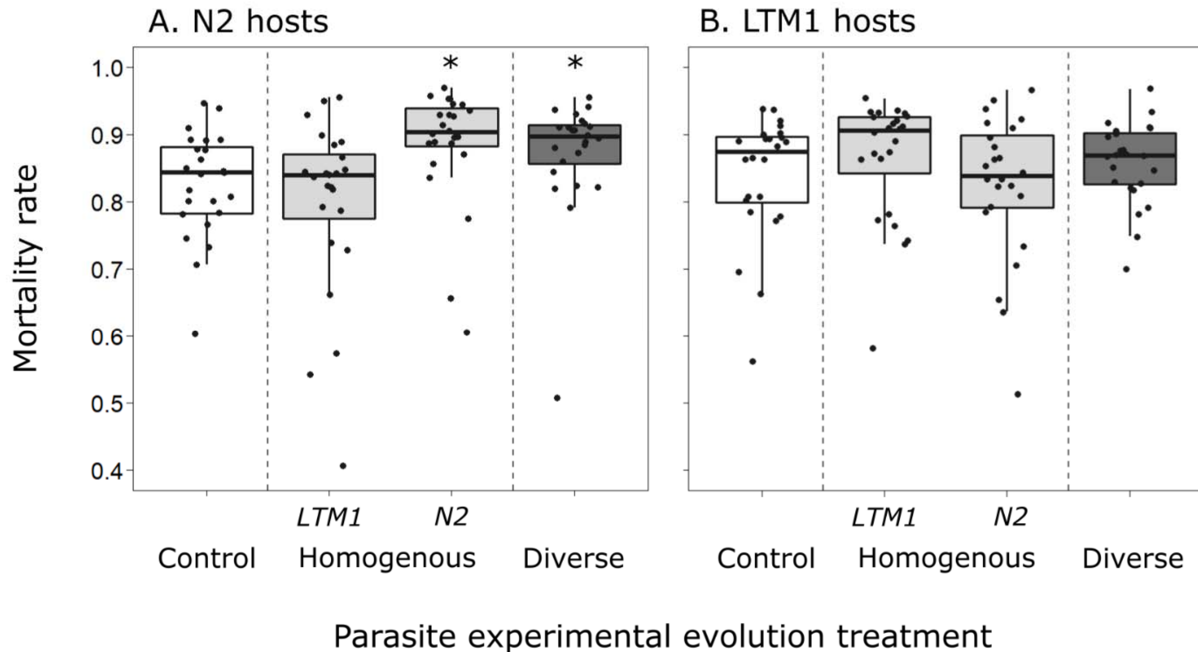

Figure 2: Virulence of experimentally evolved parasites on their sympatric host genotypes. The parasite *Serratia marcescens* was selected to kill *C. elegans* hosts in host populations that were homogeneous (100% LTM1; 100% N2) or diverse (50% LTM1: 50% N2). After 20 passages, we then tested evolved parasite lineages for their ability to kill N2 and LTM1 hosts. We compared the mortality rate of parasites against these hosts to that of control parasites, which were not selected to kill hosts and hence reflect baseline killing ability. (A) Parasites selected to kill hosts in populations that were diverse or homogeneous for N2 evolved an increased ability to kill N2 hosts, relative to control parasites or parasites selected to kill hosts in populations that were homogeneous for LTM1. (B) In contrast, parasites from different experimental evolution treatments did not differ in their ability to kill LTM1. Each box summarizes the results of 24 experimental replicates (4 replicates for each of 6 parasite lineages per treatment). Each point shows the mortality rate in a single experimental replicate, with 494 ± 26 (N2) or 498 ± 25 (LTM1) hosts tested per replicate.

275

276 *Adaptation to a novel host genotype*

277 We then evaluated the virulence of experimentally evolved parasites when paired with a
278 novel host genotype, JU1395. We initially predicted 1) an increase or maintenance of virulence
279 against the novel host genotype for parasites selected in diverse host populations and 2) a
280 decrease in virulence against the novel host genotype for parasites selected in homogeneous host
281 populations. Our results on adaptation to sympatric host genotypes subsequently suggested that

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282 support for these predictions would be strongest in comparisons of parasites selected on diverse
283 populations vs. populations homogeneous for N2.

284 The mortality rate of JU1395 was 95.6% when paired with the ancestral parasite
285 genotype. This mortality rate was slightly higher than the mortality rate of JU1395 when paired
286 with control parasite lineages after 20 experimental passages (93.6 ± 0.3% SEM) (Table S3).
287 When paired with experimentally evolved parasites, the mortality rate of JU1395 varied with
288 parasite selection treatment (Table 1C, Fig. 3).

289 We found support for our first prediction, that parasites selected in diverse populations
290 would maintain killing ability against a novel host genotype. Parasite lineages selected to kill
291 hosts in populations that were diverse showed an equivalent ability to kill JU1395 as control
292 parasites (94.1 ± 0.3%; coefficient = -0.12856, z = -0.836, p = 0.403).

293 We found partial support for our second prediction that parasites selected in
294 homogeneous host populations would lose killing ability against a novel host. Parasite lineages
295 selected to kill hosts in populations that were homogeneous for N2 showed reduced ability to kill
296 JU1395 hosts relative to control parasites (coefficient = 0.632, z = 4.075, $p < 0.001$) and to
297 parasites selected to kill hosts in diverse populations (coefficient = 0.761, z = 4.962, $p < 0.001$).
298 Specifically, the mortality rate of JU1395 was 86.1 ± 1.8% when paired with parasites selected to
299 kill hosts in populations that were homogeneous N2. Therefore, in relation to control or diverse-
300 selected parasites, survival of novel hosts was more than two-fold greater with parasites selected
301 in homogeneous N2 populations (vs. control: 2.19-fold greater; vs. 50%: 2.35-fold greater). In
302 contrast, the mortality rate of JU1395 was 92.5 ± 0.7% when paired with parasite lineages
303 selected to kill hosts in populations that were homogeneous LTM1. This did not differ
304 significantly from mortality rates of JU1395 hosts when paired with control parasites (coefficient

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305 = 0.093, z = 0.606, p = 0.544) or diverse-selected parasites (coefficient = 0.221, z = 1.442, p =
306 0.149). While counter to our prediction, this latter result corresponds to our finding that parasites
307 failed to evolve significantly increased virulence against LTM1 (Table 1B, Fig. 2B).

308 Consistent with our findings above, parasites selected in homogeneous N2 populations
309 showed the greatest between-lineage variation in performance on the novel host genotype.
310 Control parasites and diverse-selected parasites showed equivalent between-lineage variation in
311 their ability to kill the novel host, both in terms of number of survivors (coefficients of variation:
312 0.299, 95% CI [0.155,0.582] v. 0.312 [0.219,0.552], respectively) and mortality rate (0.020
313 [0.010,0.042] v. 0.020 [0.014,0.035]) (Table S4). For parasites selected in populations that were
314 homogeneous N2, between-lineage variation was substantially higher (number of survivors:
315 0.780 [0.622,0.965]; mortality rate: 0.126 [0.092, 0.169]). This variation arose from the fact that
316 virulence against JU1395 was very low for some lineages in this treatment (mortality rates: 69.5
317 ± 4.3%, 75.0 ± 4.8%) and high for others (92.9 ± 1.6%, 93.1 ± 2.5%, 93.4 ± 2.0%, 92.6 ± 1.8%).
318 For parasites selected in populations that were homogeneous LTM1, between-lineage variation
319 was somewhat elevated (number of survivors: 0.565 [0.413,0.779]; mortality rate: 0.046 [0.032,
320 0.065]), though not to the same extent as observed for parasites selected in homogeneous N2
321 populations.

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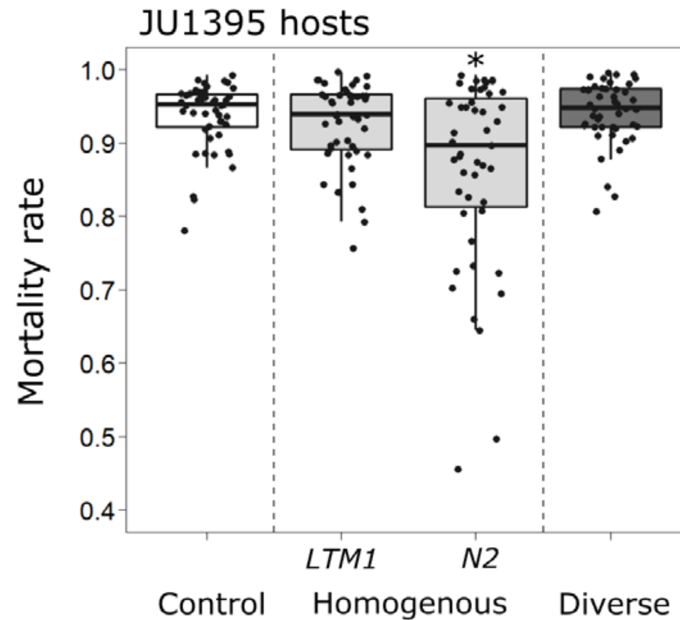

Figure 3: Virulence of experimentally evolved parasites on a novel host genotype. Here, we tested evolved parasite lineages for their ability to kill a novel host genotype, JU1395. Parasites selected to kill hosts in populations that were homogeneous for N2 (100% N2) lost their ability to kill the novel host (reduced mortality rate), consistent with specialization on the N2 genotype. In contrast, parasites selected to kill hosts in populations that were diverse (50% N2: 50% LTM1) maintained their ability to kill the novel host (equivalent mortality rate), consistent with the maintenance of generalism. Parasites selected to kill hosts in populations were homogeneous for LTM1 (100% LTM1) also showed no change in killing ability of the novel host, relative to control parasites. Each box summarizes the results of 48 experimental replicates (8 replicates for each of 6 parasite lineages per treatment). Each point shows the mortality rate in a single experimental replicate, with 270 ± 12 hosts tested per replicate.

323

324 Discussion

325 We set out to test the hypothesis that parasite host range evolves to match the variance of the
326 host environment. Specifically, we predicted that genetically diverse host populations select for
327 parasites that can infect a broad range of host genotypes, while genetically homogeneous host
328 populations select for specialist parasites. Prior studies have investigated the evolution of host
329 range under temporal variation in the host population, predominantly in virus-cell culture
330 systems. We complemented this prior body of work by testing spatial, rather than temporal,
331 variation in host diversity, in a eukaryotic host-parasite system. Consistent with our hypothesis,
332 selection in diverse host populations maintained a broad host range in parasite populations (Fig.

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333 3). However, selection in homogeneous host populations led to the evolution of specialist
334 parasites in only one of the two sympatric host genotypes (Fig. 2).

335 Selection in homogeneous and diverse host populations resulted in parasite populations
336 with increased virulence against the host genotype N2 (Fig. 2A). In fact, parasites selected in
337 diverse host populations increased in virulence to the same extent as parasites selected in
338 homogeneous N2 populations, as indicated by the statistically indistinguishable mortality rate of
339 hosts exposed to these different parasite populations. In the case of homogeneous N2-selected
340 parasites, increased killing of N2 coincided with a contraction of host range, as indicated by a
341 loss of killing against the novel host JU1395. In contrast, for diverse-selected parasites,
342 increased killing of N2 was accomplished without a contraction of host range: diverse-selected
343 parasite populations maintained genotypes with high killing ability and less between-lineage
344 performance in a novel host environment (Fig. 3). Our results suggest that genetic diversity of
345 the host population prevented the fixation of mutations that carry deleterious effects in alternate
346 host environments due to antagonistic pleiotropy or relaxed selection. We do not know the
347 extent of polymorphism in diverse-selected parasite lineages: they may be monomorphic
348 generalists or polymorphic, with some genotypes specialized on N2. These findings more
349 broadly suggest that parasite lineages from genetically diverse host populations will be more
350 likely to emerge in novel host populations.

351 Selection in homogeneous and diverse host populations did not result in parasite
352 populations with increased virulence against the host genotype LTM1 (Fig. 2B). This lack of
353 adaptation corresponded to the maintenance of a broad host range: homogeneous LTM1-selected
354 parasites showed no contraction of host range relative to control and diverse-selected parasites
355 (Fig. 3). Our experimental evolution may have provided insufficient time for adaptation to

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356 LTM1. Initially high rates of killing by ancestral parasites could have slowed fixation of
357 beneficial mutations if these are rarer in the LTM1 host environment than the N2 host
358 environment. Consistent with this hypothesis, changes in virulence against LTM1 qualitatively
359 matched the predicted changes, with slightly increased virulence of diverse-selected and
360 homogeneous LTM1-selected parasites relative to control and homogeneous N2-selected
361 parasites (Fig. 2). Compared to what we observed with adaptation to N2, these changes were
362 smaller in magnitude with more variation around the mean. Additional generations of selection
363 may produce stronger differentiation between treatments. We conclude that intrinsic differences
364 between these host genotypes altered the rate at which specialization evolved and thereby the
365 dynamics of emergence probability on novel host genotypes.

366 Prior studies of host range have similarly found that host range evolves differently
367 according to the host species encountered. Fellous et al. (2014) selected populations of the
368 spider mite *Tetranychus urticae* for growth on different plant species. They found variation in
369 host range according to the plant species: selection on rosebay produced mite populations with
370 consistent performance across plant species, while selection on tomato produced mite
371 populations with more variable performance. In Turner et al. (2010), adaptation to homogeneous
372 lines of human and canine cells reduced viral performance on novel cell lines for the human-
373 adapted viruses but not the canine-adapted viruses. They argue that performance in canine cell
374 lines is broadly correlated with performance in other host environments, such that a
375 homogeneous host environment can indirectly select for parasites with broad host range. Our
376 results suggest that the same argument may apply to host range evolution at the level of host
377 genotype.

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378 Much of our knowledge of host range evolution at the level of host genotype comes from
379 studies of coevolving bacteria-phage systems. These studies provide indirect support for the idea
380 that host populations that maintain genetic diversity select for parasites that can infect a broad
381 range of host genotypes: relative to bacteria or phage evolution alone, coevolution maintains
382 more diversity within bacterial host populations and selects for phages with broader host ranges
383 (Poullain et al. 2008; Hall et al. 2010). We prevented coevolution in our study by preventing
384 evolution of our host lines. Prior experimental coevolution studies in this system find that
385 coevolution can maintain diversity in host populations (Morran et al. 2011). Based upon our
386 results here, we then predict that, on average, parasite lineages passaged with coevolving host
387 populations will maintain broader host ranges than parasites serially passaged with host
388 populations that are homogeneous in space and time. Broadly, our results point to the
389 significance of the local host population, in terms of both the identity and diversity of host
390 genotypes present, in determining a parasite population's potential to shift to new hosts.

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