Title: The evolution of parasite host range in heterogeneous host populations

Running Title: Host heterogeneity and parasite evolution

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Abstract: Theory on the evolution of niche width argues that resource heterogeneity selects for niche breadth. For parasites, this theory predicts that parasite populations will evolve, or maintain, broader host ranges when selected in genetically diverse host populations relative to homogeneous host populations. To test this prediction, we selected the bacterial parasite *Serratia marcescens* to kill *Caenorhabditis elegans* in populations that were genetically heterogeneous (50% mix of two genotypes) or homogeneous (100% of either genotype). As predicted, parasite populations evolved a broader host range after 20 rounds of selection in heterogeneous populations: these populations gained virulence on experimental host genotypes and retained virulence on a novel host genotype. Host range shrank after selection in homogeneous populations of one experimental host genotype: these parasite populations gained virulence on this experimental host genotype and lost virulence on the novel host genotype. This result was not, however, repeated with selection in homogeneous populations of the second experimental host genotype: these parasite populations did not gain virulence on this host genotype and, accordingly, did not lose virulence on the novel host genotype. Our results indicate that host heterogeneity can maintain broader host ranges in parasite populations, which then have a greater potential to spread to new host populations. Individual host genotypes, however, vary in the degree to which they select for specialization in parasite populations.

Keywords: *Caenorhabditis elegans*, experimental evolution, generalist, genetic diversity, host heterogeneity, host range, mixture, monoculture, *Serratia marcescens,* specialist

Introduction

Genetic heterogeneity may reduce disease spread between hosts, resulting in lower parasite prevalence in genetically diverse host populations relative to genetically homogeneous host populations (rev. in Sherman et al., 1988, Mundt, 2002, King & Lively, 2012). This idea has garnered particular attention in agricultural systems, where polycultures can dramatically reduce disease levels and increase yield relative to monocultures (e.g. Zhu et al., 2000). A negative consequence of host heterogeneity has, however, received less attention: host heterogeneity should limit the spread of specialist lineages, maintaining parasite populations that are more damaging because they can attack a wider range of hosts (Groth, 1976, Lannou & Mundt, 1997). Here, we test this hypothesis by using experimental evolution to determine if parasite populations selected in heterogeneous host mixtures have a greater ability to infect a novel host genotype than parasite populations selected on a single host genotype.

This hypothesis stems from theory on the evolution of niche width. This body of theory argues that homogeneous environments select for narrow niche widths (i.e. a single specialist), while heterogeneous environments can maintain populations with larger niche widths (i.e. generalists, or a collection of specialists) (Levins, 1962, Pianka, 1966, Via & Lande, 1985, Lynch & Gabriel, 1987, Futuyma & Moreno, 1988). Specialization in a homogeneous environment is thought to arise from either antagonistic pleiotropy, where mutations that increase performance in the focal environment reduce performance in alternate environments (Rausher, 1984, Jaenike, 1990, Via, 1990), or conditional neutrality, where, for example, populations may acquire mutations that are neutral in the focal environment and deleterious in alternate environments (Schnee & Thompson, 1984, Fry, 1996, Whitlock, 1996). The probability of fixation of either of these types of mutation declines if individuals have a high

probability of encountering multiple environments, due to either temporal or fine-scale spatial heterogeneity. Experimental evolution studies of free-living systems support the maintenance of niche breadth under abiotic heterogeneity (Bennett et al., 1992, Reboud & Bell, 1997) (rev. in Kassen, 2002, Bono et al., 2017).

This body of theory has been extended to the evolution of host range in parasites. Substantial evidence now exists for the evolution of host specialization under temporal homogeneity: during serial passage, many parasites adapt to infect individual host species or genotypes and simultaneously decline in their ability to infect alternate hosts (Cunfer, 1984, Fry, 1990, Ebert, 1998). There is also evidence for the corollary, that heterogeneous host environments select for broader host ranges. This evidence derives from experimental evolution of viruses under extreme temporal variation in the host environment: broader host ranges evolve when viral lineages are alternated between cell lines of different host species (Weaver et al., 1999, Turner & Elena, 2000, Vasilakis et al., 2009, Turner et al., 2010, Coffey & Vignuzzi, 2011).

It is not clear how applicable these studies are to the subtler form of heterogeneity characterized by genetically diverse host populations, where host genotypes vary finely in space. One set of experimental evolution studies imposed spatial variation in host genotype, demonstrating that intraspecific host heterogeneity can limit viral specialization (Bono et al., 2013, Bono et al., 2015). A separate body of work has tested the association between host genetic diversity and parasite host range in the field. After seeing the potential for polycultures to reduce disease spread in agricultural fields, researchers set out to determine if this short-term benefit of polycultures would be counteracted by a long-term cost of selection in polycultures for crop parasites able to attack a wider range of host cultivars (Leonard, 1969, Groth, 1976,

Marshall, 1989, Lannou & Mundt, 1997). These concerns were supported by field studies of the powdery mildew fungus *Blumeria graminis*: parasite strains with broader host ranges reached higher prevalence in fields with mixtures of barley cultivars than in monocultures (Chin & Wolfe, 1984, Huang et al., 1991, Huang et al., 1994, Huang et al., 1995). Thrall and Burdon (2003) found a similar pattern in a wild plant-pathogen system: broader host ranges were found for isolates of the rust fungus *Melampsora lini* sampled from genetically diverse populations of *Linum marginale*. The combination of these experimental and field studies raises the questions: does genotypic heterogeneity of hosts alter the evolutionary trajectory of parasite populations, and what are the consequences for the emergence of disease on novel hosts?

Here, we build on these studies by testing the hypothesis that, relative to genetically homogeneous populations, heterogeneous populations of a metazoan host maintain bacterial populations that can kill a broader range of host genotypes. Beginning with a single genotype of *Serratia marcescens*, we used experimental evolution to select for increased virulence (i.e. killing rate) in populations of the nematode host *Caenorhabditis elegans* that varied in their composition. Some host populations were genetically heterogeneous (an even mix of two genotypes), creating fine-scale spatial variation in the host environment. Others were genetically homogeneous (one of two possible genotypes). We then compared the breadth of host ranges across treatments by evaluating the virulence of evolved parasite populations on a novel host genotype. We predicted that 1) parasite populations selected in heterogeneous host populations would maintain or increase in virulence on a novel host genotype, consistent with selection for a broad host range, and 2) parasite populations selected in homogeneous host populations would show reduced virulence on the novel host, consistent with specialization and limited potential for a host shift. We found mixed support for these predictions.

Materials and Methods

The raw data and R codes for statistical analyses are available in the Dryad Digital Repository (http://dx.doi.org/TBD) and the GitHub repository TBD.

Host and parasite genotypes

For experimental evolution, we used two genotypes of the nematode *Caenorhabditis elegans*: N2 and LTM1. Slowinksi et al. (2016) described the origins of the LTM1 line, which is a single genotype derived from ethylmethane sulfonate mutagenesis of the CB4856 genotype. We selected these two host genotypes for experimental evolution because 1) N2 and CB4856 are among the most genetically divergent genotypes within *C. elegans* (Barrière & Félix, 2005), and 2) preliminary assays demonstrated that the parasite *Serratia marcescens* is equally virulent to N2 and LTM1 (Fig. S1).

For surveying host range, we also included the host genotype JU1395. JU1395 is relatively divergent from both N2 and LTM1 (see Andersen et al., 2012, Cook et al., 2017 for phylogenies). Hence we limited the potential that genetic proximity alone would generate differences between parasites adapted to N2 vs. LTM1 in their virulence against JU1395. Assays with JU1395 allowed us to compare the host range of evolved parasite populations and their potential to spread to a new genotype. We subsequently refer to JU1395 as the novel host genotype and to N2 and LTM1 as sympatric host genotypes, because parasite lineages encountered one or both of these host genotypes during experimental evolution.

We initiated replicate parasite lineages from Sm2170, a genotype of the bacterial parasite *Serratia marcescens*. Sm2170 is known to be highly virulent towards *C. elegans* hosts (Schulenburg & Ewbank, 2004). The interaction of *C. elegans* and Sm2170 is a novel host-

parasite interaction constructed in the lab: there is no evidence that *C. elegans* encounters this particular strain of *S. marcescens* in the wild, and Sm2170 had not previously been experimentally evolved with *C. elegans*. Hosts acquire infection while feeding.

Parasite selection treatments

We established four treatments, each with six replicate parasite lineages (Fig. 1). In three of these treatments, we subjected replicate parasite lineages to selection for increased virulence (killing rate) against host populations that differed in their composition. In the first two treatments, parasites were selected to kill hosts in homogeneous host populations. These host populations comprised either 100% N2 or 100% LTM1. In the third treatment, parasites were selected to kill hosts in heterogeneous mixtures. These populations were 50% N2 and 50% LTM1. There is no indication of host choice in *S. marcescens*, so parasites passaged in heterogeneous host populations had an equal probability of encountering an N2 or LTM1 host each round of selection.

We did not allow for host evolution during experimental evolution. Hence, each passage, parasites were re-exposed to host populations of the same make-up as the prior round. We limited host evolution by maintaining stock populations of N2 and LTM1 at 15°C. Every few weeks, we refreshed these stocks by thawing hosts archived at -80°C. Our experimental treatments therefore limited temporal host heterogeneity in order to contrast spatial host heterogeneity with homogeneity.

The fourth treatment was the control treatment, where we did not directly select for increased virulence. We designed this treatment to serve as the baseline against which to measure evolutionary change in the prior three treatments. In this treatment, we passaged

bacteria without hosts; in doing so, we subjected bacterial populations to genetic drift and to non-focal selection pressures of the experiment in the absence of selection for increased virulence.

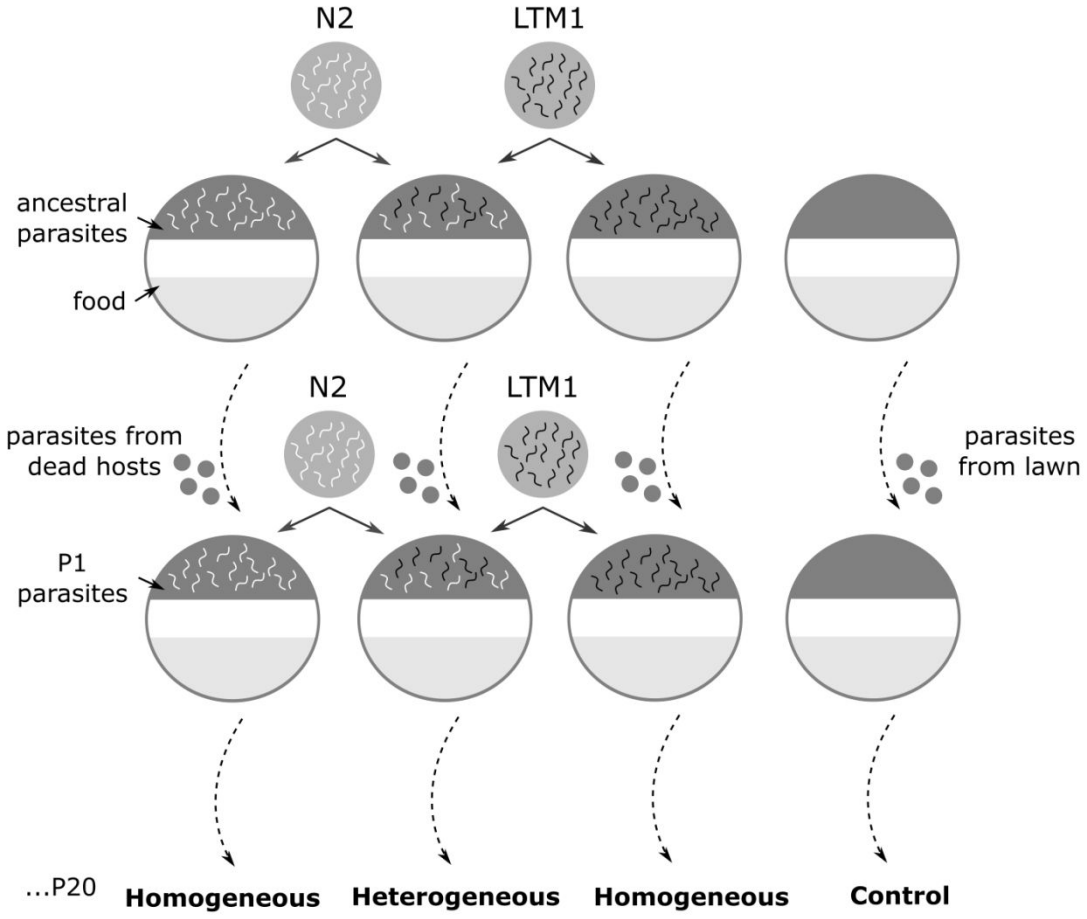

Figure 1: Experimental evolution scheme. We initiated experimental evolution by adding 500 *C. elegans* hosts to *Serratia* selection plates seeded with a lawn of Sm2170, the ancestral parasite genotype (dark lawn on upper portion of plates). For homogeneous selection, we added 100% N2 (left, white) or 100% LTM1 (right, black). For heterogeneous selection, we added 50% N2 and 50% LTM1. 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 selected 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.

Experimental evolution design

Selection was performed using *Serratia* selection plates, as in Morran et al. (2009) (Fig. 1). Under this design, we seeded 100 mm petri dishes of Nematode Growth Media (NGM-lite, United States Biological) with 35 uL of a liquid culture of bacterial parasites (*Serratia marcescens*) on one side of the plate and 35 uL of a liquid culture of food (*Escherichia coli*, strain OP50) on the other. Adding nematodes to the *Serratia* lawn forced interaction between hosts and parasites. Hosts could then migrate towards the lawn of food. We used this particular design in order to maintain the conditions of prior evolution experiments (Morran et al., 2009, Morran et al., 2011, Slowinski et al., 2016) and thereby facilitate comparison with their results.

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

We maintained these plates for 24 hours at 20°C. We then selected the most virulent parasites by isolating and transferring those that killed hosts rapidly, within 24 hours. To accomplish this, we picked 20-30 dead hosts from the Sm2170 lawn of each plate. We removed external bacteria from these hosts by repeated rinsing, then crushed the hosts to extract the internal bacteria that had killed them (Morran et al., 2011). We grew these bacteria on NGM-lite plates at room temperature (~22°C) for 48 hours, then maintained them at 4°C for 48 hours. We

then randomly selected 40 colonies from each plate for overnight growth in five mL of LB media at 28°C. These liquid cultures were used to produce the next round of *Serratia* selection plates, to which we added the same host population encountered by the parasite lineage in the prior passage.

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

The treatments outlined here are a subset of the treatments included in a larger experimental evolution scheme, which was not initially developed to address the focal questions of the study described here.

Survival assays of parasite virulence

We measured parasite virulence as the mortality rate of a host genotype after 48 hours of exposure to a parasite lineage. Virulence, or killing ability, served as a measure of parasite performance, because our experimental evolution design selected for parasites that killed rapidly. In setting up the assays, we replicated the experimental passaging scheme. For each host genotype tested, we added a fixed volume of L4 larvae (100% focal host genotype) to multiple replicate *Serratia* selection plates of all 24 parasite lineages. We determined the mean number of L4 larvae added to *Serratia* selection plates by adding this same volume to 10 standard plates seeded with OP50 and counting the number of hosts after 24 hours. We maintained *Serratia* selection plates at 20°C for 48 hours, then counted the number of live worms that had migrated

out of the *Serratia* lawn. The mortality rate was then obtained from the survival rate, which we calculated as the number of live hosts divided by the mean number added. We elected to calculate mortality rate this way, based upon the number of live hosts, because deep red, virulent *Serratia* strains (like Sm2170) obscure and rapidly degrade nematode carcasses, reducing the accuracy of mortality rates derived from counts of dead bodies.

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

Statistical Analyses

All statistical analyses were performed in R (ver. 3.5.3; R Core Team, 2013). We conducted three separate analyses, one for each host genotype tested in the survival assays, in order to compare the virulence of parasite lineages from different experimental treatments on a given host genotype. Statistical analyses with the N2 and LTM1 genotypes served to evaluate adaptation of parasite lineages to their sympatric host genotypes. The statistical analysis with the JU1395 genotype served to evaluate the host range of selected parasite lineages, by testing their ability to kill a novel host genotype. In our mortality assays with JU1395, we were able to assay twice as many replicates (n=8) as for the sympatric host genotypes N2 and LTM1 (n=4 replicates each). We first conducted the JU1395 analysis with the full eight replicates, then repeated the analysis with a random subset of four replicates. Halving the replicate number had no effect on the results, so we report the results of the analysis with the full eight replicates.

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

We then evaluated parasite selection treatment as a predictor of variation in the number of surviving hosts by using likelihood ratio tests to compare models with and without the treatment factor. For models in which treatment was a significant predictor of variation in survival, we examined model coefficients to compare between treatments. In analysis of sympatric host genotypes, we tested the prediction that parasite lineages evolved increased virulence against hosts with which they were passaged during experimental evolution. In analysis of the novel host genotype, we tested the prediction that parasite lineages selected in heterogeneous host populations would have higher virulence against a novel host than parasite lineages selected in homogeneous host populations, consistent with a larger host range for parasites selected in heterogeneous host populations.

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

Results

Adaptation to sympatric host genotypes

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

The mortality rate of N2 was 82.3% when paired with the ancestral parasite genotype. This closely matched the mortality rate of N2 when paired with control parasite lineages after 20 experimental passages (Table 1A). The mortality rate of N2 varied with parasite selection treatment (Table 2A, Fig. 2A). Consistent with our prediction, survival of N2 hosts declined by approximately a third from control levels when paired with parasites selected to kill N2 (see Table S1 for coefficients). Parasites selected in homogeneous N2 or heterogeneous populations had increased killing rates against N2, and N2 mortality rates did not differ between these two parasite selection treatments (GLMM, number of surviving hosts: coefficient = 0.19, z=1.417, p=0.157). In contrast, parasites selected in homogeneous LTM1 populations killed N2 at a rate equivalent to control parasites.

When paired with the ancestral parasite genotype, the mortality rate of LTM1 was 82.2%, identical to that of N2 hosts (Table 1, Fig. S1). This mortality rate was slightly lower than the mortality rate of LTM1 when paired with control parasite lineages after 20 experimental passages (Table 1B). Counter to our prediction, and in contrast to the results obtained for the N2 host genotype, the mortality rate of LTM1 did not vary significantly with parasite selection treatment (Table 2B, Fig. 2B). The changes in virulence qualitatively matched those observed with N2: parasites selected in homogeneous LTM1 or heterogeneous populations had slightly

272 increased killing rates against LTM1 relative to control parasites and parasites selected in
273 homogeneous N2 populations (Table S2).

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Table 1: Mean virulence of experimentally evolved genotypes on sympatric and novel host genotypes

			No. survivors		Mortality rate (%)	
Host genotype		**Parasite treatment**	*Mean*	*SE*	*Mean*	*SE*
A.	N2	Ancestor	87.25		82.33	
		Control	85.96	4.10	82.59	0.83
		100% LTM1	100.38	10.22	79.68	2.06
		Heterogeneous	62.96	3.67	87.26	0.74
		100% N2	57.17	6.67	88.43	1.35
		Total added	*494*	*26*		
B.	LTM1	Ancestor	88.50		82.23	
		Control	80.54	2.68	83.83	0.54
		100% LTM1	67.46	4.17	84.45	0.84
		Heterogeneous	70.75	3.11	85.79	0.63
		100% N2	88.17	7.48	82.30	1.50
		Total added	*498*	*25*		
C.	JU1395	Ancestor	11.75		95.65	
		Control	17.14	0.85	93.65	0.32
		100% LTM1	20.23	1.91	92.51	0.71
		Heterogeneous	16.01	0.83	94.07	0.31
		100% N2	37.59	4.89	86.08	1.81
		Total added	*270*	*12*		

We calculated the mean values for number of surviving worms and mortality rate by averaging the mean values obtained for the six parasite lineages per treatment, which were in turn obtained by averaging the values obtained for the four experimental replicates per lineage. Standard errors of the mean therefore reflect variation across the six parasite lineages (hence the lack of standard error for the ancestor, which is a single lineage). We calculated mortality rate as the number of living worms divided by the total added, which was calculated as the mean number of worms counted on 10 standard plates.

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Table 2: Parasite selection 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 selection treatment (homogeneous N2, homogeneous LTM1, heterogeneous, 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 parasite selection treatment as a predictor.

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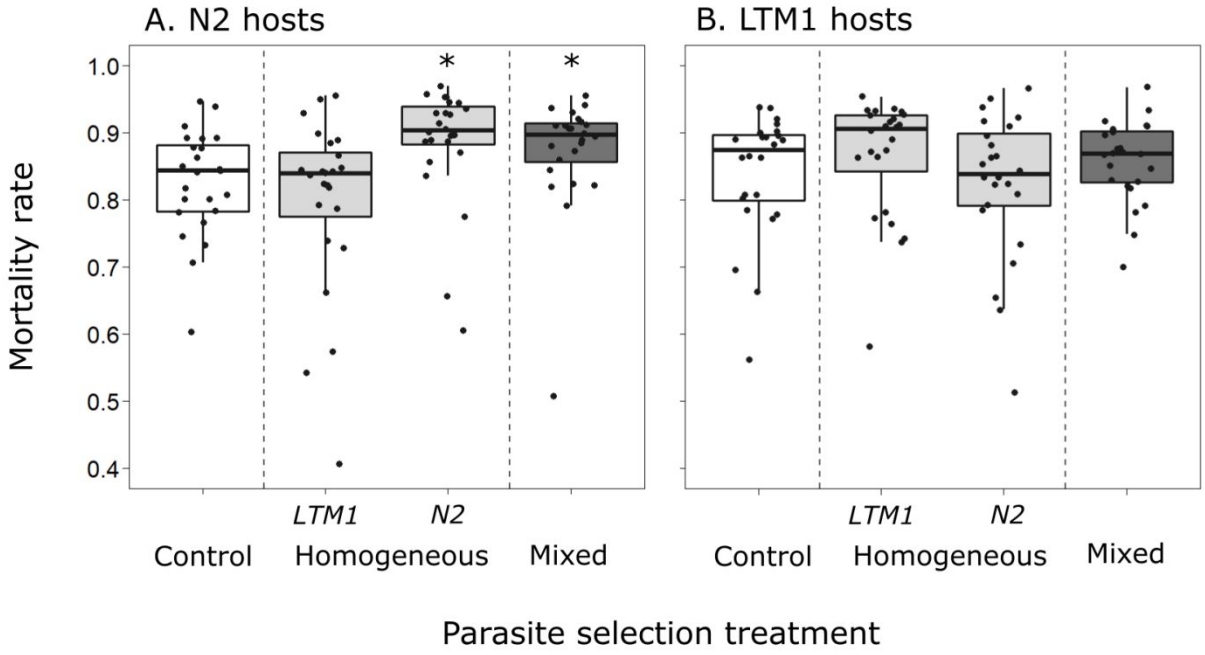

Figure 2: Virulence of experimentally evolved parasites on their sympatric host genotypes. The parasite *S. marcescens* was selected to kill *C. elegans* hosts in host populations that were homogeneous (100% LTM1; 100% N2) or heterogeneous (mixed: 50% LTM1: 50% N2). After 20 passages, we tested evolved 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 reflected baseline killing ability. (A) Parasites selected to kill hosts in populations that were heterogeneous or homogeneous for N2 evolved an increased ability to kill N2 hosts, relative to control parasites and parasites selected to kill hosts in populations that were homogeneous for LTM1. (B) In contrast, experimental selection did result in increased killing of LTM1 hosts relative to control parasites. 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.

Adaptation to a novel host genotype

We then evaluated the virulence of experimentally evolved parasites when paired with a novel host genotype, JU1395. We initially predicted 1) an increase or maintenance of virulence against the novel host genotype for parasites selected in heterogeneous host populations and 2) a decrease in virulence against the novel host genotype for parasites selected in homogeneous host populations. Our results on adaptation to sympatric host genotypes subsequently suggested that support for these predictions would be strongest for parasites selected on N2.

The mortality rate of JU1395 was 95.7% when paired with the ancestral parasite genotype. This mortality rate was slightly higher than the mortality rate of JU1395 when paired with control parasite lineages after 20 experimental passages (Table 1C). The mortality rate of JU1395 varied with parasite selection treatment (Table 2C, Fig. 3).

We found support for our first prediction, that parasites selected in heterogeneous populations would maintain virulence against a novel host genotype. Parasites selected in heterogeneous host populations showed an equivalent ability to kill JU1395 as control parasites (Fig. 3, Table S3).

We found partial support for our second prediction that parasites selected in homogeneous host populations would lose killing ability against a novel host. Parasite lineages selected in homogeneous N2 populations showed reduced ability to kill JU1395 hosts (Fig. 3). Compared to control or heterogeneous-selected parasites, survival of novel hosts was more than two-fold greater on parasites selected in homogeneous N2 populations (Table 1C). In contrast, parasites selected in homogeneous LTM1 populations killed JU1395 hosts at the same rate as control (Table S3) and heterogeneous-selected parasites (coefficient = 0.221, z=1.442, p=0.149.)

309 While counter to our prediction, this latter result aligns with our finding that parasites failed to
310 evolve increased virulence against LTM1 (Table 2B, Fig. 2B).

311 Consistent with our findings above, parasites selected in homogeneous N2 populations
312 showed the greatest between-lineage variation in performance on the novel host genotype.
313 Control and heterogeneous-selected parasites showed equivalent between-lineage variation in
314 their ability to kill the novel host, both in terms of number of survivors (coefficients of variation:
315 0.299, 95% CI [0.155,0.582] v. 0.312 [0.219,0.552], respectively) and mortality rate (0.020
316 [0.010,0.042] v. 0.020 [0.014,0.035]) (Table S4). For parasites selected in homogeneous N2
317 populations, between-lineage variation was substantially higher (number of survivors: 0.780
318 [0.622,0.965]; mortality rate: 0.126 [0.092, 0.169]). This variation arose from the fact that
319 virulence against JU1395 was very low for some lineages in this treatment (mortality rates: 70-
320 75%) and high for others (92-93%). For parasites selected in homogeneous LTM1 populations,
321 between-lineage variation was elevated (number of survivors: 0.565 [0.413,0.779]; mortality
322 rate: 0.046 [0.032, 0.065]), though not to the same extent as for parasites selected in
323 homogeneous N2 populations.

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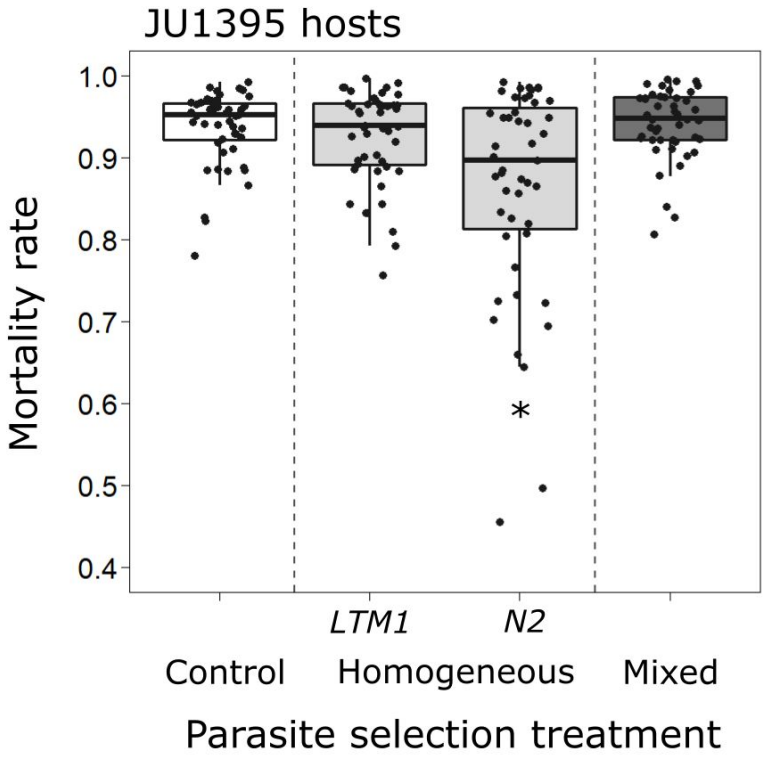

Figure 3: Virulence of experimentally evolved parasites on a novel host genotype. Here, we tested evolved parasite lineages for their ability to kill a novel host genotype, JU1395. Parasites selected to kill hosts in populations that were homogeneous for N2 lost their ability to kill the novel host (reduced mortality rate), consistent with specialization on the N2 genotype. In contrast, parasites selected to kill hosts in populations that were heterogeneous maintained their ability to kill the novel host, consistent with the maintenance of a broad host range. Parasites selected to kill hosts in populations were homogeneous for LTM1 also killed the novel host at the same rate as 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.

Discussion

We tested the hypothesis that parasite populations from genetically heterogeneous host populations maintain larger host ranges than parasite populations from homogeneous host populations. Consistent with our hypothesis, parasites selected in heterogeneous host populations had relatively high virulence against both sympatric and novel host genotypes (Fig. 2,3). Our results therefore provide some support for prior theoretical and empirical findings that heterogeneous host populations can select for more broadly damaging parasites (Groth, 1976, Chin & Wolfe, 1984, Thrall & Burdon, 2003, Bono et al., 2013). However, they also show that host homogeneity does not consistently limit parasite host range: selection for parasite specialization varied with host genotype (Fig. 2,3). Overall, these findings coincide with those of prior experimental evolution studies of host range, where viruses were alternated between host species, typically as cell lines (e.g. Weaver et al., 1999, Turner & Elena, 2000, Turner et al., 2010, Coffey & Vignuzzi, 2011). Our study of a bacterial parasite under genotypic heterogeneity of a whole-organism host thus serves to generalize the experimental study of host range evolution beyond viral systems.

Selection in homogeneous and heterogeneous host populations resulted in parasite populations with increased virulence against the host genotype N2 (Fig. 2A). In fact, selection in heterogeneous host populations increased virulence to the same extent as selection in homogeneous N2 populations, as indicated by the statistically indistinguishable mortality rate of N2 hosts exposed to these different parasites. In the case of homogeneous N2 selection, increased killing of N2 coincided with a contraction of host range, as indicated by a loss of killing against the novel host JU1395. In contrast, for heterogeneous selection, increased killing of N2 was accomplished without a contraction of host range: heterogeneous-selected parasite

populations maintained high killing ability and less between-lineage performance in a novel host environment (Fig. 3, Table S4). Our results suggest that host heterogeneity prevented the fixation of mutations that carry deleterious effects in alternate host environments. We do not know the extent of polymorphism following heterogeneous selection: parasite lineages may be monomorphic generalists or polymorphic, with some genotypes specialized on N2. Regardless, these findings suggest that host genetic heterogeneity maintains parasite populations that are more likely to emerge in novel host populations.

Selection in neither homogeneous nor heterogeneous host populations resulted in parasite populations with increased virulence against the host genotype LTM1 (Fig. 2B). This lack of adaptation corresponded to the maintenance of a broad host range: homogeneous LTM1-selected parasites showed no loss of virulence against JU1395 (Fig. 3). Our experimental evolution may have provided insufficient time for adaptation to LTM1. Initially high rates of killing by ancestral parasites could have slowed fixation of beneficial mutations, if these are rarer for LTM1 than for N2. Consistent with this hypothesis, changes in virulence against LTM1 qualitatively matched the predicted changes, with slightly increased virulence after heterogeneous and homogeneous LTM1 selection relative to control and homogeneous N2 selection (Fig. 2). Additional rounds of selection may produce stronger differentiation between treatments. We conclude that intrinsic differences between these host genotypes altered the rate at which specialization evolved and thereby the dynamics of emergence probability on a novel host genotype.

Prior studies have similarly found that host range evolves differently according to the host encountered (Flores et al., 2011, Fellous et al., 2014). After selection of *Tobacco etch potyvirus* on five ecotypes of *Arabidopsis thaliana*, Hillung et al. (2014) found substantial

variation in the host range of evolved virus lineages. Viral lineages evolved narrow host ranges on the most susceptible host genotypes and broader host ranges on the most resistant host genotypes. Our two host genotypes did not differ in susceptibility to ancestral parasites, so differences in initial host resistance cannot explain the different evolutionary trajectories for parasites selected on N2 vs. LTM1. In Turner et al. (2010), vesicular stomatitis virus (VSV) evolved reduced performance on novel cell lines following adaptation to human cells but not canine cells. They argued that performance in canine cell lines is broadly correlated with performance in other host environments, such that a homogeneous host environment can indirectly select for parasites with broad host range. Morley et al. (2016) also pointed to correlated performance across host cell lines for VSV lineages. Our results suggest that the same argument may apply to host range evolution at the level of host genotype.

Much of our knowledge of host range evolution at the level of host genotype comes from studies of coevolving bacteria-phage systems. These studies provide indirect support for the idea that host populations that maintain genetic diversity select for parasites that can infect a broad range of host genotypes: relative to bacteria or phage evolution alone, coevolution maintains more diversity within bacterial host populations and selects for phages with broader host ranges (Poullain et al., 2008, Hall et al., 2010). We prevented coevolution in our study by preventing evolution of our host lines. Prior experimental coevolution studies in this system find that coevolution can maintain diversity in host populations (Morran et al., 2011). Based upon our results here, we then predict that, on average, parasites passaged with coevolving host populations will maintain broader host ranges than parasites serially passaged with host populations that are homogeneous in space and time. Broadly, our results point to the

401 significance of the local host population, in terms of both the identity and diversity of host
402 genotypes present, in determining a parasite population's potential to shift to new hosts.
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Supplement

Statistical Analyses

Our analysis of variation in survival of LMT1 hosts produced a mixed-effects model with a singular fit. A singular fit can arise from overfitting. To address this problem, we treated parasite lineage as a fixed effect, as opposed to a random effect. We then re-fit the model as a negative bionomial regression using the glm.nb function in the package MASS (45). Treatment remained an insignificant predictor of variation in mortality of LTM1 ($D = 4.37$, $df = 3$, $p = 0.224$).

Supplemental Tables

Table S1: Coefficients of generalized linear mixed model of number of surviving N2 hosts following exposure to experimentally evolved parasite populations

	Coefficient	SE	z value	Pr(>|z|)
Intercept (Control)	4.454	0.155	28.746	<0.001
100% LTM1	0.068	0.138	0.494	0.621
Heterogeneous	-0.315	0.138	-2.287	0.022
100% N2	-0.514	0.140	-3.677	<0.001

We included parasite lineage (six independent lineages per experimental evolution treatment) as a random effect. The effects of parasite populations selected under homogeneous or heterogeneous host conditions are referenced against the control treatment.

Table S2: Coefficients of generalized linear mixed model of number of surviving LTM1 hosts following exposure to experimentally evolved parasite populations

	Coefficient	SE	z value	Pr(>|z|)
Intercept (Control)	4.378	0.117	37.312	<0.001
100% LTM1	-0.188	0.148	-1.268	0.205
Heterogeneous	-0.112	0.149	-0.756	0.450
100% N2	0.077	0.148	0.522	0.602

The model was constructed as described in Table S1.

Table S3: Coefficients of generalized linear mixed model of number of surviving JU1395 hosts following exposure to experimentally evolved parasite populations

	Coefficient	SE	z value	Pr(>|z|)
Intercept (Control)	2.866	0.153	18.792	<0.001
100% LTM1	0.093	0.153	0.606	0.544
Heterogeneous	-0.129	0.154	-0.836	0.403
100% N2	0.632	0.155	4.075	<0.001

The model was constructed as described in Table S1.

Table S4: Variation in virulence of experimentally evolved parasite lineages against novel host genotype JU1395

	No. survivors		Mortality rate	
Parasite treatment	*CV*	*95%CI*	*CV*	*95%CI*
Control	0.299	(0.155,0.582)	0.020	(0.010,0.042)
100% LTM1	0.565	(0.413,0.779)	0.046	(0.032,0.065)
Heterogeneous	0.312	(0.219,0.552)	0.020	(0.014,0.035)
100% N2	0.780	(0.622,0.965)	0.126	(0.092,0.169)

Coefficients of variation are calculated across the six parasite lineages within each treatment.

Supplemental Figures

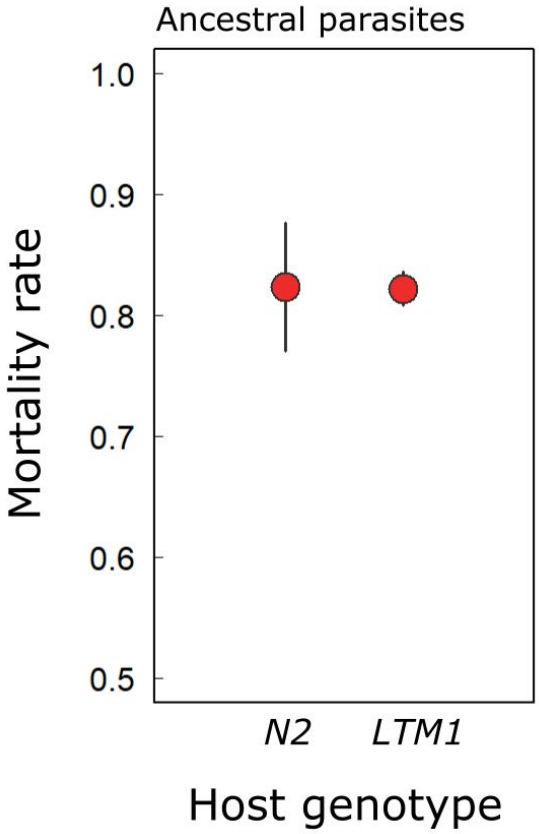

Figure S1: Virulence of ancestral parasites on experimental host genotypes. We selected the host genotypes N2 and LTM1 because they have the same mortality rate following exposure to ancestral Sm2170. Points show the mean mortality across four experimental replicates, and error bars show standard error of the mean.