Host heterogeneity mitigates virulence evolution

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Abstract

Parasites often infect genetically diverse host populations, and the evolutionary trajectories of parasite populations may be shaped by levels of host heterogeneity. Mixed genotype host populations, compared to homogeneous host populations, can reduce parasite prevalence and potentially reduce rates of parasite adaptation due to tradeoffs associated with adapting to specific host genotypes. Here, we used experimental evolution to select for increased virulence in populations of the bacterial parasite *Serratia marcescens* exposed to either heterogeneous or homogeneous populations of *Caenorhabditis elegans*. We found that parasites exposed to heterogeneous host populations evolved significantly less virulence than parasites exposed to homogeneous host populations over several hundred bacterial generations. Thus, host heterogeneity impeded parasite adaptation to host populations. While we detected tradeoffs in virulence evolution, parasite adaptation to two specific host genotypes also resulted in modestly increased virulence against the reciprocal host genotypes. These results suggest that parasite adaptation to heterogeneous host populations may be impeded by both tradeoffs and a reduction in the efficacy of selection as different host genotypes exert different selective pressures on a parasite population.

Introduction

Hosts and parasites are ubiquitous in nature. A long-standing goal in evolutionary biology is to understand the reciprocal selective pressures exerted by host and parasite interactions [1]. Theoretical and empirical studies point to multiple factors that can determine the rate and magnitude of parasite adaptation to hosts. These factors include host genetic heterogeneity [2,3], host spatial structure [4,5], competition [6,7], and migration and gene flow [8,9]. Of particular interest is how host genotypes influence the evolutionary trajectory of parasites populations as they adapt to host populations.

Historically, host heterogeneity has been overlooked in theoretical models of infection dynamics [10,11], yet host heterogeneity is both biologically relevant and a potential source of selection driving parasite evolution. Host homogeneity is generally rare in natural populations, even in many asexual hosts [12,13]. Theoretical models of host heterogeneity predict that specialization on similar host genotypes results in reduced transmission between dissimilar genotypes, which leads to lower parasite prevalence [3,14]. Due to this trade-off, parasite prevalence tends to be mitigated compared with homogeneous populations, known as the monoculture effect [15]. Evidence for the monoculture effect has been found in agriculture systems [16–19] and natural populations [20–27], in which prevalence differs between homogeneous and heterogeneous populations.

Heterogeneous populations may impede parasite adaptation and thus limit virulence. In some cases, host genetic diversity can even prevent parasite adaptation altogether [3]. Host diversity reduces the average rate at which parasites successfully infect hosts [28], thereby limiting specialization on a single host genotype. Here, we asked whether heterogeneity per se is sufficient to alter parasite evolution by examining virulence in populations with different ratios of host genotypes. Further, if homogeneity leads

to greater virulence, is there a cost of adapting to one specific genotype when parasitizing novel hosts, resulting in a fitness loss?

We used experimental evolution to select for virulence while passaging parasites through either genetically homogeneous or heterogeneous host populations. We predicted that heterogeneous host populations would impede virulence evolution and that parasites evolved in homogeneous host populations would evolve greater virulence by specializing on a single host genotype. Further, we expected to see a cost of specialization when infecting a new host genotype. To test these predictions, we evolved a clonal bacterial parasite, *Serratia marcescens* (Sm2170), in two genotypes of the host *Caenorhabditis elegans*. The *C. elegans* genotypes used were CB4856 and ewIR 68 [29]. The two strains have genetically diverse backgrounds but identical portions of chromosome V, where many innate immune loci reside. CB4856 and ewIR 68 were chosen to minimize tradeoffs of specialization as a means to better isolate heterogeneity as a variable. For our experimental treatments, we varied the ratio of the host genotypes in each host population. We then compared the mortality rates of the evolved parasites from each treatment to the ancestral parasites by infecting each host genotype separately.

Methods

(a.) Experimental Evolution

Using experimental evolution, we imposed selection for increased virulence on *S. marcescens* Sm2170 parasites exposed to either homogeneous or heterogeneous host populations. Hosts were the *C. elegans* strains ewIR 68 and CB4586. *S. marcescens* infection occurs upon feeding. Some live bacterial cells survive ingestion [30] and infect the host [31]. We measured virulence as infection-induced host mortality rate, and imposed selection for increased virulence by passaging Sm2170 only from hosts that died after 24 hpi (see electronic supplementary material, figure S1 for detailed experimental design). Thus, parasite genotypes that facilitated rapid killing were favored. We passaged Sm2170 populations through 5 different host treatments and a control in which parasites were passaged in the absence of hosts (*in vitro*, 0-0) (figure S2).

For each passage of experimental evolution, we plated 1,000 worms on a *Serratia* selection plate (figure S1) and allowed the worms to consume Sm2170 for 24 hours [32,33]. We then isolated 30 dead worms from the Sm2170 lawn. Dead worms were identified by a lack of movement in response to provocation with a platinum wire [34]. Then, we extracted Sm2170 from the hosts, cultured them in standard lab

conditions (28ºC shaker overnight), and inoculated an unseeded nematode growth media (US Biological, Salem, MA) plate to grow colony forming units (CFUs) for 48 hours at room temperature. From these plates, we randomly picked 40 CFUs per Sm2170 population, to inoculate the next passage. New naïve (non-evolved) hosts (from homozygous host lines kept at -80ºC) were then placed on the evolved bacteria and the process was repeated. For our *in vitro* control (0-0), 40 CFUs of Sm2170 were picked from the bacterial lawn. This treatment served as our control for passage conditions. The selection experiment concluded at the end of 10 passages (totaling hundreds of bacterial generations). At the end of each passage, a subset of the evolved bacteria was stored at -80ºC.

(b.) Mortality Assays

Mortality assays were used to determine virulence at the beginning and end of the experiment. Bacteria from passage 10 were used to infect homogeneous groups of either host genotype, and mortality rates were compared to the ancestral bacteria. The steps outlined in the creation of the *Serratia* selection plates were identical to those of the mortality assays (see figures S1-S2).

We placed 200 worms from one genotype on a mortality assay plate (figure S2, step 1). After 48 hours at 20ºC, the number of dead worms on each plate were counted (figure S2, step 2). Mortality rates were calculated as the proportion of dead worms divided by the number plated. When performing mortality assays, each replicate population had 3-6 technical replicates for a total of 360 mortality assay plates (figure S2, step 3). Ancestral mortality assays were performed both at the outset of the experiment and again when performing evolved Sm2170 mortality assays at the end of the experiment (figure S2 and S3).

Statistical Analysis

To assess mean changes in mortality rate between ancestral and evolved populations, we used JMP Pro 14 (SAS, Cary, NC) to perform a generalized linear model (GLM) with a link logit function and normal distribution. Factors in the model include treatment (e.g., homogeneous, heterogeneous, *in vitro*), host genotypes in mortality assays (ewIR 68 or CB4856), and the interaction. We did not detect overdispersion using a Pearson test. Post-analysis Tukey contrast tests were used to determine significance of pair-wise comparisons. We report our values as chi-squared statistics and corresponding p-values. Multiple comparisons were corrected for using a Bonferroni correction of $p < 0.025$ ($p < a/k$, where $a = 0.05$, $k = 2$ comparisons: host genotype and parasite treatment).

Results

The ancestral populations of Sm2170 bacteria tested at the beginning of experimental evolution produced a mean mortality rate of 49.51% (SEM ± 0.03) in host strain ewIR 68 and 64.32% (SEM ± 0.04) in host strain CB4856 [35]. As predicted, we found that selection for virulence resulted in an increase in mortality when experimental populations were assayed concurrently with the ancestral population. Parasites evolved in both homogeneous host populations were significantly more virulent than the *in vitro* controls (CB4856: $X_2 = 29.13$, $p < 0.0001$; ewIR 68: $X_2 = 14.68$, $p = 0.0001$, figure 1, table S2-S3). Parasites evolved in CB4856 hosts had a 29% increase in mortality rate in CB4856 populations compared to the ancestor, while parasites evolved in ewIR 68 had a 19% increase in mortality rate in ewIR 68 populations compared to the ancestor.

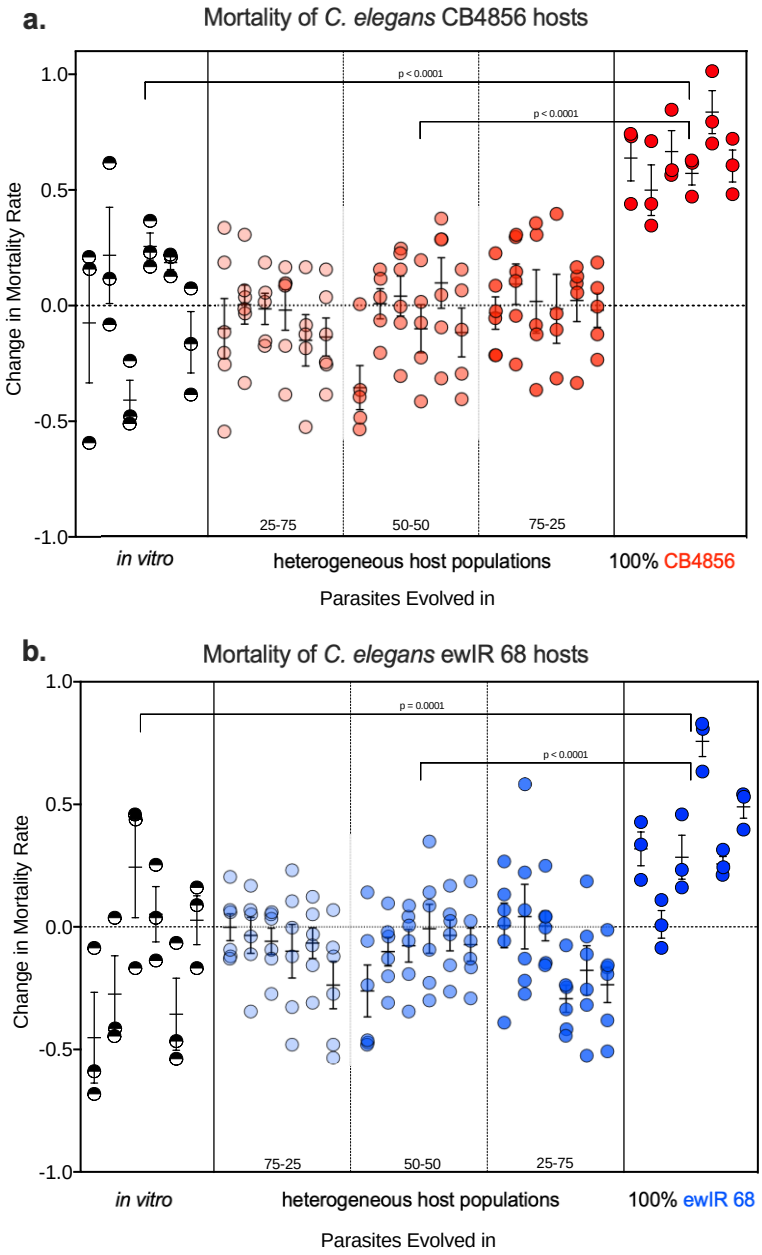

Figure 1. (a,b) Mean change in host mortality rate relative to the ancestral para- sites in C. elegans host strains CB4856 (a) and ewIR 68 (b). All experimental populations shared a common ancestor, and thus, any change from the ancestral data is indicative of relative virulence. Parasites were evolved in heterogeneous host populations, homogeneous host populations or in vitro (no hosts), and then tested for changes in virulence. The heterogeneous populations, from left to right, are 75– 25, 50–50 and 25–75. Circles represent the mean change within each technical replicate (18–36 each). Bars represent ± s.e.m. (Online version in colour.)

There were no significant differences in mortality induced by parasites in either host between any of the pairs evolved on 75-25, 50-50, or 25-75 (figure 1). When tested in CB4856 hosts, parasites evolved in heterogeneous host populations did not differ significantly in mortality rate from the *in vitro* parasites (X_2 = 0.0023, p = 0.96, figure 1*a*, table S2), indicating little to no adaptation to the CB4856 host

genotype. Further, the same parasites exhibited no significant increase in mortality rate compared to *in vitro* parasites when tested in ewIR 68 hosts (X_2 = 0.00002, p = 0.99, figure 1*b*, table S3). Parasites evolved in homogeneous ewIR 68 populations caused greater virulence in ewIR 68 than parasites evolved in heterogeneous populations (X_2 = 18.37, p < 0.0001, figure 1*b*, table S3). Additionally, parasites evolved in homogeneous CB4856 populations caused greater virulence in CB4856 hosts than parasites evolved in heterogeneous populations (X_2 = 52.99, p < 0.0001, figure 1*a*, table S2). Overall, these results demonstrate that host heterogeneity impedes parasite adaptation relative to host homogeneity.

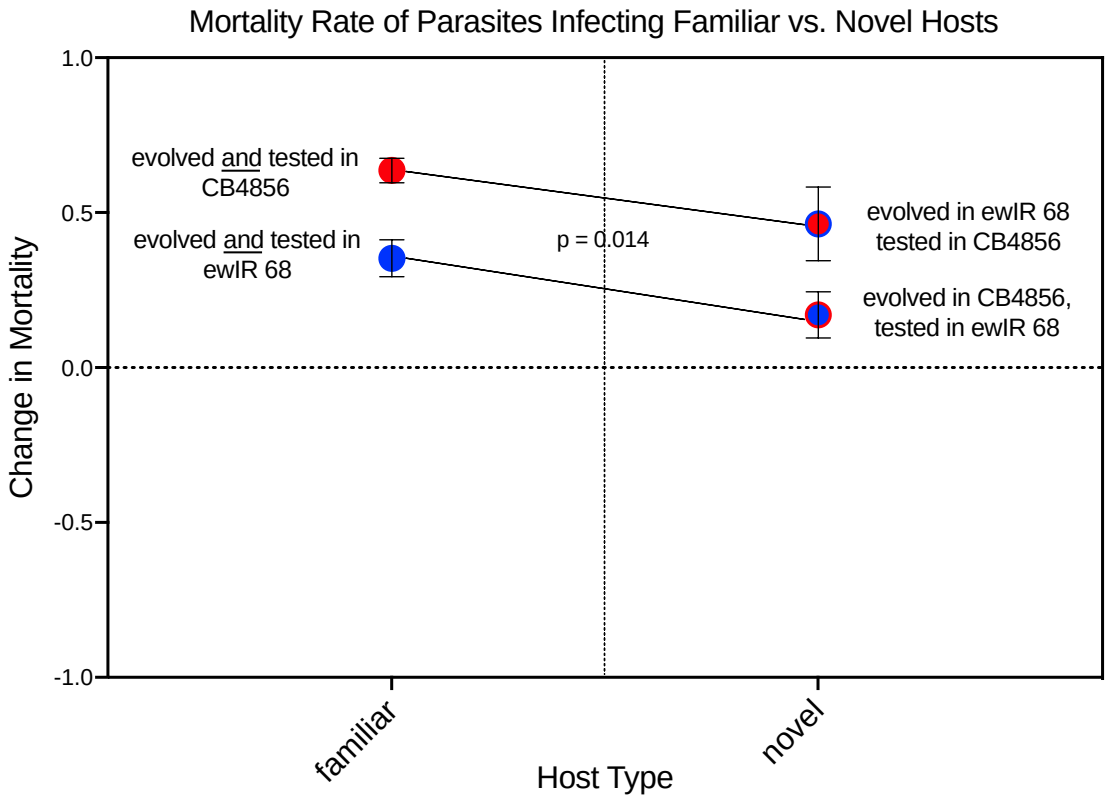

Figure 2. Each dot represents the treatment's average change in mortality rate of all populations and replicates relative to ancestral parasites. All experimental populations shared a common ancestor, and thus, any change from the ancestral data is indicative of relative virulence. The x-axis shows the type of host infected: either familiar to the parasite or novel. The p-value is based on a post-GLM Tukey contrast test between all familiar hosts (left panel) and all novel hosts (right panel) (χ^2 = 6.04, p = 0.01). In both cases, although all treatments had an increased mortality rate relative to the ancestor, novel hosts had a lower mortality rate than do the familiar hosts. Bars around mean represent s.e.m. (Online version in colour.)

Next, we determined if parasites that were evolved on homogeneous hosts and then exposed to a novel host exhibited reduced virulence, and thus lowered fitness, as predicted by trade-off theory. In both cross-infections there was an increase in mortality rate relative to the ancestral strain and relative to the *in vitro* controls (figure 2, table S1). Further, cross-infections were significantly different from one

147 another (X_2 = 6.04, p = 0.014, figure 2, table S1), indicating that although parasites caused high
148 mortality in novel hosts, they did not increase to the same extent as parasites in familiar hosts. Despite
149 this difference, the result overall is in accordance with the previous finding: that heterogeneous host
150 populations limit the evolution of parasite virulence and indicate a trade-off imposed by host
151 heterogeneity.
152
153 **Discussion**
154
155 In our selection regime, higher host mortality equates to higher virulence, and thus greater parasite
156 fitness. Our results show that parasites selected in homogeneous host populations evolved substantial
157 increases in virulence when infecting those same hosts (for both ewIR 68 and CB4856) as compared to
158 *in vitro* controls (figure 1). However, parasites that were selected in mixed genotype host populations
159 and then tested on homogeneous host genotypes exhibited limited increases in virulence (figure 1),
160 despite strong selection favoring increased virulence. We found no differences in the mortality rates of
161 hosts infected by parasites evolved with any mixed host population on either host – neither comparing
162 between each mixed treatment nor compared with the *in vitro* control. Thus, exposure to heterogeneous
163 host populations impeded virulence evolution relative to exposure to homogeneous hosts. Further,
164 parasites evolved in homogeneous populations and then used to cross-infect the other (novel) host
165 genotype exhibited smaller increases from the ancestral virulence than when infecting their familiar host
166 (figure 2). Therefore, we observed tradeoffs in virulence due to specialization on the parasites' familiar
167 host genotype.
168
169 Tradeoffs in parasite virulence due to specialization on a particular host genotype are often invoked as a
170 reason that heterogeneous host populations may impede parasite adaptation. Here, we found that
171 heterogeneous host populations impeded the evolution of parasite virulence and we found evidence of
172 tradeoffs in parasite virulence (figure 1). However, the evolved tradeoffs in parasite virulence that we
173 observed are not sufficient to explain the limited virulence evolution in parasites evolved with
174 heterogeneous host populations. Despite parasite specialization (greater virulence) on familiar
175 homogeneous hosts, parasites evolved in homogeneous host populations still exhibited increased
176 virulence against novel hosts relative to the *in vitro* control parasites (figure 2). Therefore, any potential
177 cost of a tradeoff should have been mitigated in the heterogeneous host populations, as adaptation to
178 either host genotype still resulted in increased virulence against the other host genotype. Yet, we still

observed a limited response to selection for increased virulence in parasites evolving in heterogeneous host populations (figure 1).

One possibility for the lack of a substantial tradeoff cost (i.e. a decline in parasite fitness) may be that the *C. elegans* genotypes used, CB4856 and ewIR 68, share an identical region of chromosome V, which harbors loci associated with innate immunity [36]. It is likely that parasites evolved in either genotype were under strong selection to evolve in response to that particular region of the genome. Despite the genetic similarity of the strains at many innate immune system loci, heterogeneous populations still impeded parasite adaptation relative to homogeneous populations. While it is plausible that tradeoffs in virulence slowed parasite adaptation in the heterogeneous host populations to some degree, tradeoffs alone are insufficient to explain the lack of increase in virulence exhibited by heterogeneous-selected parasites when infecting CB4856 hosts (figure 1). We hypothesize that this lack of response to selection was likely driven by a reduction in the efficacy of selection in the heterogeneous host populations relative to the homogeneous hosts. Selection imposed by different host genotypes can act on different groups of loci in the parasite genome [37]. As a result, the efficacy of selection on a particular set of loci in the parasites may be reduced in the heterogeneous hosts as parasites encounter different host genotypes with each infection [38]. Although a portion of our host genomes were identical, the diverse genetic backgrounds of the CB4856 and ewIR 68 strains may have imposed fluctuating selection on the parasite populations, resulting in limited parasite adaptation within heterogeneous host populations. Another possibility is that specialization on a single host, as opposed to a generalist strategy, may lead to a stronger strength of selection over time. Thus, our results at passage 10 may be the result of stronger selection in homogeneous populations as specialization increases [39].

Heterogeneous host populations are shown to be common in nature [40–44], and our results demonstrate that heterogeneity can alter the trajectory of parasite evolution. Importantly, parasites are capable of adapting to heterogeneous host populations [45]. Nonetheless, our results indicate that parasite adaptation can be impeded by heterogeneous relative to homogeneous host populations. While we observed little cost to host specialization in our experiment, tradeoffs are likely to impede rates of parasite adaptation in heterogeneous host populations [46]. We anticipate that changes in the efficacy of selection imposed by heterogeneous host populations may also contribute to reduce rates of parasite adaptation. Therefore, we believe it is critical to understand the implications of host heterogeneity for disease evolution. The ability to manage parasite virulence in both human infectious diseases, agriculture, and in the conservation of wildlife has long been a goal of research on parasite evolution

[47]. Our results indicate that increasing host heterogeneity may not only be useful for decreasing disease prevalence and spread, but also for hindering parasite adaptation and virulence evolution.

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