Title:

An experimental test of parasite adaptation to common vs. rare host genotypes

Running title:

Parasites on rare vs. common hosts

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Abstract

In adapting to specifically infect common host genotypes, parasites impose negative frequency-dependent selection that favors rare host genotypes. This parasite-mediated advantage of rarity is key to the idea that parasites maintain genetic variation and select for outcrossing in host populations. Here, we report the results of an experimental test of parasite adaptation to common vs. rare host genotypes. We selected on the bacterial parasite *Serratia marcescens* to kill *C. elegans* hosts in uneven mixtures of host genotypes. To examine the effect of commonness itself, independent of host identity, each of four host genotypes was represented as common or rare in experimental host mixtures. After experimental selection, we evaluated a parasite line's change in virulence, the selected fitness trait, on its rare and common host genotypes. Our results were consistent with a slight advantage for rare host genotypes: on average, parasites lost virulence against rare genotypes, but not against common genotypes. The response varied substantially, however, with distinct patterns across host genotype mixtures. These findings support the potential for parasites to impose negative frequency-dependent selection, and they emphasize that the cost of being common may vary with host genotype.

Keywords: coevolution, negative frequency-dependent selection, Red Queen hypothesis, experimental evolution**,** *Serratia marcescens, Caenorhabditis elegans*

Introduction

Models of host-parasite coevolution often find, or assume, that parasites adapt to infect locally common host genotypes. It is an intuitive idea: selection to exploit frequently encountered resources should be much stronger than selection to exploit rare resources (Whitlock 1996). This process explains why parasites are thought to impose negative frequency-dependent selection: the fitness of a host genotype should decline as it becomes common, because it becomes more infected than the population mean. In contrast, parasites fare poorly against rare hosts, which should increase in frequency. Hence this targeting of common over rare host genotypes lies at the heart of major evolutionary hypotheses, including the Red Queen hypothesis and parasite-mediated maintenance of genetic diversity (Bell 1982; Haldane 1949; Hamilton 1980; Hamilton et al. 1990; Hutson and Law 1981; Jaenike 1978).

In strong support of this process, Chaboudez and Burdon (1995) found that, in 13 of 16 populations, the most common clone of rush skeletonweed (*Chondrilla juncea*) was the only clone or one of just two clones infected by the rust fungus *Puccinia chondrillina.* Infection of the most common clone in a single population would be expected by random chance; what is remarkable is the replication of this finding across multiple populations with distinct clones. This study, however, is one of very few reported instances of frequency-dependent infection (see also Lively and Dybdahl 2000). Perhaps this dearth reflects the variety of possible manifestations of parasite adaptation in natural populations. With parasite-mediated changes in host genotype frequencies and time lags in parasite adaptation, ongoing parasite adaptation can generate different relationships between host frequency and infection or susceptibility over time (Wolinska and Spaak 2009).

Holding the host population constant reveals that parasites can rapidly specialize on individual host genotypes. Experimental evolution studies have clearly shown that parasites will adapt to specifically infect an individual host genotype after serial passage in homogeneous

populations of the host (e.g. Little et al. 2006; Nidelet and Kaltz 2007; Yourth and Schmid-Hempel 2005). There is limited evidence, however, that parasites target common host genotypes during serial passage in genetically heterogeneous host populations, like those parasites encounter in the wild. The results of Koskella and Lively (2009) are suggestive: over five generations of experimental coevolution, the trematode *Atriophallophorus winterbourni* (formerly *Microphallus* sp.: Blasco-Costa et al. 2019) evolved to specifically attack the most common clone in a diverse population of the snail *Potamopyrgus antipodarum*. The next critical step is to determine if this result is repeatable across different host genotypes: in heterogeneous host populations, parasites should evolve to attack common over rare host genotypes, regardless of the host genotypes in question.

Here, we used experimental evolution to compare adaptation of parasites to common and rare host genotypes in experimental host populations that varied in the identity and frequency of the common and rare host genotypes. We assembled genetically heterogeneous host populations by mixing two genotypes of the nematode *Caenorhabditis elegans* such that one genotype was common (90% or 75%) and one rare (10% or 25%). We repeated this across different combinations of genotypes, so four genotypes were represented as both rare and common in experimental populations. Beginning with a single genotype of the bacterial parasite *Serratia marcescens*, we selected for increased virulence (i.e. host mortality) against hosts in these mixed populations for 10 or 20 rounds of selection, accounting for several hundred bacterial generations. Our results suggest that parasites confer a slight advantage on rare host genotypes.

Methods

Heterogeneous host populations

We assembled heterogeneous host populations using four genotypes of *C. elegans*: the canonical divergent lab genotypes, N2 and CB4856, plus LTM1 and ewIR68 (Doroszuk et al. 2009; Gibson et al. 2020). The ancestral bacterial strain Sm2170 showed substantial variation in virulence against these genotypes, with mortality far higher for N2 and LTM1 than CB4856 and ewIR68 (Fig. S1) (Gibson et al. 2020; White et al. 2019). We established six host heterogeneity treatments (Fig. 1). In two treatments, N2 was common (90% or 75%) and LTM1 rare (10% or 25%). Two additional treatments reversed these frequencies, with LTM common and N2 rare. The final two treatments paired CB4856 and ewIR68 at 75%:25% and 25%:75%. These experimental treatments are a subset of the treatments included in two larger experimental evolution studies (Gibson et al. 2020; White et al. 2019), which demonstrated that Sm2170 can adapt to specifically attack these host genotypes when selected in homogeneous host populations (Fig. S2). As a result, the LTM1/N2 treatments differ from the CB4856/ewIR68 treatments in the duration of experimental evolution and the number of technical replicates in mortality assays. For this study, we do not draw comparisons between treatments that differed in mixing frequency (90:10 vs. 75:25). For our purposes, these treatments served only as alternate approaches to establishing host mixtures with a rare and a common host genotype.

Experimental evolution

Our experimental design imposed selection for increased virulence (i.e. parasite-induced host mortality) in heterogeneous host populations by passaging those parasites that killed hosts rapidly (Fig. 1) (based on Morran et al. 2011; Morran et al. 2009). We established the ancestral parasite lineage from a single colony of Sm2170, a genotype of *S. marcescens* that is virulent to *C. elegans*. We then subjected this lineage to 10 (CB4856/ewIR68) or 20 (N2/LTM1) rounds of selection. Each round of selection, we added ~1000 (for CB4856 and ewIR68 mixtures) or ~500 (for N2 and LTM1 mixtures) L4 larvae to the parasite lawn of a *Serratia* selection plate. After

24 hours, we selected 20-30 dead hosts from the parasite lawn and extracted their parasites. We cultured these bacteria and randomly selected 40 colony-forming units per experimental line to establish the parasite lawn for the next round of selection. The selection process was repeated by adding naive hosts to this lawn. We prevented evolution in the host lines by continually thawing stock collections archived at -80°C. Experimental selection produced 36 independent parasite lines (6 replicate lines x 6 heterogeneity treatments), which were frozen at -80°C.

Mortality assays

Our experimental evolution design selected for parasites that killed their hosts rapidly, so we measured the response to selection by assaying the mortality of hosts when paired with experimentally evolved parasites. Generation 10 parasite lines (for CB4856/ewIR68) and generation 20 parasite lines (for N2/LTM1) were tested against homogeneous groups of both the rare and the common host genotype with which they were evolved. The ancestral parasite population was also tested against homogeneous groups of each host genotype.

We constructed mortality assay plates as described for selection plates during experimental evolution (Fig. 1). A known number of hosts was added to the parasite lawn of a plate and incubated at 20°C. At 48 hours post exposure, we counted the number of surviving hosts on the plate and from this calculated the proportion of dead hosts (as in Gibson et al. 2020; Penley et al. 2017; White et al. 2019). For each of the 24 parasite lines in the N2/LTM1 pairing, ~500 hosts were added per mortality assay plate, with 8 technical replicates (four per each of two independent assays). For each of the 12 parasite lines in the CB4856/ewIR68 pairing, ~200 hosts were added per mortality assay plate, with six technical replicates. Virulence of the ancestral parasite was assayed with six (CB4856/ewIR68) or twelve (N2/LTM1) technical

141 replicates. The difference in replication arose because these data were collected as part of two
142 larger experimental evolution projects.

143 *Statistical analysis*

144 We conducted all statistical analyses in R v.3.6.0 (R Core Team 2013) and plotted data using
145 ggplot2 (Wickham 2016). To compare adaptation of parasites to common vs. rare host
146 genotypes, we examined the change in virulence (i.e. change in proportion of dead hosts) from
147 the ancestral mean for each host genotype. Using the packages lme4 (Bates et al. 2015) and afex
148 (Singmann et al. 2019), we fit a linear mixed model to the change in virulence for each mortality
149 assay replicate. To account for the structure of the experiment, we specified random effects for
150 mortality assay date (3 separate blocks), parasite line (1-6), and experimental treatment (six
151 combinations of host genotypes and frequencies, Fig. 1), with line nested in treatment. The
152 predictor variables were the host genotype assayed (N2, LTM1, ewIR68, or CB4856), the
153 frequency of the assayed host during experimental evolution of the parasite line being tested
154 (rare or common), and an interaction of frequency and assayed host genotype.

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159 **Results**

160 After experimental selection of parasites in uneven host mixtures, we evaluated adaptation to
161 rare and common host genotypes by measuring change from the ancestor in the focal fitness trait,
162 virulence. We found different patterns of virulence evolution across host mixtures. In two cases,

parasites lost virulence against rare host genotypes: averaged across replicate parasite lineages, parasite-induced mortality declined 8.78 ± 3.27% (SEM) from the ancestor on rare genotype ewIR68 and 3.86 ± 3.85% on rare genotype N2, with no change against the respective common genotypes CB4856 (1.00 ± 1.70%) and LTM1 (1.35 ± 1.87%) (Fig. 2A,2C). In another case, parasites gained virulence against the common host genotype: mortality increased 4.71 ± 1.80% from the ancestor on the common genotype N2, with no change against the rare genotype LTM1 (0.36 ± 2.62%) (Fig. 2D). In the final case, parasites lost virulence against both the rare (CB4856: -6.57 ± 5.72%) and the common (ewIR68: -11.42 ± 5.72%) host genotypes (Fig. 2B).

To test the focal hypothesis, we evaluated the effect of host frequency on virulence evolution independent of the host genotypes in question. We found that a host genotype's frequency (rare/common) during experimental selection of a parasite line was an important predictor of virulence evolution ($F=9.547$, $df=1$, $p=0.002$) (Table S1B). Specifically, change in virulence from the ancestor was reduced on host genotypes that had been rare during selection, relative to host genotypes that had been common (coefficient: -0.031, SE=0.010, $p=0.002$) (Table S1C). In spite of the differences in virulence evolution noted above, we did not find that the effect of host genotype frequency varied significantly with the assayed host genotype (insignificant interaction: $F=0.587$, $df=3$, $p=0.680$) (Table S1A), so we dropped this term from the model. The difference between rare and common hosts was slight: on average, the mortality of rare host genotypes declined -3.73 ± 1.74% from the ancestor, while mortality of common host genotypes was unchanged (0.28 ± 1.55%).

Discussion

We set out to test the critical idea that parasites evolve to target common host genotypes over rare host genotypes. We aimed to test the effect of frequency itself, independent of host identity

and initial resistance level. Hence, we used multiple combinations of host genotypes that varied in their resistance to ancestral parasites. Host genotypes were represented as both rare and common across experimental host populations (Fig. 1). Overall, our results are consistent with a slight, parasite-mediated advantage of rare host genotypes (Fig. 2): the mean evolutionary change from the ancestor in the focal fitness trait, virulence, was lower when parasite lines were tested on their rare host genotype than on their common host genotype. On average, virulence was maintained against common host genotypes but declined against rare host genotypes.

This effect did not vary significantly with the host genotype in question: the interaction of assayed host genotype and frequency did not contribute to variation in virulence evolution (Table S1). Nonetheless, there is obvious variation in the relative performance of parasite lines on rare vs. common host genotypes (Fig. 2). Most notably, for the host mixture of CB4856 (rare) and ewIR68 (common), parasites evolved reduced virulence against both rare and common hosts (Fig. 2B). Among the genotypes tested, the CB485 and ewIR68 host genotypes are the most resistant to infection (Fig. S1), with the common genotype, ewIR68, the most resistant of the group. It is plausible that small parasite effective population sizes due to high host resistance and reduced efficacy of selection to attack a single host genotype resulted in the rapid fixation of deleterious mutations in these parasite populations. Parasites can substantially increase in virulence when selected in homogeneous populations of CB4856 and ewIR68 (Fig. S2), but mixing them at a range of frequencies severely limits the response to selection (White et al. 2019). Regardless of the explanation, this outlier response suggests that host genetic background determines if, and to what extent, parasites evolve to target common vs. rare hosts.

Our results support the idea that, on average, rare genotypes have an advantage over common genotypes in the presence of parasites, but the differences we observed were small. The mean proportional change in virulence (i.e. change in host mortality from the ancestor)

against a common host genotype exceeded the change for its paired rare host genotype by only ~four percentage points. The Red Queen hypothesis and related ideas rest upon the idea that parasite adaptation to common host genotypes confers a fitness advantage on rare host genotypes that is substantial enough to drive rapid oscillations in host genotype frequency and counterbalance the cost of outcrossing (Howard and Lively 1994; May and Anderson 1983). In support of the Red Queen, coevolving *S. marcescens* prevents the invasion of selfing lineages into obligately outcrossing populations of *C. elegans* (Morran et al. 2011; Parrish et al. 2016; Slowinski et al. 2016). This consistent pattern suggests that *S. marcescens* targets common, selfed genotypes, giving rare, outcrossed genotypes a fitness advantage that outweighs the costs of outcrossing. Those studies used the same design and similar genetic backgrounds as ours. Yet, given that ancestral virulence was relatively even between paired host genotypes (Fig. S1), the average rare-common differential we observed here likely would not translate into substantial parasite-mediated variation in fitness.

Why might the advantage of being rare be so low in our experiment? We can rule out the possibility that insufficient genetic variation limited adaptation to common host genotypes - selection in homogeneous populations resulted in large changes from ancestral virulence, at least on CB4856 and ewIR68 (Fig. S2) (Gibson et al. 2020; White et al. 2019). We can also rule out insufficient time as a primary explanation – parasites were serially passaged for half as long on CB4856/ewIR68 mixtures relative to LTM1/N2 mixtures yet we observed the largest difference in performance on rare and common host genotypes for parasites selected in mixtures of ewIR68 (rare) and CB4856 (common) (Fig. 2). It is possible that surveying the mean virulence of each parasite line masked relevant variation in the parasite population. According to this hypothesis, we would expect to find substantial variability in virulence between individual clones within each parasite line, with some showing especially high virulence against the common host

genotype or especially low virulence against the rare host genotype. Indeed, some individual parasite lines showed fairly large differences between proportional mean change from the ancestor on common vs. rare host genotypes, with an upper quartile ranging from 10.17 to 25.16 percentage points. We also prevented coevolution in our experiment. Coevolution can generate and maintain more genetic variation in parasite populations than parasite evolution alone (Morran et al. 2011; Pal et al. 2007; Paterson et al. 2010; Schulte et al. 2013). Thus we expect that coevolution would accelerate virulence evolution and enhance the rare-common differential.

Coevolutionary models rest upon the process of adaptation by parasites to infect common host genotypes, at the expense of infection of rare host genotypes. Thus far, this theoretical finding lacked direct experimental support. Using an experimental evolution approach with fully reciprocal, uneven combinations of multiple host genotypes, we found that parasites targeted common over rare hosts. This resulted from a mean loss of virulence against rare genotypes. We also found suggestive evidence that the effect of host frequency may vary with host genotype (though this interaction was insignificant). The variation we observe across parasite lines emphasizes the value of complementing field studies, which survey ongoing adaptation, with controlled experiments, which can parse the contributions of host frequency and genotype to divergence in evolutionary trajectories. Finally, based on our findings, we hypothesize that coevolution, as opposed to parasite evolution alone, would strengthen the covariance of parasite fitness and host genotype frequency.

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Data Accessibility Statement

Data and analysis scripts associated with this study are provided as a supplemental file for review. Upon acceptance, we will make these files publicly available on the Dryad Digital Repository.

Author contributions

AKG conceived and directed the study, performed experimental evolution and assays, collected data, analyzed data, and wrote the manuscript. PSW contributed to experimental design, performed experimental evolution and assays, and collected data. MJP assisted in experimental evolution and collected data. JCdR contributed to developing and guiding the study and critically revised the manuscript. LTM conceived the study, provided guidance, collected data, and critically revised the manuscript. *All authors gave final approval for publication.*

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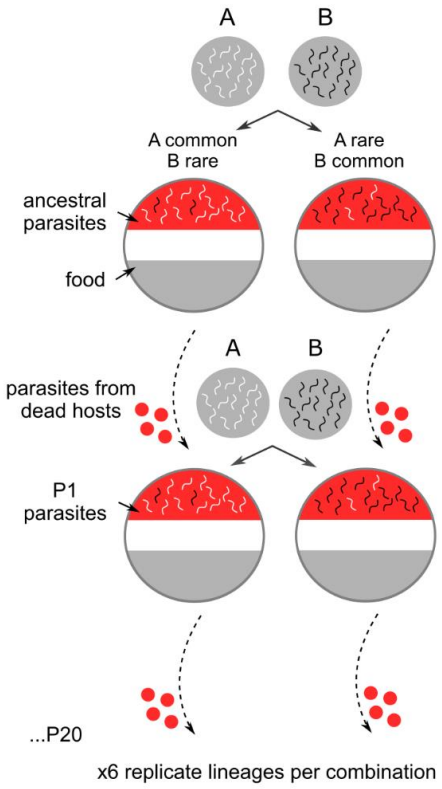
Figures



Combinations	Rare	Common
9 N2: 1 LTM1	LTM1	N2
3 N2: 1 LTM1	LTM1	N2
1 N2: 3 LTM1	N2	LTM1
1 N2: 9 LTM1	N2	LTM1
3 CB4856: 1 ewIR68	ewIR68	CB4856
1 CB4856: 3 ewIR68	CB4856	ewIR68

Figure 1: Experimental evolution design. A single genotype of *Serratia marcescens* was used to initiate 36 independent parasite lines. These lines were selected for increased killing of hosts in heterogeneous host populations, in which one host genotype was common and one rare. Each round of selection, non-evolving hosts were added to a lawn of parasites, dead hosts were isolated at 24 hours, and parasites were extracted. These parasites - those that killed their hosts rapidly - were then passaged to the next round of selection. Four host genotypes were paired in different combinations and frequencies. There were six replicate parasite lines for each combination of host genotypes.

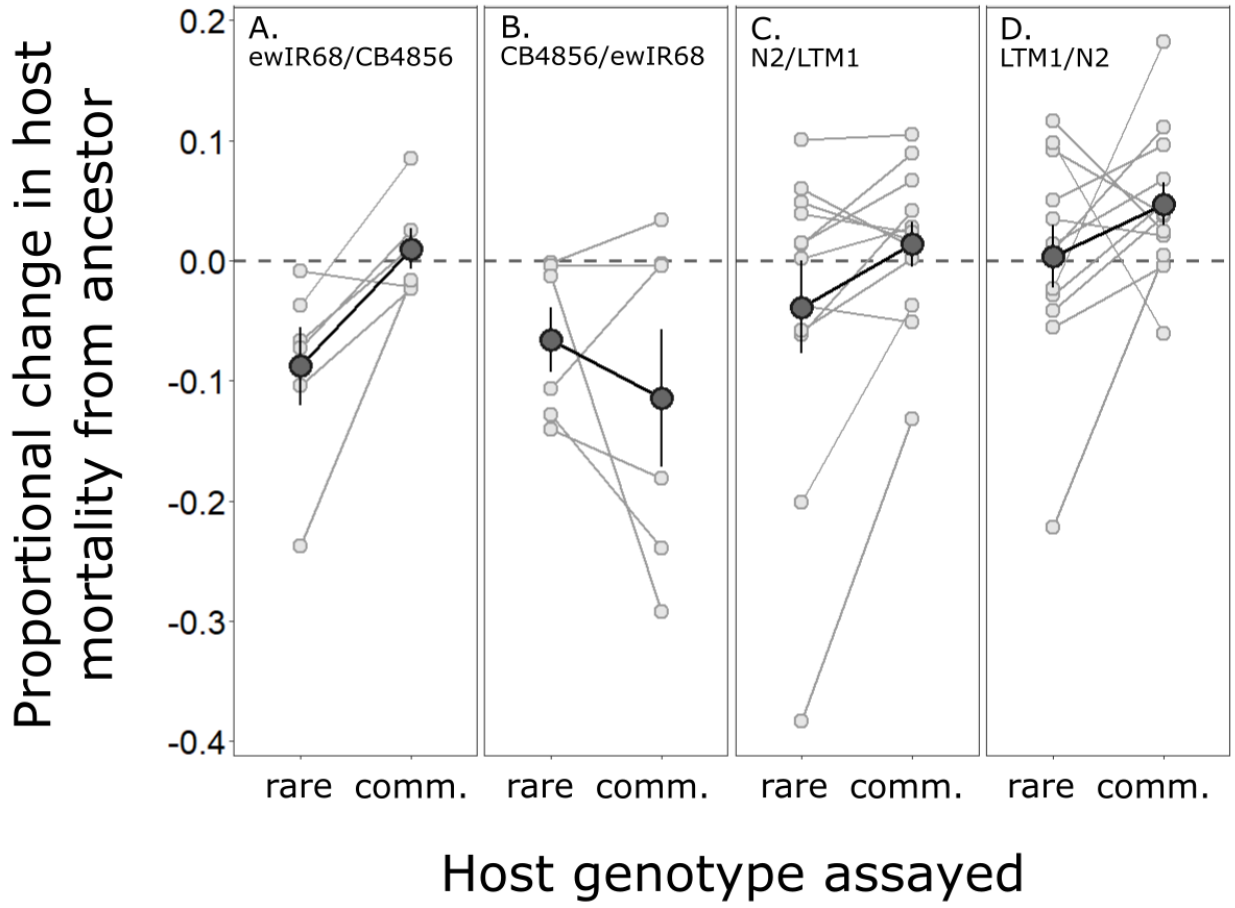


Figure 2: Change in virulence against rare vs. common host genotypes. We assayed the virulence (host mortality) of each parasite line against the rare (left) and common (right) host genotypes with which it was evolved. We used these estimates to calculate the proportional change from ancestral virulence (Fig. S1) against each host. The dashed line indicates no change from the ancestor. Each dark point represents the mean proportional change of 12 (N2/LTM1 combinations) or six (CB456/ewIR68 combinations) evolved parasite lines, with standard error of the mean. Shaded points show the mean estimates for each individual parasite line; these mean estimates were calculated from eight (N2/LTM1) or six (CB456/ewIR68) technical assay replicates per line.

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