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Caenorhabditis elegans and *Serratia marcescens* system

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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Parasites are known to vary in their host range. Some are limited to infecting a single host genotype, while others infect multiple host genotypes, even multiple host species. One hypothesis to explain this variation is that parasite lineages differ in their evolutionary history: parasites that consistently encounter a genetically homogenous host population may experience selection to become specialists, while parasites that often encounter a heterogeneous host population may experience selection to become generalists. Here, we utilized experimental evolution to impose selection on *Serratia marcescens* bacterial parasites to kill the nematode host *Caenorhabditis elegans.* Parasites were selected to kill hosts in homogeneous (one host genotype, N2 or CF3) or heterogeneous populations (an equal mix of two genotypes, N2 and CF3). After 20 passages of selection, we assessed the evolution of parasite host range by measuring the parasite populations' ability to kill hosts of the N2 genotype, CF3 genotype and a novel genotype, JU1395. We predicted that parasites selected to kill homogeneous host populations will evolve a more limited host range than parasites selected to kill heterogeneous host populations. We found mixed support for this prediction. Parasites passaged with N2 exhibited preferential adaptation to N2, in the form of increased mortality rates of N2 hosts. As predicted, these parasites showed reduced ability to kill the novel host genotype relative to control parasites. In contrast, parasites passaged with CF3 did not exhibit preferential adaptation to the CF3 host. Accordingly, these parasites showed no loss of ability to kill the novel host. These results demonstrate that both the diversity and genotypes of local hosts can influence the evolution of host range in parasites.

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Table of Contents

Introduction

Organisms encounter a multitude of parasites, many of which are unsuccessful at establishing infections in a given host or host population (Perlman and Jaenicke 2003; Longdon et al. 2011). This is because parasites have a limited breadth of species that they can infect. Parasite lineages vary in how limited this host range is (Ebert 2005). Some are limited to infecting a narrow range of genotypes, while others infect a broad range of host genotypes, potentially even infecting hosts from different species. One hypothesis to explain this variation is that parasite lineages differ in their evolutionary history: parasites that encounter a genetically homogenous host population may experience selection to become specialists, while parasites that encounter a heterogeneous host population may experience selection to become generalists. Much evolutionary theory argues that a population's niche width evolves to match the degree of variation in the environment (reviewed in Kassen 2002). From this theory, we predict that a parasite lineage will evolve a broader host range if there is a large amount of variation in the host population (i.e. a heterogeneous environment). Conversely, we predict that a parasite lineage will evolve a narrow host range if there is minimal variation in the host population (i.e. a homogenous environment).

There is ample support for the notion that continual selection in a specific environment results in specialization. In an experimental evolution project conducted by Travisano and Lenski (1996), lines of *E. coli* were passaged in glucose-limited environments. They then compared the performance of the passaged *E. coli* lines to the ancestor in novel, single-nutrient environments. They observed that the more the nutrient environment differed from glucose in its physiological uptake pathway, the greater the fitness cost experienced by the passaged *E. coli*. Little et al. (2006) conducted experimental evolution where they selected the parasite *Pasteuria ramosa* to infect *Daphnia magna*, its crustacean host. They passaged two lines of parasites in two different clones of *D. magna*. They found that each parasite line evolved greater infectivity in the host genotype with which they were passaged. Then, as predicted, they found that both parasite lines lost the ability to infect the alternate host genotype. Similar results have been found with fungal parasite models (Birnbaum and Gerardo 2016), insect parasite models (Henter and Via 1995; Schmid-Hempel 2004) and mammal parasite models (Smith et al. 1999).

To explore the evolution of niche breadth, Reboud and Bell (1997) selected *Chlamydomonas reinhardtii*, a single-celled green alga, to grow in either light or dark environments for about 1000 generations. They then transferred the cultured populations to the opposite environment. They found that the transferred *C. reinhardtii* did not grow well in the alternative environments. The *C. reinhardtii* populations had specialized on the environment they were selected for and lost the ability to grow in the alternative environment. They also selected *C. reinhardtii* to grow in an environment that varied between light and dark for about 150 generations. These lines evolved to be generalists: they grew just as well as in both environments as the lines that were selected for one condition or the other.

These studies of the maintenance and expansion of ecological breadth in free-living systems have clear relevance to the evolution of host range in host-parasite systems. There are few studies that look at the expansion of host range and disease emergence of novel hosts. Those studies that do exist focus on bacteria and bacteriophage (Turner et al. 2010 and Ciota et al. 2007). Thus there is limited breadth to the literature on the evolutionary expansion of host range. In our study, we address this gap by asking the question: Do parasites that have been selected to kill two host genotypes retain a broader host range than parasites selected to kill one host?

In this study, we selected the bacterial parasite *Serratia marcescens* to kill the nematode *Caenorhabditis elegans* via experimental evolution in the lab. Experimental evolution is a technique used to study the evolutionary process by tracking laboratory populations through time and manipulating selection pressures in a controlled environment (Kawecki and Ebert 2004). In this experiment, we used experimental evolution to select on parasite populations to kill populations of hosts that varied in their composition. Parasites were selected to kill hosts in homogeneous (one host genotype, N2 or CF3) or heterogeneous populations (an equal mix of two genotypes, N2 and CF3). After 20 passages (several hundred bacterial generations), we assessed the evolution of parasite host range by measuring parasite populations' ability to kill hosts of the N2 genotype, CF3 genotype and a novel genotype, JU1395. Based on the hypothesis that the host range of parasites is positively correlated with the diversity of their host population, we predicted that parasites selected to kill homogeneous host populations would have a more limited host range than parasites selected to kill heterogeneous host populations.

Methods

Strains used

Hosts – We used the N2 and CF3 WT-INV *Caenorhabditis elegans* strains for the experimental evolution. We obtained the N2 genotype from the Caenorhabditis Genetics Center at the University of Minnesota. The CF3 genotype, described in Slowinski et al. 2016, is an ethylmethane sulfonate mutagenized population with a CB4856 background. We started with a single clonal lineage from this population and used the same clonal lineage for all subsequent lineages in the experimental evolution. These two genotypes were chosen for the passaging because N2 and CB4856 are two of the most genetically divergent *C. elegans* strains (Barriere 2005). They are also equally susceptible to the parasite *S. marcescens*. The JU1395 strain was used as the novel genotype in the subsequent survival assay because it is equally genetically divergent from both N2 and CF3 (E. Andersen, personal communication). Therefore, we expect no bias in our results due to differences in genetic similarity between sympatric (N2 or CF3) and novel (JU1395) hosts. We also started with a single clonal lineage from this novel host population. The strains were maintained in a 20°C incubator and bleach synchronized to L4 larval stage prior to use in the experiment.

Parasite – The bacterial parasite that was passaged was the Sm2170 strain of *Serratia marcescens*. This parasite strain had not been previously adapted to *C. elegans*, therefore, it is naïve to the nematodes.

Serratia Selection Plates (SSPs)

We followed the Morran et al. (2009) protocol for making *Serratia* Selection Plates (SSPs). The plates were prepared by pouring 24mL of an autoclaved solution of Nematode Growth Medium Lite (US Biological, Swampscott, MA) into a 10 cm Petri dish. The plates were then horizontally divided into thirds. The middle section was left empty. The other two sections were seeded with 35 μl of *E. coli* (OP50), a laboratory food source for *C. elegans*, and 35 μl of *S. marcescens* respectively. The inocula were spread evenly with sterile spreaders. This setup provides the surviving hosts with a place to escape to after initially being plated on the side with

S. marcescens. We maintained this approach because it is the standard experimental evolution design in the lab. Altering this setup may have potentially altered the projected evolutionary trajectory of the treatments. Prior to seeding the SSPs, the inocula were grown in test tubes containing 5mL of Lysogeny broth (LB). The tubes were incubated at 28°C for 24 hours. After seeding the SSPs, the plates were incubated at 28°C for 24 hours to allow for ample lawn development.

Preparing treatments

Starting with the ancestral strain of *S. marcescens*, Sm2170, we created four different selection treatments. In the 0-0 treatment (control), parasites were passaged without hosts to control for drift and non-focal selection on the parasite (e.g. selection due culturing in a laboratory environment). In the 0-100 treatment, parasites were selected to kill host populations that were 0% N2 and 100% CF3. In the 50-50 treatment, parasites were selected to kill host populations that were 50% N2 and 50% CF3. In the 100-0 treatment, parasites were selected to kill host populations that were 100% N2 and 0% CF3. The host ratios were obtained by calculating the volume needed for 500 total worms to be added to each SSP. Both N2 and CF3 worms were washed from L4 synchronized stocks. The concentrations of the solutions were then calculated by aliquoting 20uL of the solution into 6 glass wells. The worms were counted and averaged to find the concentration of worms per 20uL. A total of 500 worms were plated on each SSP. The various strains were added to the SSPs according to the percentages dictated by the treatment.

Passage of parasites

For 20 passages, we isolated and transferred the parasites that killed their hosts rapidly, within 24 hours. According to Kurz et al. 2003, the mean killing time for the *S. marcescens* strain we used is roughly 40 hours. By passaging only *S. marcescens* isolated from hosts that died after 24 hours of exposure, we were imposing strong selection for killing. We then picked 20-30 dead worms from each treatment and placed them in microcentrifuge tubes containing 1mL of M9 buffer. The worms were then spun in a centrifuge at 3000 rpm for 1 minute. The solution was rinsed six times, leaving 100uL of solution after the last rinse. The worms were then crushed using a motorized pestle. The contents were poured onto an unseeded NGM plate and the bacteria were streaked using a sterile spreader. The selected parasites were left to grow at room temperature for 48 hours then maintained at 4°C for 24 hours. Forty colonies were picked from each treatment line and inoculated in 5 mL of LB. Two colonies of OP50 from a lab stock were inoculated in the same manner. The cultures were then grown in a 28°C shaker for 24 hours. These parasite populations were then used to make the next batch of SSPs.

Survival assay and scoring

To test *S. marcescens* virulence, we replicated the experimental passaging scheme exactly, then measured mortality after 48 hours. We plated about 270 worms per plate, then the SSPs were placed in the 20°C incubator for 48 hours. The total number of worms added to the SSPs was estimated by plating the calculated volume of worms needed onto 10 OP50-seeded NGM plates at the same time that worms were added to the SSPs. These plates were then incubated at 15°C for 24 hours. The plates were scored and averaged to obtain an estimate of the worms added to each plate in the assay. Forty-eight hours after adding them to the SSPs, the number of worms that survived (indicated by the number of worms that migrate to the side with OP50) were scored. The killing rate of the *S. marcescens* was determined by subtracting the fraction that survived (number of live worms divided by total worms plated) from 1.

Statistical Methods

Virulence of each treatment, as demonstrated by killing rate, was analyzed using a generalized linear mixed model approach. This approach was chosen over linear regression because the data were not normally distributed. Count data follow a Poisson distribution, and our data were over-dispersed (variance substantially exceeded the mean), so we fit a negative binomial distribution, the proper distribution when count data is over-dispersed. We verified that this distribution was a significantly better fit to our data than a Poisson distribution. We fit model treatment (Ancestor, 0-0, 0-100, 50-50, 100-0) as a predictor of the total number of surviving worms on each replicate plate. In no case did we see a significant difference between the ancestor and the 0-0 treatment. As a result, the ancestor treatment was excluded from the final analyses. We included parasite lineage as a random effect. We ran three separate models of the same structure, one for N2, one for CF3 and one for JU1395. If treatment was a significant predictor of variation in the number of surviving worms, we examined the coefficients of the model to make comparisons between treatments. All statistics were conducted in the R programming software.

Results

N2 Assay

Host treatment significantly contributed to the variance we observed in our count data for N2 (generalized linear mixed model, treatment: $\chi^2 = 22.93$ df = 3, $P < 0.001$). We found evidence that parasites from the 100-0 and 50-50 treatments adapted to become better at killing N2. Killing rate against N2 hosts was significantly higher with 100-0 parasites relative to 0-0 (Fig. 1; coefficient = 0.51 ± 0.14, $z = 3.68$, $P < 0.001$) or 0-100 (Fig. 1; coefficient = 0.58 ± 0.14, $z = 4.23$, $P < 0.001$) parasites. Killing rate against N2 hosts was also significantly higher with 50-50 parasites relative to 0-0 (Fig. 1; coefficient = 0.32 ± 0.14, $z = 2.29$, $P = 0.022$) or 0-100 (Fig. 1; coefficient = 0.38 ± 0.14, $z = 2.76$, $P = 0.006$) parasites. There was no statistical difference between survival against 100-0 and 50-50 parasites. Our data shows the 100-0 parasites may have evolved a higher virulence against N2 hosts relative to 50-50 parasites, but the mean difference between treatments is neither substantial from a biological perspective, nor is it statistically significant (Fig. 1; coefficient = 0.20 ± 0.14, $z = 1.42$, $P = 0.157$).

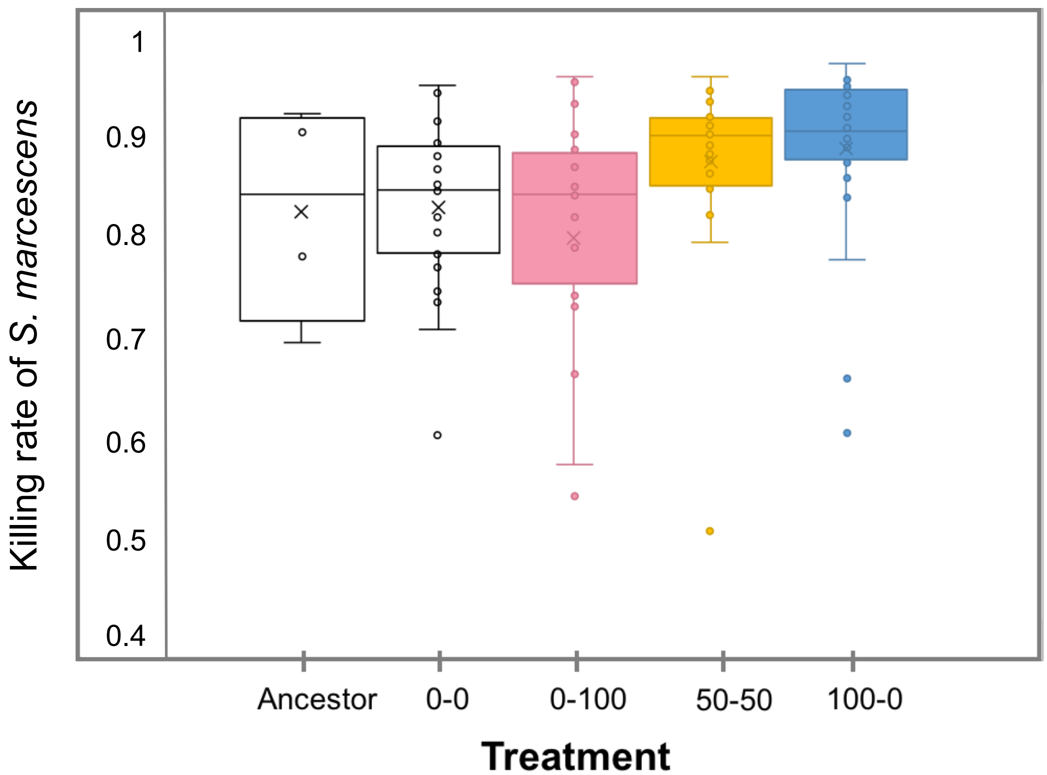

Figure 1. Mean killing rate of N2 host populations exposed to ancestral Sm2170, control (0-0), CF3-selected (signified by pink), CF3-N2 heterogeneous mix (signified by yellow), and N2-selected (signified by blue) parasite treatments over 20 generations. The x-axis represents the treatment group and the y-axis represents the killing rate of *S. marcescens*. Twenty-four replicates of the various treatment groups and ten replicates of the ancestral strain were assayed against populations of approximately 500 N2 nematodes.

There was no difference in survival against 0-0 and 0-100 parasites (Fig. 1; coefficient = 0.07 ± 0.14, $z = 0.50$, p=0.621). Relative to the 0-0 control, the 0-100 killing rate decreased by 2.9% against N2 hosts (Table 1). Relative to the 0-0 control, the 50-50 treatment's killing rate was 4.7% higher against N2 hosts (Table 1).

Table 1. Mean killing rate in N2 assay

Treatment	***Ancestor***	***0-0***	***0-100***	***50-50***	***100-0***
Mean Killing Rate	0.823	0.826	0.797	0.873	0.884

CF3 Assay

We found no evidence that CF3 hosts exposed to parasites from different treatments vary in their survival. Treatment did not significantly contribute to the variance we observed in our count data (Fig. 2; generalized linear mixed model, treatment: $\chi^2 = 3.73$, df = 3, P = 0.292). We have no evidence of preferential parasite adaptation to kill CF3 in either the 0-100 or 50-50 treatments, the treatments where we expected to see increased killing rates.

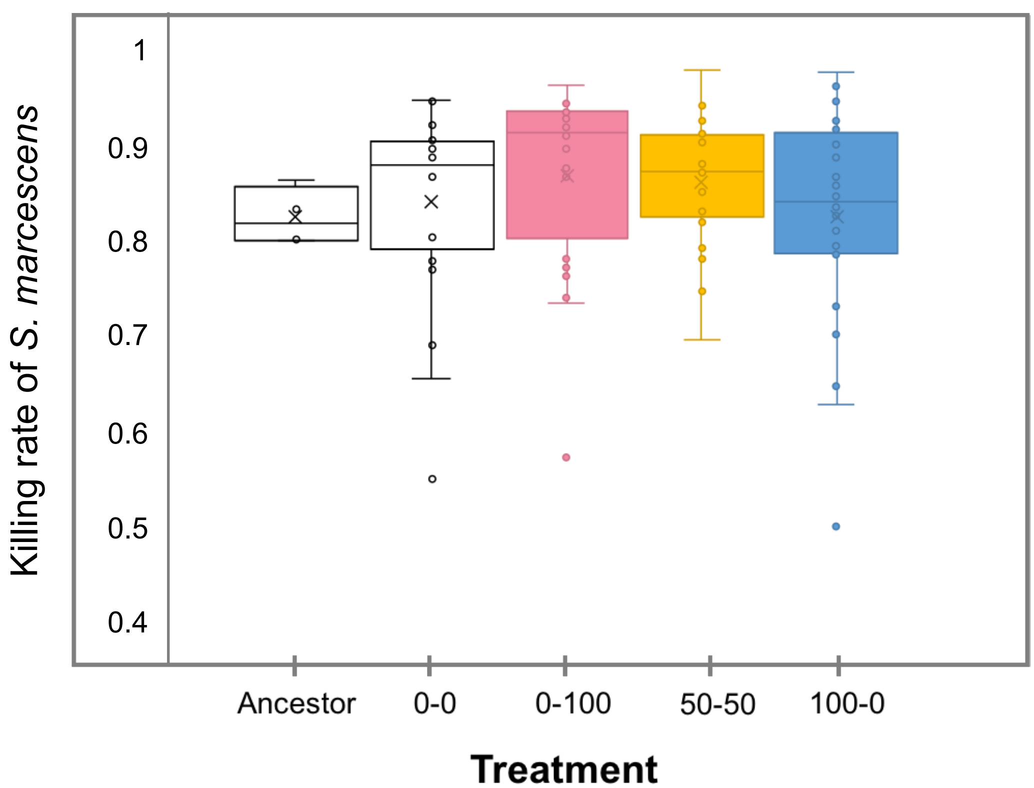

Figure 2. Mean killing rate of CF3 host populations exposed to ancestral Sm2170, control (0-0), CF3-selected (signified by pink), CF3-N2 heterogeneous mix (signified by yellow), and N2-selected (signified by blue) parasite treatments over 20 generations. The x-axis represents the treatment group and the y-axis represents the killing rate of *S. marcescens*. Twenty-four replicates of the various treatment groups and ten replicates of the ancestral strain were assayed against populations of approximately 500 CF3 nematodes.

When faced with the CF3 host, the mean killing rate of the 0-100 treatment was 2.7% percent higher than the killing rate of the 0-0 control (Table 2). While this decrease may be biologically relevant, it is not statistically relevant.

Table 2. Mean killing rate in CF3 assay

Treatment	***Ancestor***	***0-0***	***0-100***	***50-50***	***100-0***
Mean Killing Rate	0.822	0.838	0.865	0.858	0.823

JU1395 Assay

Host treatment significantly contributed to the variance we observed in our count data for the novel host, JU1395 (generalized linear mixed model, treatment: $\chi^2 = 3.01$, df = 3, $P < 0.001$). On JU1395, we see a significant loss in killing ability of 100-0 parasites relative to 0-0 (coefficient = -0.63 ± 0.15, $z = -4.08$, $P < 0.001$) and 50-50 (coefficient = -0.76 ± 0.14, $z = -4.96$, $P < 0.001$) parasites, consistent with a decrease in host range. There was no statistical difference between survival against 0-0 and 50-50 parasites (coefficient = 0.13 ± 0.15, $z = 0.836$, $P = 0.402$). Killing rate against JU1395 hosts with 0-100 parasites did not differ from that with 0-0 (coefficient = -

0.09 ± 0.15, z = -0.61, p=0.544) or 50-50 (coefficient = -0.22 ± 0.15, z = -1.44, P = 0.149) parasites.

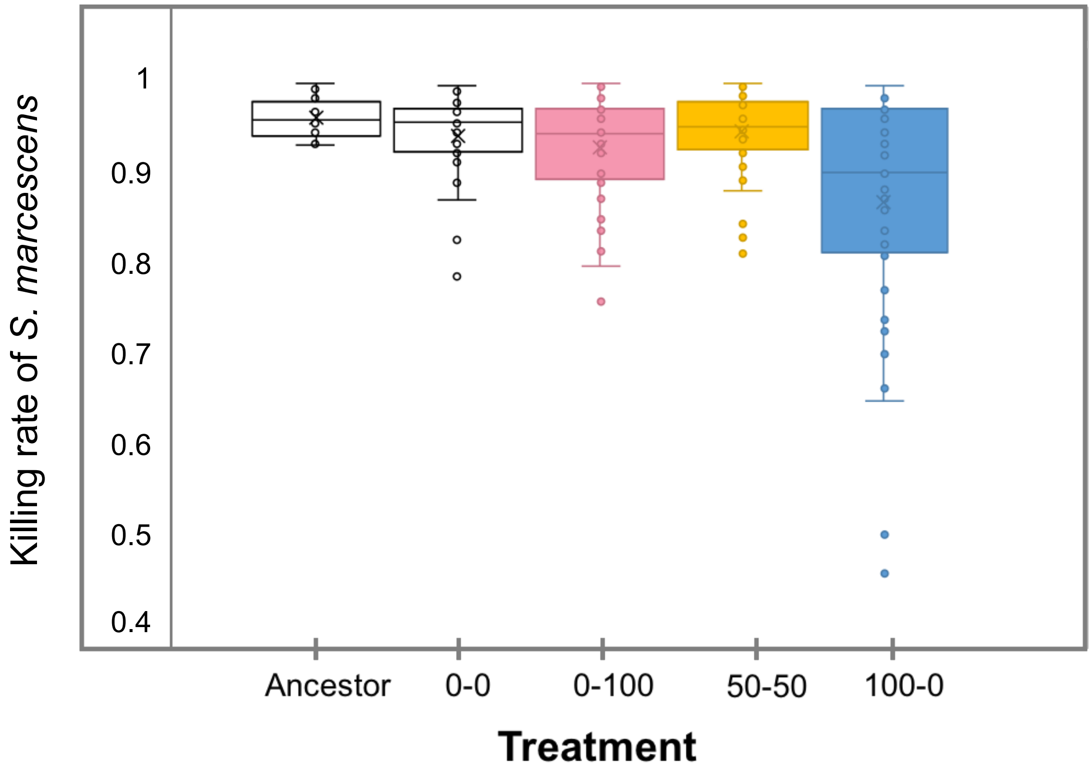

Figure 3. Mean killing rate of JU1395 host populations exposed to ancestral Sm2170, control (0-0), CF3-selected (signified by pink), CF3-N2 heterogeneous mix (signified by yellow), and N2-selected (signified by blue) parasite treatments over 20 generations. The x-axis represents the treatment group and the y-axis represents the killing rate of *S. marcescens*. Forty-eight replicates of the various treatment groups and 16 replicates of the ancestral strain were assayed against populations of approximately 270 JU1395 nematodes.

The killing rate of the 100-0 parasites decreased by 7.5% compared to the 0-0 control (Table 3). Regardless of the treatment type, the parasite was better at killing JU1395 hosts than N2 and CF3.

Table 3. Mean killing rate in JU1395 assay

Treatment	*Ancestor*	*0-0*	*0-100*	*50-50*	*100-0*
Mean Killing Rate	0.956	0.936	0.925	0.941	0.861

Discussion

As a response to the limited literature on the evolutionary expansion of host range, we set out to explore if parasites that have been selected to kill two host genotypes retain a broader host range than parasites selected to kill one host. After experimentally evolving parasites to infect both homogenous and heterogeneous populations of hosts, we conducted survival assays to assess changes in killing ability. We found evidence that parasites from the 100-0 (selected to kill populations of 100% N2) and 50-50 (selected to kill a population with an even mix of N2 and CF3) treatments have adapted to killing N2 hosts (Fig. 1). When faced with a novel host, the 100-0 parasites experienced a significant decrease in killing ability compared to the other parasite treatments, consistent with specialization following selection against a homogenous host population. In contrast, the 50-50 treatment did not exhibit a loss in killing ability relative to other treatments (Fig. 3), consistent with the maintenance of generalism following selection in response to a heterogeneous host population. These findings support the hypothesis that parasites who encounter homogenous host populations evolve to become specialists and parasites who encounter heterogeneous host populations evolve to be generalists. In contrast, parasites from the 0-100 and 50-50 treatments did not adapt to killing CF3 (Fig. 2), suggesting a limited ability for preferential adaptation to CF3 hosts. We also found no evidence of a loss of ability to kill a novel host for 0-100 parasites. We accordingly conclude that a lack of preferential adaptation to CF3 hosts prevented the loss of host range that we predicted to accompany selection in homogenous host populations.

These results demonstrate that both the heterogeneity and genotypes of local hosts can influence the evolution of host range in parasites. Results from selection on N2 hosts support the hypothesis that host range varies according to the parasite's history of host contact. The parasites in the 100-0 treatment had a history of solely interacting with N2 hosts. As a result, they adapted a greater ability to kill N2 hosts. This increase in killing ability came at the expense of losing the capability to kill other hosts. The 0-100 treatment did not exhibit this same trend. The 0-100 treatment's failure to adapt to kill CF3 at a higher rate supports the idea that the genotype of the host population may play a role in host range dynamics. Specifically, some host genotypes may select for, or allow for, the evolution of greater parasite specificity than other host genotypes. Despite being exposed to only CF3 hosts throughout the course of the experimental evolution, the 0-100 treatment did not adapt to it and did not lose the ability to kill other hosts. In the context of CF3, host range does not appear to be determined by the diversity of hosts encountered. Rather, the evolutionary trajectory of host range here was defined by host genotype, with the CF3 host genotype failing to promote a shift towards parasite specialization in the same manner as the N2 genotype.

Our study brings up the question of why parasites selected to kill CF3 hosts do not evolve to be specialists, and further did not evolve greater virulence toward CF3 hosts. There are multiple potential explanations for this result. First, it may reflect a lack of genetic variation in the parasite population. Twenty passages of experimental evolution may not be enough time for sufficient *de novo* mutations to arise and permit greater killing ability of CF3. In particular, if CF3 hosts require a more specialized parasite genotype for successful infection, relative to N2, then more evolutionary time may be required for parasite adaptation and specialization. Second, there may be a lack of specialized parasite defense for CF3, which could lead to reduced strength of selection on parasite populations, relative to N2 hosts. As a result, parasites would adapt to and specialize on CF3 hosts at slower rates. Perhaps with more time, we would see stronger differences emerge in the CF3 selected parasites.

Another area for further investigation may be exploring host range in the *S. marcescens* and *C. elegans* parasite-host system in a coevolutionary study. Our study involved evolving only parasites. If both the parasites and hosts are passaged together, we would expect to see a stronger drive towards specialization. Experimental coevolution with bacteria and bacteriophage have demonstrated that, because both the parasite and host are being passaged together, adaptations in one player may alter selection on the other. This then leads to more rapid divergence between populations of hosts and parasites (Buckling and Rainey 2002; Meyer et al. 2012; Morgan et al 2005). In a study conducted by Morran et al. (2014), experimental coevolution was used examine the effect of host mating system, either obligate outcrossing or mixed mating, on the pace of evolutionary change in *C. elegans* hosts and *S. marcescens*. They found that most parasites evolved to specialize on their local hosts, but that specialization was more substantial and more frequent in parasite populations that were passaged with obligately outcrossing hosts, which exhibited greater rates of adaptation than mixed mating hosts. Therefore, it appears that intense antagonistic coevolutionary interactions may select for increased specialization and reduced host range, a hypothesis that can be readily tested in the *C. elegans-S.marcescens* system.

Our finding that the evolution of host range in response to heterogeneous environments is seen in some strains and not others adds complexity to the dialogue on parasite host breadth. It highlights the importance of studying other factors that contribute to parasite local adaptation, such as parasite specificity and virulence. Gandon (2002) analyzed a coevolutionary model where he found that higher parasite specificity and higher virulence typically resulted in higher levels of local adaption. A broader understanding of parasite host range can also be useful for predicting and preventing disease spillover. Parasites moving to novel hosts can lead to the emergence of new diseases (Longdon et al. 2011). Determining which parasite species or genotypes are more likely to undergo shifts in host range can be utilized for disease threat prevention. Study of parasite host range also has implications in biological pest control. Parasites are often introduced to suppress crop pests in agriculture (Simberloff and Stiling 1996). Understanding more about the potential of parasites to shift their host range can inform pest control innovation and regulation. The maintenance of parasite host range is an important topic that can contribute greatly to our understanding of host-parasite interactions on both a micro and macro level.

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