Do parasites adapt to infect common hosts? An experimental evolution test

499 Progress Report after 2 Semesters

Abstract

How do parasites adapt to their host populations? A prominent hypothesis argues that parasites adapt to infect the most common host genotype in a population. A few field-based studies support this hypothesis by using natural host-parasite systems. We build on previous work by directly testing this hypothesis through experimental evolution in the lab, using two genetically distinct strains of the model nematode *Caenorhabditis elegans* and a bacterial parasite, *Serratia marcescens*. First, we created experimental treatment groups that differed in which host genotype was common and in how common that host genotype was. Over ten generations of experimental evolution, we selected for *S. marcescens* that successfully killed hosts in these different populations. We will now measure the ability of selected *S. marcescens* lineages to kill the two different host genotypes. We predict that parasites selected under conditions where one host genotype was more common will be more effective at killing that genotype, in comparison to the genotype that is rare. The results of this study will help us understand how parasites adapt to infect their host populations.

Introduction

Parasites are widespread organisms in nature that place selection pressures on host populations. Hosts in turn adapt to resist these parasites, generating a constant struggle for survival. How do parasites continually adapt to infect their host populations?

One hypothesis proposes that parasites adapt to infect the most common host genotype in a population. Parasites that infect the common host genotype in a population have a greater amount of resources and produce a higher number of offspring relative to parasites that infect rare host genotypes. This hypothesis gives a survival advantage to the rare host genotype, which maintains genetic variation in host populations (Haldane, 1949). Three prior studies have supported this hypothesis in natural systems (Chaboudez & Burdon, 1995, Wolinska & Spaak, 2009, Koskella & Lively, 2009).

The idea that parasites infect the most common host genotype is crucial in evolutionary biology. It is a major assumption of the Red Queen hypothesis, one of the most important postulates for the evolutionary maintenance of sex (Jaenike, 1977). Furthermore, this concept has implications for fields beyond evolutionary biology, including agriculture. A study on disease control in rice planting found that genetically diverse rice fields had higher yields and a reduced incidence of rice blast disease relative to genetically homogenous rice fields, which only consisted of a single genotype (Zhu et al., 2000). Thus, having an abundance of one genotype in a population can be detrimental, indicating that the study of common host genotype infection has both theoretical and practical applications.

There are no direct tests of the common clone hypothesis. A direct test of this hypothesis, in a controlled laboratory system, is required in order to determine if

preferential infection of the common host genotype truly derives from its commonality or simply because it is particularly susceptible to parasitism. A controlled experimental setting also allows us to ask the question of which threshold frequency (90%, 75%, etc.) makes a host genotype a common target for parasite adaptation. Our study provides a direct test of the common clone hypothesis and addresses these questions through experimental evolution in a controlled lab setting.

In this experiment, we selected the parasite *Serratia marcescens* to infect and kill two genetically distinct host genotypes of the nematode *Caenorhabditis elegans* that have equivalent initial levels of resistance to the parasite. *C. elegans* nematodes are ideal for experimental evolution experiments because of their short generation time and ability to act as host for a range of pathogens (Aballay & Ausubel, 2002, Gray & Cutter, 2014). *S. marcescens* is a highly virulent pathogen that infects the intestine of *C. elegans*, causing high mortality rates (Schulenburg & Ewbank, 2004).

There are two parts to our experimental process. In the first part, we used experimental evolution to select for parasites that successfully infected and killed host populations which varied in the identity and frequency of the common host genotype. In the second part of the experiment, we will conduct a survival assay by exposing the two hosts genotypes to the parasites we selected during experimental evolution. We will measure the survival rate of the hosts to determine whether the parasites have adapted to preferentially kill the most common host genotype in the populations. According to the hypothesis, we predict that *S. marcescens* will successfully infect the common host genotype in a population. In addition, we predict that the difference in killing rates of the common and rare host genotypes will be highest for parasites selected to kill host

populations where the common host genotype was most frequent (e.g. 90% of the host population). We expect to observe this outcome regardless of which lineage of *C. elegans* is used as the common host genotype. Conversely, if we see that *S. marcescens* evolves to kill one lineage of *C. elegans* both when it is the common host genotype and when it is the rare host genotype, we would reject our hypothesis. The latter result would suggest that one host genotype is simply more susceptible to parasitism, regardless of its frequency.

Materials and Methods

Host-parasite system

In this experiment, we are using the Sm2170 strain of *S. marcescens* to infect two genetically distinct host strains of *C. elegans:* N2 and CF3-*wt-inv*. N2 is the most common strain used in laboratory studies (Félix & Braendle, 2010). We obtained it from the *Caenorhabditis* Genetics Center, the international repository for *Caenorhabitis* strains. CF3-*wt-inv* is a derivative of the Hawaiian strain CB4856. CB4856 is another very common strain used in laboratory studies and, importantly, is one of the strains that is most genetically distinct from N2 (Thompson et al., 2015). CF3-*wt-inv* was created in the Morran lab in conjunction with another project. We make use of it here because it is equally susceptible to killing by Sm2170 as is N2, while CB4856 is quite resistant. The strain Sm2170 is highly virulent, leading to high mortality rates of both N2 and CF3-*wt-inv*. Our experimental evolution was conducted in the Morran Lab at Emory University in Atlanta, GA.

Experimental evolution and selection of virulent parasites

The selection followed a seven-day schedule, with one generation of selection per week. The process was repeated for ten generations of selections, equivalent to ten weeks. On the first day of each week, the host lines were chunked separately onto agar plates seeded with the OP50 strain of *Escherichia coli*. This strain of bacteria is universally used as food for *C. elegans*. These plates were maintained in a 20°C incubator for 48 hours. On day three, the nematodes and eggs were washed off the plates with M9 solution and treated with a 2:1 mixture of bleach and 5M NaOH. This treatment killed and digested all but the eggs, resulting in populations of worms at a single life stage. The eggs were placed on plates of OP50 and stored at 20°C. Forty ancestral Sm2170 colonies were also picked on day three and used to inoculate tubes containing 5 mL of Luria-Bertani broth (LB), a liquid medium used to grow bacteria. Two tubes of LB were each inoculated with a colony of OP50. All the tubes were placed in a 28°C shaker to grow overnight. On day four, 48 *Serratia* selection plates (SSPs) were prepared as described in Morran et al., 2011, except no ampicillin was used and the volume of bacterial food and ancestral parasite culture was increased to 35 μL (Morran et al., 2011). The plates were divided into three sections, with a lawn of *S. marcescens* spread on one side of the plate and OP50 on the opposite side, separated by an empty section in between. One ancestral line of *S. marcescens* was spread on all 48 plates during the first generation and then in following generations each plate was kept as a separate bacterial line.

On day five of the schedule, the 48 SSPs were divided into six replicate groups that each contained eight different treatment plates. The eight treatment plates in each

group would receive varying proportions of the two host strains, as shown in the following table.

Table 1 Eight treatment plates with differing frequencies of N2 and CF3-wt-inv strains, each plate was replicated six times for a total of 48 plates

	Treatment Plate #							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Frequency of N2 nematodes on plate	0%	100%	90%	75%	50%	25%	10%	0%
Frequency of CF3-*wt-inv* nematodes on plate	0%	0%	10%	25%	50%	75%	90%	100%
	Control	**N2 Common**				**CF3-*wt-inv* Common**		

The 0%-0% group with no nematodes acted as a control to represent non-focal selective effects on *S. marcescens*, when there was no selection pressure on the parasite to kill nematodes. The other plates represented a reciprocal gradient of commonness between two genetically distinct, but equally susceptible hosts. L4 nematodes for both host lines were washed off with M9 from previously prepared plates and centrifuged at 1000 rpm for 30 seconds. The supernatant was removed, 10 μL M9 was added, and the centrifugation process was repeated twice more. The concentrations of nematodes in 20 μL were estimated for each host line. From these counts, we calculated the volumes needed to add 500 worms to each SSP at the varying proportions of each host line specified in the table above. We then added these volumes to the Sm2170 side of each

SSP and kept them at 20°C for 24 hours. During the 24 hours, the nematodes either died on the Sm2170 side or survived and crawled to the OP50 side.

On day six, 30 dead nematodes were picked from the *Serratia* side of each of the 48 plates and placed into respective tubes with 1 mL of M9. Thirty colonies were picked from the 0%-0% plates into M9 solution to mimic the population sizes of bacteria selected in the other treatments. The 48 tubes were spun at 3000 rpm for 3 minutes, the supernatant was discarded, and more M9 was added. This rinsing process was repeated 5 times to remove external parasites. Afterwards, the nematodes in each tube were crushed in about 100 µL of M9 using a motorized pestle to extract the internal parasites responsible for host-killing. By selecting dead nematodes and extracting the bacteria in their innards, we aimed to select for *S. marcescens* that successfully killed their hosts. The resulting bacteria were streaked across unseeded plates and placed at room temperature for two nights, then moved to 4°C for another two nights.

The parasite lineages were then subjected to this 7-day selection process for an additional 9 weeks, for a total of 10 generations of selection. Following each generation of selection, the bacteria were preserved for future assays by adding 500 µL of each parasite culture to cryotubes containing 500 µL of glycerol. The cryotubes were stored in a -80°C freezer.

Survival assay

The same schedule described above will be repeated, except that only 200 worms will be added to each plate and the two host strains will be exposed to the evolved parasite lines for 48 hours instead of 24 hours. Therefore, the counting of worms will take

place on day seven of each generation, rather than on day six. On day seven of each generation, we will count the number of nematodes found alive on the OP50 side of the SSPs after 48 hours and compare it to the number of nematodes initially added. This will determine the proportion of nematodes that were killed by the bacteria. A lower number of nematodes on the OP50 side will indicate a higher mortality and successful bacterial killing rate. Conversely, a higher number of nematodes on the OP50 side will indicate a lower mortality and a weak bacterial killing rate.

Statistical analysis

We will use the statistical program R to build a generalized linear model. We will do statistical analysis based upon the basic framework of an ANOVA to determine differences in host mortality across host line, parasite line, and their interactions.

Results and Discussion

At this time, the experiment is ongoing and we have not collected any results yet. Here are some expected results.

Predicted outcomes

We will include data showing that the two host strains have similar survival rates when exposed to ancestral Sm 2170. We expect that *S. marcescens* selected under treatments in which N2 was common will be better at killing N2 relative to CF3-*wt-inv*. Conversely, *S. marcescens* selected under treatments in which CF3-*wt-inv* was more common will be better at killing CF3-*wt-inv* nematodes than N2. We expect *S.*

marcescens to have the highest killing rate in plates containing only N2 or only CF3-*wt-inv*. The ability of the bacteria to kill the most common host genotype will decline as its frequency decreases to 50-50 and there are equal numbers of both hosts.

We will discuss whether results supported or contradicted the hypothesis that *S. marcescens* would successfully infect the most common host genotype in the population. We will also discuss how the frequency gradient and differing proportions of the two host strains influenced our results. Potential errors and future steps will be mentioned as well as the significance of our results and how it builds on previous research.

Implications of this experiment

This experiment carries importance in investigating the hypothesis of whether parasites adapt to infect the common host genotype as a way of evolving with their host populations. It builds on previously conducted research and will directly test the common clone hypothesis, which has not been done before. The concept that parasites infect common host genotypes in populations will have implications not only in evolutionary biology but also in other far-reaching fields such as medicine and agriculture.

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