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Kylie Measimer April 10, 2023

Assessing the presence of antagonistic fluctuating selection in a non-coevolved host-parasite system

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An abstract of
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of Emory University in partial fulfillment
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Bachelor of Science with Honors

Biology

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Abstract

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While the maintenance of outcrossing in nature has puzzled scientists for several decades, the mechanisms that drive this phenomenon are still unclear. Theory posits that Red Queen dynamics in coevolving host-parasite systems lead to parasites imposing antagonistic fluctuating selection on hosts that favors recombination. While previous experiments have shown evidence of antagonistic fluctuating selection in *Caenorhabditis elegans* populations coevolved with *Serratia marcescens*, explicit tests had not been performed to determine if this type of selection is unique to coevolving populations. In this study, I performed tests that analyzed whether evidence of antagonistic fluctuating selection was present in *C. elegans* populations evolved in the presence of changing, non-coevolving *S. marcescens* strains. While significant host adaptation was noted in these populations, there was a lack of evidence of antagonistic fluctuating selection. In addition, nematodes exposed to non-coevolving, changing parasite strains experienced weaker selection than nematodes coevolved with the parasite. Therefore, it is likely that strong, antagonistic fluctuating selection is a driving force of the maintenance of outcrossing in coevolving host-parasites systems, while weaker directional selection does not select for outcrossing in non-coevolving systems.

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Acknowledgements

Special thanks to all members of the Morran lab for their continued support and mentorship during my time in the lab. Thanks to Michelle McCauley for her advice and for completing the experiments from which my project is based. Thank you to Levi for being an awesome mentor and advisor for the past two years. Thanks to my committee members, Dr. Koelle and Dr. Sinykin, for their advice and guidance for the duration of this project. This project was supported by NSF grant DEB 1750553.

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Introduction

The prevalence of sexual reproduction in nature is puzzling due to the inherent increased energy cost of sexual reproduction in comparison to self-fertilization or asexual reproduction (Maynard-Smith, 1971). There are energy costs associated with finding and reproducing with a mate, and males cannot produce offspring alone. Therefore, sexual reproduction must provide an evolutionary advantage greater than the cost of self-fertilization to persist (Maynard-Smith, 1971). One proposed mechanism for the maintenance of outcrossing, or mating between genetically distinct individuals, is the Red Queen hypothesis. This hypothesis posits that coevolution between hosts and parasites creates an evolutionary struggle for survival that leads to outcrossing in hosts being favored over self-fertilization (Hamilton, 1980; Bell, 1982).

As hosts adapt to resist infection, the parasites often in turn adapt to become more infective, and this cycle continues as both species adapt to resist or infect (Hamilton, 1980). Therefore, during host-parasite coevolution, it is predicted that antagonistic fluctuating selection is imposed on the host as the parasite population continuously adapts and changes (Hamilton et al., 1980; Peters & Lively, 1999). Indeed, parasites are predicted to evolve to infect the most common host genotype, thus imposing negative frequency dependent selection on the host (Hamilton et al., 1990; Dybdahl & Lively, 1998). Therefore, coevolutionary host-parasite interactions can potentially lead to selection on both host and parasite that consistently changes the most common genotype within a population as the opposing species adapts (Hamilton et al., 1990). It has been theorized that this mechanism of selection drives the maintenance of genetic diversity, and it is thus predicted that outcrossing would be more favorable than self-fertilization for host populations (Murray, 1972; Hamilton, 1980).

While self-fertilization significantly increases the number of progeny over time in comparison to outcrossing, individual lineages within self-fertilizing populations tend to lack genetic variation, and there is no opportunity for novel beneficial alleles to be shared between lineages (Lande & Schemske, 1985; Morran et al., 2011). On the contrary, outcrossing facilitates the creation of novel or rare combinations of host genotypes, and these genotypes can potentially confer increased parasite resistance to an individual, thus increasing the individual's fitness (Muller, 1932; Morran et al., 2011). This increased fitness provided by sexual reproduction of hosts in the presence of coevolving parasites is posited to increase outcrossing rates. Accordingly, the Red Queen hypothesis predicts that coevolving parasites favor host outcrossing via fluctuating selection. However, it is unclear if coevolution is necessary for antagonistic fluctuating selection, or if a changing parasite environment alone can induce this type of selection. Could exposure to different parasite genotypes over time, in the absence of coevolutionary change, be sufficient to drive fluctuating selection in host populations?

Model organisms that exhibit mixed mating systems, such as *Caenorhabditis elegans,* provide an opportunity to study mating dynamics and host-parasite interactions in experimental evolution studies. *C. elegans* are androdiecious (males and hermaphrodites), and the hermaphrodites can reproduce sexually with males or through self-fertilization (Riddle et al., 1997). Therefore, by evaluating the rate of outcrossing in *C. elegans* populations within varying environmental conditions, factors that contribute to the maintenance of outcrossing can be determined. In addition, survival assays can be performed on *C. elegans* populations to determine relative parasite resistance between populations as a way to test for the presence of fluctuating selection.

Experimental evolution projects have been conducted to determine how the presence of *Serratia marcescens*, a parasitic bacterium, impacts the maintenance of outcrossing in *C. elegans*. It was found that the presence of *S. marcescens* leads to the maintenance of outcrossing in populations of *C. elegans* hosts (Morran et al., 2009). In addition, the presence of coevolving *S. marcescens* increases the frequency of outcrossing in populations of *C. elegans* (Morran et al., 2011; Figure 1). The Red Queen hypothesis provides a potential explanation for this phenomenon. This hypothesis predicts that *C. elegans* coevolving with *S. marcescens* must constantly adapt to resist infection as a response to antagonistic fluctuating selection from an ever-evolving parasite (Morran et al., 2011).

An experimental evolution project previously conducted in the Morran lab evolved *C. elegans* populations either in the presence of one coevolving *S. marcescens* strain, one of three non-coevolved *S. marcescens* strains, or a changing environment in which different, non-coevolved parasite strains were used and switched every four generations (McCauley et al., in prep). A total of three different *S. marcescens* strains were used in the "switch" treatment. The goal of this project was to determine if the presence of changing parasitic bacteria imposed fluctuating selection that favored the maintenance of outcrossing, or if the presence of coevolving parasites was integral to this process, in accordance with the Red Queen hypothesis. It was determined that *C. elegans* populations that were coevolved with *S. marcescens* exhibited the highest rate of outcrossing, while populations from the "switch" treatment had low outcrossing rates. However, the exact mechanism of the selective advantage of outcrossing in hosts in coevolving populations is unclear.

To assess the prediction that this advantage is conferred by the ability of outcrossing to provide novel genotypes when a population is exposed to antagonistic fluctuating selection, time

shift experiments were performed after creating inbred lineages of coevolved populations (McCauley et al., in prep). It was found that the survival rates between genotypes varied, and that different genotypes showed increased survival against coevolved *S. marcescens* from different points in time. This suggests that antagonistic fluctuating selection was acting on the coevolved hosts, causing genotypes with greater fitness to become less fit over time as the parasite evolved to infect the most common genotype. However, it is unclear whether this effect of antagonistic fluctuating selection is unique to parasites that change due to coevolution, or simply any changing parasite genotype.

To test the prediction of fluctuating antagonistic selection being a driving force for the maintenance of outcrossing in *C. elegans* in coevolved populations, these same tests must be performed on *C. elegans* populations that were not coevolved with *S. marcescens* and instead experienced changing parasitic environments during the experimental evolution project. Since antagonistic fluctuating selection can occur as a result of a changing parasite, it is possible that *C. elegans* evolved in the presence of different *S. marcescens* strains at different points in time may be subjected this type of selection. In a population such as this, individual genotypes within a host population would be expected to exhibit varying survival rates against different strains of the parasite that they were exposed to. For example, if antagonistic fluctuating selection occurred, one genotype would be expected to have relatively high survival rates in the presence of one strain, but not the other strains, while another genotype may have high survival rates against a different strain. While this pattern was found in populations of *C. elegans* coevolved with *S. marcescens,* it is unknown if antagonistic fluctuating selection is also present in host populations exposed to different bacterial strains at different points in time.

This study aims to detect signals of antagonistic fluctuating selection by determining if survival rates in the presence of different parasite strains vary between individual genotypes within *C. elegans* population replicates evolved in the presence of changing, non-coevolving *S. marcescens* strains. It is hypothesized that survival rates between the genotypes will not vary significantly because the lack of coevolution resulted in directional selection as opposed to fluctuating selection. This aligns with the finding that outcrossing rates in these populations were reduced after approximately eight generations, due to outcrossing no longer conferring a selective advantage over self-fertilization (McCauley et al., in prep; Figure 1). Ultimately, I predict that evidence for antagonistic fluctuating selection within the nematode populations that were subjected to a changing bacterial environment will not be found. Instead, I postulate that antagonistic fluctuating selection imposed by parasitic bacteria on host populations is unique to coevolving systems, and this interaction drives the maintenance of outcrossing in *C. elegans* by selecting for the frequent emergence of novel genotypes.

Methods

This project was completed using the four replicate *C. elegans* populations from the "switch" treatment of the experimental evolution described previously. The nematodes used in this evolution experiment were derived from the Hawaiian CB4856 strain of *C. elegans* and mutagenized as described by Morran et al. (2011). The "switch" treatment involved transferring nematode populations to a new *Serratia* Selection Plate that contained non-coevolving bacteria once per generation. Further, every four generations, the strain of *S. marcescens* used was switched. A previous model of antagonistic fluctuating selection determined that a fluctuating selective force must change every two to five generations to impose strong enough selection for recombination to be advantageous (Peters & Lively, 1999). Therefore, the strain was switched every four generations to mimic these findings. The nematodes were exposed to *S. marcescens* strain SM2170 first, then strain SMD1, and finally strain CoF 1-20 before starting the cycle over again. These specific strains were chosen because significant cross-strain resistance was not previously found after evolving *C. elegans* with one strain (Lynch et al., 2018). In addition, these strains are highly virulent and are thus able to impose strong selection (Lynch et al., 2018). During the evolution process, every fourth generation of nematodes was frozen and kept in a -80ºC freezer. Replicate populations from the nineteenth generation were thawed and used for this project. The nineteenth generation of nematodes was used for this project because previous analyses were performed on the same generation of the coevolved treatment. Since assays were performed on the nineteenth generation of nematodes, it is important to note that the order and recency of the bacterial environments may impact results. For example, since the nematodes were exposed to SM2170 first, all nematodes susceptible to infection by that strain may have not survived to reproduce beyond the first few generations. In addition, since the nematodes were

most recently exposed to SMD1, it is possible that increased resistance to this parasite strain may be noted.

This project occurred in two phases. The first phase involved isolating individual lineages from each replicate population of the original experimental evolution project (Figure 2). This allowed for survival assays to be performed to determine if survival rates after exposure to each parasite varied in between lineages of the same replicate population. To isolate genotypes, a single hermaphrodite nematode was transferred, or "picked," from a thawed replicate population and placed onto a 60mm NGM-lite plate seeded with *Escherichia coli* OP50. There were five original replicate populations, each labeled T7 and then a number between 1 and 5 (e.g., T7-1). However, the third replicate population was not assayed in coevolved treatments, so only T7-1, T7-2, T7-4, and T7-5 were used in this experiment (McCauley et al., in prep). Five different lineages were isolated from each population for a total of 20 distinct lineages. For each population, the five replicates were labeled R1-5, so the final name for each lineage was, for example, T7-1 R1. Each generation, approximately every three to four days, one nematode from each lineage was randomly selected and transferred to a new plate. This process was repeated for six generations. After several generations of inbreeding, the hermaphrodite populations became homozygous and thus representative of a single genotype within the original replicate population (Lynch & Walsh, 1998). Once the twenty lineages were isolated, all lineage populations were bleach-synced to kill any bacterial or fungal contamination and synchronize the life stage of the population to help prepare the nematodes for freezing (Stiernagle, 2006). Then, several samples of nematodes from each lineage population were frozen and stored in a -80ºC freezer to prevent further aging and population growth.

Phase two of this project involved performing survival assays on all lineages of *C. elegans* using *Serratia* Survival Plates (SSPs) with a strain of parasitic bacteria on one side and *E. coli* OP50 on the other side (Figure 3). The survival rates of nematodes in the presence of *S. marcescens* SMD1, SM2170, or CoF-120 was measured. Three replicate SSPs of each bacterial strain were used per lineage, and nematodes from each lineage were also plated onto three control plates containing *E. coli* OP50 to validate total population count. To determine overall survival rate, the number of nematodes that survived and crawled to the middle or OP50 side of the SSP were counted, and then this value was divided by the average control plate nematode count. Approximately 200 nematodes were placed onto each SSP or control plate.

To contextualize the results of this experiment, the survival rates of the lineages from the "switch" treatment will be compared to the survival rates of lineages from the coevolved treatment of the original experimental evolution project mentioned previously (McCauley et al., in prep). For the coevolved treatment, nematodes were evolved on *Serratia* Selection Plates and transferred to a new *Serratia* Selection Plate once per generation. However, each generation, new *Serratia* Selection Plates were seeded with *S. marcescens* found in the gut of dead *C. elegans* from the current generation to impose selection on parasites for increased virulence. Thus, the parasitic bacteria and host reciprocally influenced the evolution of one another in this treatment. The same inbreeding and survival assay protocols used in my experiment were completed using four replicate populations from the nineteenth generation of the coevolved treatment, except survival assays were performed using the coevolved *S. marcescens* from generations fifteen, nineteen, and twenty-three to determine if evidence of antagonistic fluctuating selection was present at these time points (McCauley & Morran, n.d.).

The survival assay protocol is based on protocol from Morran et al., 2011. Since each survival assay was a five-day process, two to four lineages were tested per week against all three *S. marcescens* strains due to time constraints, and lineages were thawed as needed. Initially, lineages of nematodes were given two weeks to thaw and repopulate to have enough nematodes for survival assays, but this was reduced to one week for the last month of the project since it was determined that the extra week was not necessary for adequate proliferation. Because the populations were presumed to be homogenous, this minor change in protocol was not expected to change results. In addition, reducing the amount of time between thawing and survival assays decreased the chance of contamination. The five-day protocol for survival assays is detailed below.

Survival Assay Protocol

Day 1:

Nematode lineages for each week's assay were bleach-synced to kill any potential bacterial or fungal contamination and synchronize the life-stage of nematodes assayed. Three Luria broth (LB) tubes were seeded with either *S. marcescens* SM2170, SMD1, or CoF-120 and placed in the 28ºC incubator. Lineages for assays taking place in 1-2 weeks were thawed. Thawed nematodes were plated onto large, NGM lite agar plates seeded with *E. coli* OP50. All nematodes, including bleach synched and thawed lineages, were stored in a 20ºC refrigerator.

Day 2:

SSPs were created. One third of each plate was seeded with one strain of S. marcescens, and then a third of plate on the opposite side was seeded with *E. coli* OP50. A one-inch-long space was left in the middle of the plate and was expected to remain relatively bacteria-free (Figure 3). All SSPs were placed in the 28ºC incubator for proliferation of bacteria.

Day 3:

Nematodes were plated onto control plates and SSPs. First, all lineages were washed off "holdover" plates. Once washed, each lineage population of nematodes temporarily remained in a centrifuge tube with M9 buffer. To determine the density of nematodes in the liquid, 20 µL of liquid was plated into three wells of a watch glass, and the number of nematodes were counted. If necessary, additional M9 buffer was added to the centrifuge tube of nematodes such that there

were approximately 30-60 nematodes per 20 µL. After counting the number of nematodes in each of the three wells, the volume of M9 that contained approximately 200 nematodes was calculated. Next, prior to plating the nematodes onto SSPs and control plates, 20 µL of 200 ug/µL of ampicillin was pipetted onto the center of all SSPs such that a line of antibiotic divided the two sides of bacteria (Figure 3). The purpose of this is to slow the spread of *S. marcescens* to the *E. coli* side of the plate by nematodes. Finally, the appropriate volume of M9 was pipetted onto each plate to plate 200 nematodes. For SSPs, nematodes were pipetted onto the edge of the plate, towards the center, on the side containing *S. marcescens* to ensure exposure to the parasite. For control plates, nematodes were simply pipetted onto the center of the plate. In addition, the centrifuge tube of nematodes was inverted prior to pipetting and once every four plates to ensure the mixture was homogenous. After all nematodes were plated onto SSPs and control plates, all plates were placed in the 20ºC refrigerator.

Day 4:

The number of nematodes on each control plate was counted and documented.

Day 5:

The number of alive nematodes in the middle of the plate and the OP50 side of each SSP was counted and documented.

Statistical Analysis Methods

The percent host survival was calculated for each replicate assay of each lineage from the "switch treatment" against each parasite strain. The mean percent survival was then calculated for each lineage against each parasite strain. Then, I compared switch treatment lineages mean survival against survival data from the ancestral strains, lineages from the coevolved treatment, and compared survival within and across lineages against the 3 parasite strains of the switch treatment. I used a GLM fitted with a normal distribution and identity function. In the comparison with the ancestor, I tested the effect of treatment (ancestor vs experimentally evolved) on percent survival. In the comparison with the coevolved populations, I tested the effect of treatment (coevolution vs switch) on percent survival. Then, I tested the effects of lineage, parasite strain, and lineage x parasite strain on percent survival within each population of the switch treatment. I then performed post-hoc contrast tests to compare within the lineage x parasite strain interactions.

Results

I performed survival assays on isolated genotype lineages from generation nineteen of *C. elegans* populations that evolved in the presence of changing, non-coevolved *S. marcescens* strains. Then, I obtained survival rate data of each lineage when exposed to *S. marcescens* strains SMD1, SM2170, or CoF-120. First, the survival rates in the presence of SMD1, CoF-120, or SM2170 of all "switch" treatment host lineages were compared to the survival rates of the ancestral strain of hosts from which they evolved. The evolved lineages exhibited significantly higher survival rates than the ancestral nematodes for all three *S. marcescens* strains (Figure 4, ($X^2_1 = 45.46$, $p < 0.001$). This indicates that the host populations evolved to resist infection by parasites.

Next, survival rates of the "switch" treatment lineages were compared to survival rates of lineages from the coevolved treatment, in which nematodes were coevolved with *S. marcescens* as opposed to being exposed to different strains of bacteria at different periods of time. Survival assays for the lineages from the "switch" treatment used the same bacterial strains the nematodes were exposed to during the evolution experiment. Meanwhile, the assays for the lineages from the coevolved treatment used coevolved bacteria from generations fifteen, nineteen, and twenty-three of coevolution. Therefore, the survival data specifically represents survival against relevant strains from the evolution experiment. While the survival assays did not use the same strains for both the coevolved and "switch" treatment, comparing this data provides context for the performance of generation nineteen nematodes against bacteria their ancestors were exposed to (generation fifteen), current bacteria (generation nineteen), and bacteria from a future generation (generation twenty-three). By using a time-shift experimental approach, evidence for selection that fluctuates over time can be detected. I found that the average survival rate for the "switch" treatment (mean survival = 47%) was significantly higher than the average survival rate for the

coevolved treatment (mean survival 31%) (Figure 5, ($X^2_1 = 83.94$, $p < 0.001$). Therefore, selection imposed by parasites in the coevolved treatment was stronger than the "switch" treatment.

To determine if evidence of antagonistic fluctuating selection was present in the "switch" treatment lineages, several within-population analyses were completed (Figure 6). In populations experiencing antagonistic fluctuating selection, we would expect to see parasite strain by lineage effects in which different lineages exhibit varying strain-specific adaptation. Lineage effects, parasite strain effects, and parasite strain by lineage effects were analyzed per population. For populations T7-1 and T7-2, no significant differences were found in survival rates across lineages, regardless of parasite strain ($X^2_8 = 3.68$, $p = 0.885$, $X^2_8 = 6.63$, $p = 0.578$). In population T7-4, significant lineage effects were noted ($X^2_4 = 18.74$, $p < 0.001$, but no parasite strain effects by lineage were found ($X^2_8 = 8.25$, $p = 0.409$). Population T7-5 was the only population where lineage, parasite strain, and parasite strain by lineage effects were found ($X^2_8 = 18.76$, $p = 0.0163$). However, survival rates for the three most resistant lineages within T7-5 (R2, R3, and R5) when exposed to strain SMD1 were not significantly different ($X^2_1 = 0.183$, $p = 0.668$), and there was also not significant variance of survival rates for exposure to strain CoF-120 ($X^2_1 = 0.445$, $p = 0.505$). For strain SM2170, lineage T7-5 R5 exhibited significantly greater survival when compared to the other four T7-5 lineages ($X^2_1 = 17.89$, $p < 0.0001$). However, lineage T7-5 R5 exhibited relatively high survival rates for all three bacterial strains. Therefore, this lineage likely evolved a broad resistance mechanism as opposed to adapting to a single parasite strain.

Discussion

In accordance with the Red Queen hypothesis, coevolving host-parasite systems are expected to increase the rate of outcrossing in hosts. Antagonistic fluctuating selection imposed by parasites onto hosts in coevolving populations likely provides a mechanism for the maintenance of outcrossing, and it is likely that these effects are unique to coevolving populations.

In host populations exposed to non-coevolved, changing parasite strains, evidence for antagonistic fluctuating selection was not found. However, host adaptation did occur over time in the "switch" treatment, as reflected by the increased average survival rates in evolved populations when compared to the ancestor (Figure 4). While the presence of adaptation indicates that selection occurred, within-population analyses of genotype survival data did not provide evidence for antagonistic fluctuating selection. For populations T7-1 and T7-2, all lineages within each population exhibited similar survival rates for all three *S. marcescens* strains (Figure 6). This indicates that the nematodes in these populations acquired parasite resistance that was effective against all three strains. This likely limited the potential for antagonistic fluctuating selection if the mechanism of resistance did not experience negative selection during switches to a different parasite strain. In these populations, it may be that a fitness peak was reached, and the resistant genotype spread across the population. In population T7-4, while there were some differences in survival across the five lineages, each lineage exhibited similar survival rates against the three parasite strains (Figure 6). Certain lineages had higher survival rates than other lineages from the same population in the presence of all three parasite strains, thus indicating cross-resistance as opposed to strain-specific resistance. Therefore, while some

variation between lineages existed within this population, the data did not provide sufficient evidence for antagonistic fluctuating selection.

Population T7-5 had the most variation between lineages (Figure 6), with significant parasite, lineage, and parasite by lineage effects being present. However, when examining the survival rates across lineages for each parasite, markers for antagonistic fluctuating selection are not present. If antagonistic fluctuating selection were a strong driving force of evolution in this population, we would expect to see lineages with resistance specific to one parasite strain, while other lineages would present with relatively high survival in another strain. Adaptation to a single parasite strain in the presence of antagonistic fluctuating selection would be expected to cause low resistance to other strains, as seen in the coevolved treatment (McCauley et al., in prep; Figure 5). However, in population T7-5, the survival rates for strain CoF-120 are similar across lineages, and the three most fit populations exhibit similar survival rates for SMD1 (Figure 6). The only parasite strain where one lineage has significantly increased resistance over others is SM2170 in lineage T7-5 R5 (Figure 6). However, this lineage exhibited similar survival rates for all three strains (Figure 6). Thus, population T7-5 did not show the varying, strain-specific parasite resistance expected in populations experiencing antagonistic frequency dependent selection.

While the lack of antagonistic fluctuating selection in the "switch" treatment was expected given the host outcrossing rates (Figure 1; Curtis and Lively, 1999), comparing the survival rates to data from the coevolved treatment provided intriguing results. *C. elegans* lineages exposed to changing bacterial strains exhibited higher average survival rates than nematodes coevolved with *S. marcescens* (Figure 5). The lineages from the "switch" treatment also showed greater survival rates than the ancestral strain. Initially, we expected poor adaptation

within the “switch” treatment lineages due to low outcrossing rates and the rapidly changing parasite environment. Instead, it is likely that directional selection led to high levels of cross-strain parasite resistance arising in the “switch” population, thus making the evolution of novel genotypes unnecessary. These results are consistent with the findings that the first eight generations of the “switch treatment” exhibited increased outcrossing rates before falling to ancestral rates (McCauley et al., in prep; Figure 1). It is possible that directional selection in “switch” populations favored recombination in the short-term, but once cross-resistance was achieved, outcrossing was no longer favorable. This aligns with previous quantitative models and predictions that weaker selection would not favor outcrossing (Bell & Maynard-Smith, 1987; Peters & Lively, 1999). In the coevolved populations, however, the constantly evolving parasite likely imposed strong antagonistic fluctuating selection, which is predicted by the Red Queen hypothesis. Therefore, in coevolving populations, a reproduction method that allows for the creation of novel genotypes would be favored due to the selection inflicted by a coevolving parasite.

While this study supported the hypothesis that antagonistic fluctuating selection drives the maintenance of outcrossing of hosts in coevolving host-parasite systems, it does not explicitly test for negative frequency dependent selection. The theoretical framework of this project predicts that the fluctuations in selection imposed by parasites is a result of positive selection on parasites that can infect the most common host genotype (Hamilton et al., 1990). In turn, the parasite imposes negative frequency dependent selection on the most common host genotype (Hamilton et al., 1990). Therefore, future studies that test the presence of negative frequency dependent selection in host-parasite systems would provide further insight into the selective pressures that may influence the maintenance of outcrossing.

Overall, this study provided a deeper insight into the mechanisms of the maintenance of outcrossing in nature. The coevolution that occurs within host-parasite systems likely causes antagonistic fluctuating selection that acts on the host, thus making sexual reproduction advantageous in hosts. The data from this project provided evidence that antagonistic fluctuating selection is unique to *C. elegans* coevolved with *S. marcescens*, and a changing parasitic environment alone does not impose antagonistic fluctuating selection.

The results of this study may provide insight into the maintenance of outcrossing in nature, as it is expected that antagonistic fluctuating selection imposed on hosts during host-parasite coevolution leads to increased outcrossing rates. However, further studies are needed to fully understand the advantages of outcrossing and the mechanisms of this advantage. While there is evidence that antagonistic fluctuating selection likely plays a significant role, parasites may not be necessary to impose this type of selection. A previous study found that predator-prey interactions may also lead to antagonistic fluctuating selection that increases the frequency of sexual reproduction in prey (Koch et al., 2020). Therefore, general antagonistic symbiosis may be integral to the ubiquity of sexual reproduction. Since my project determined that antagonistic fluctuating selection is likely exclusive to coevolving host-parasite systems, future studies that analyze this type of selection in other coevolving systems may provide further insight into the maintenance of outcrossing in nature.

Figures

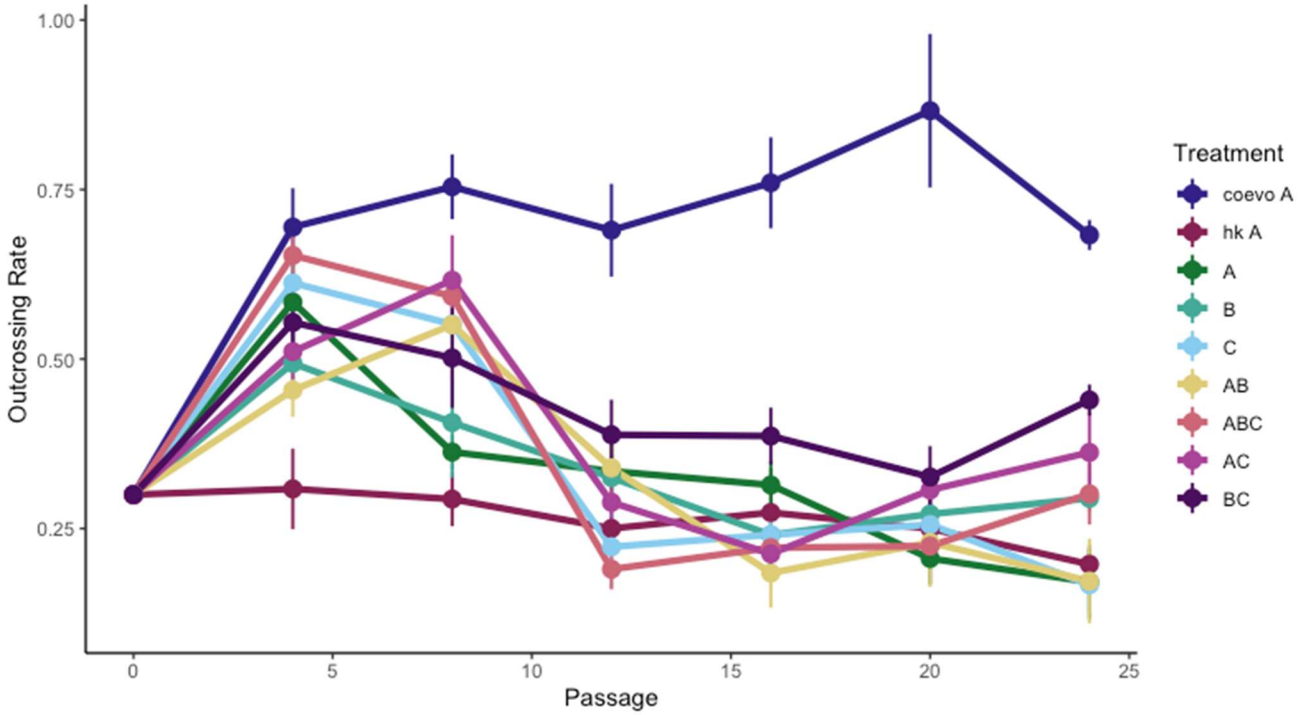

***Figure 1. Caenorhabditis elegans* coevolved with *Serratia Marcescens* experience higher outcrossing rates (McCauley et al., in prep).**

Outcrossing rates of *C. elegans* populations evolved in the presence of different parasitic bacterial environments were determined. Coevolved nematodes exhibited significantly higher outcrossing rates than all other treatments over time, while all other experimental treatments showed a temporary increase in outcrossing that decreased over time. "Coevo A" refers to nematodes coevolved with *S. marcescens*. "hk A" denotes *C. elegans* evolved with heat-killed, avirulent bacteria to function as a control. Treatments A, B, and C involved evolution with one of three different, non-coevolving bacterial strains. Treatments AB, ABC, AC, and BC were "switch" treatments in which nematodes were exposed to different, non-coevolved *S. marcescens* strain that were switched every four generations. For two-lettered treatments, only two different strains were used, and all three strains were used for treatment ABC. Treatment ABC is the treatment population from which the nematodes used in this experiment originated.

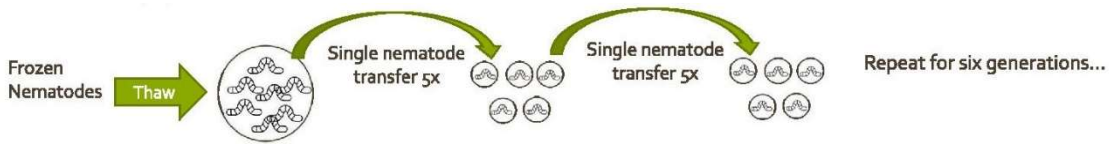

Figure 2. **Phase 1 Protocol Schematic**

Phase 1 of this experiment involved isolating individual genotypes within replicate populations from the "switch" treatment of experimental evolution. Five hermaphrodites were randomly and individually transferred from each population to a new plate. Then, once per generation or every three to four days, a single hermaphrodite from each plate of the current generation was transferred to a new plate. This was repeated for six generations to create a homogenous lineage representative of a single genotype within each original population.

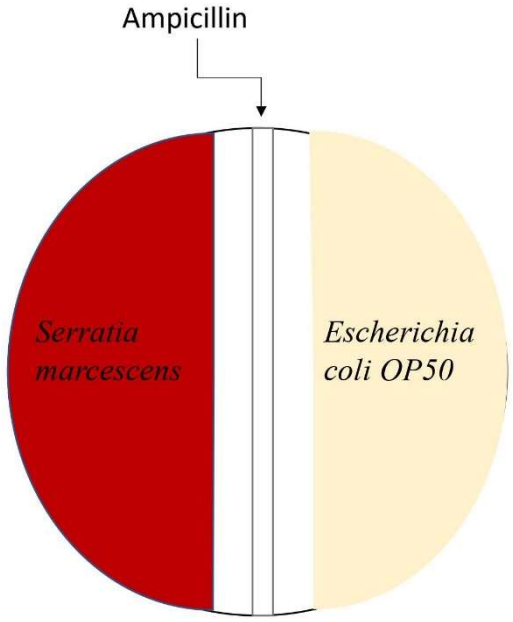

Figure 3. Serratia **Survival Plate (SSP) Schematic**

Nematodes were plated onto a *Serratia* Survival Plate (SSP) during survival assays. A one-inch-wide segment of the plate was left in the middle and expected to remain relatively bacteria free. Then, one side of the plate was seeded with *S. marcescens*, and the other side was seeded with *Escherichia coli* OP50. 20 µL of 200 mg/µL ampicillin was poured down the middle of each SSP.

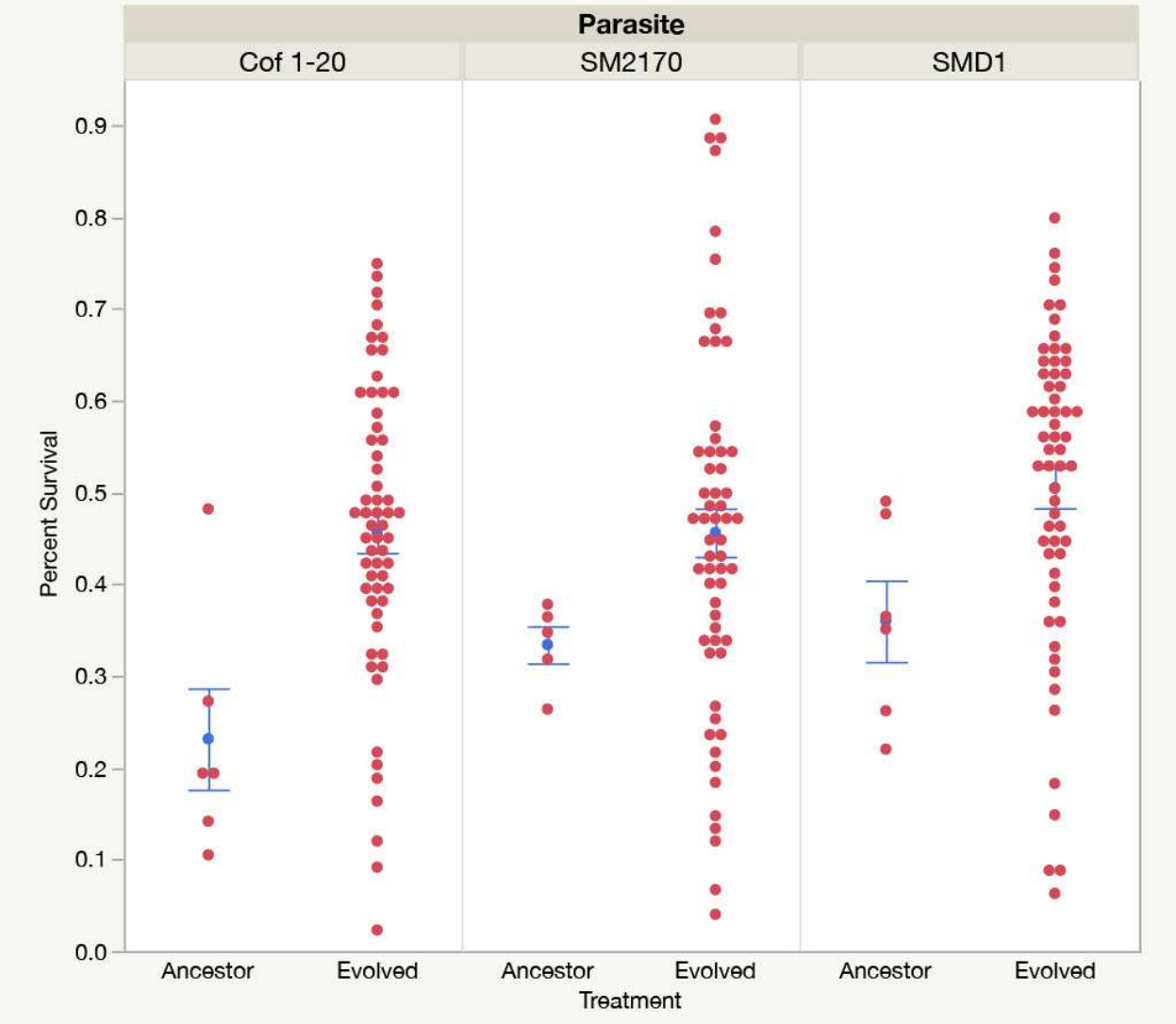

***Figure 4. Caenorhabditis elegans* evolved in the presence of changing, non-coevolved *Serratia marcescens* strains exhibited higher survival rates than the ancestral nematode strain.**

The average survival of *C. elegans* populations before and after evolution in the presence of changing, non-coevolved *S. marcescens* strains was compared. Evolved nematodes exhibited increased survival rates in survival assays with *S. marcescens* when compared to ancestral nematodes, thus providing evidence for host adaptation. Average percent survival of each replicate is represented by a red dot. Error bars represent the standard error. The ancestral nematode populations are represented by "ancestor" columns, and generation nineteen of evolved populations are denoted by the "evolved" columns. *C. elegans* were evolved in the "switch" treatment, which involved changing the parasite strain used for *Serratia* Survival Plates every four generations. Strains Cof-120, SMD1, and SM2170 were used during evolution, so survival assays were performed using these three strains.

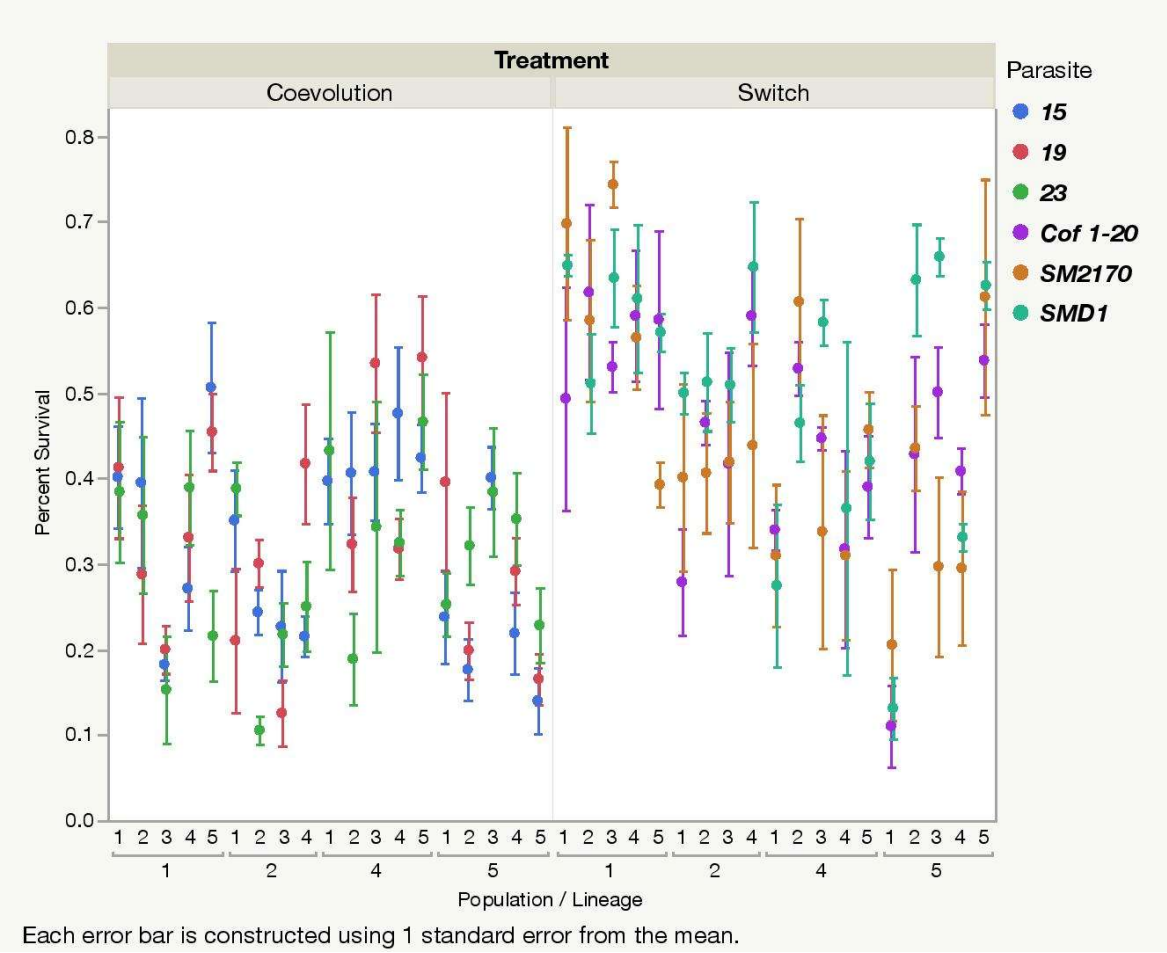

***Figure 5. Caenorhabditis elegans* lineages from the "switch" treatment exhibited higher survival rates than lineages from the coevolved treatment.**

Host survival data of individual lineages from *C. elegans* populations coevolved with *S. marcescens* was compared to data from survival assays of individual lineages of *C. elegans* populations evolved in the presence of changing, non-coevolved *S. marcescens* strains was compared. It was determined that nematodes from the nineteenth generation of the "switch" treatment exhibited higher average survival rates than nematodes that were coevolved with parasitic bacteria. Therefore, stronger selection was acting on coevolved host populations. Survival assays on coevolved *C. elegans* used coevolved *S. marcescens* from generation fifteen, nineteen, and twenty-three of the evolution experiment. Survival assays on nematodes from the "switch" treatment used S. marcescens strains Cof-120, SM2170, and SMD1. Therefore, all survival assays were performed using bacteria that was present during each population's evolution, except for parasite 21, which was from a future generation. While the same parasite strains were not used for survival assays of both treatments, the purpose of this analysis was not to directly compare fitness between populations. Instead, survival rates were used as a measure of selection, with lower survival rates indicating stronger selection imposed by parasites. Therefore, to determine selection imposed by each treatment, strains that were present during the evolution of each population were used for survival assays. By using parasite strains from different points in time, evidence for fluctuating selection could be detected.

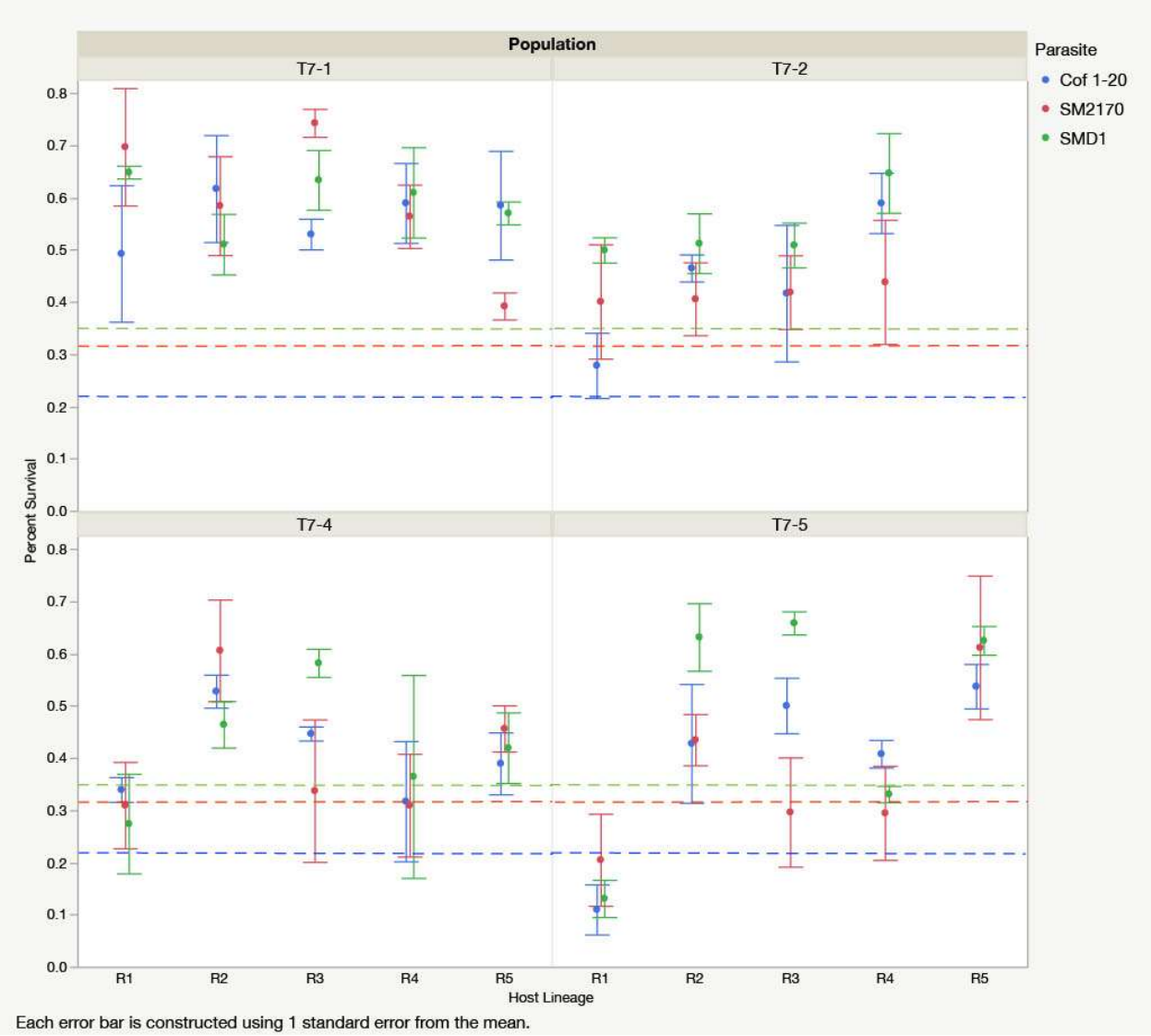

***Figure 6*. Analyses of genotypes within "switch" treatment population replicates do not provide evidence of antagonistic fluctuating selection.**

Survival assays were performed on individual lineages from *C. elegans* populations evolved in the presence of changing, non-coevolved *Serratia marcescens* strains. Analyses of the survival data did not find varying, strain-specific resistance across lineages within a single population; therefore, evidence of antagonistic fluctuating selection was not found. Each dot represents the mean percent survival of a lineage against one parasite strain. Error bars represents the standard error. Parasite strain is denoted by color. The colored, dotted lines denote the average survival rate of the ancestral nematode population.

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