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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Abstract

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Sexual reproduction is evolutionary costly, but its prevalence has generated theories which attempt to explain its maintenance in nature. One of these theories is the Red Queen hypothesis which postulates that as hosts are coevolving with parasites, they maintain a biparental mode of sexual reproduction when the costs of the parasite outweigh the benefits of asexual reproduction. The Hamilton-Zuk hypothesis extends this coevolving dynamic to parasite-mediated sexual selection in which females use males' secondary sexual characters to evaluate male vigor and resistance to parasites. To explore the intersection of sexual conflict and selection for parasite resistance, we use male *C. elegans* to test the role of selective pressures imposed by coevolving parasites in the evolution of male competition. Here, we competed experimental males with differing evolutionary histories (coevolved, evolved, and non-evolved on the parasite *S. marcescens*) against RFP-labeled tester males to reveal changes in male competition. The effect of the treatments on male competition across the populations was not consistent. On a population level, we observed more experimental male competitiveness in sexual conflicts against the RFP-labeled tester males, suggesting that population was a greater predictor of the change in male competitive ability than treatment. Characterizing male competition demonstrates the evolutionary role of male *C. elegans* in hosts exposed to parasites, framed by the Red Queen and Hamilton-Zuk hypotheses. More investigation is necessary to elucidate the connection between male tail morphology and male sexual behavior to analyze the genetic basis of parasite resistance and male competition.

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Introduction

Sex is widespread in the animal kingdom: it can generate genetic diversity to facilitate adaptations to a changing fitness landscape. While "sex" can describe different biological phenomena, such as anisogamy or meiosis, we focus on "sex" as genetic recombination resulting from the fusion of two dissimilar gametes from separate, dioecious parents (Orive, 2020). Sex is evolutionarily costly when accounting for: the cost of (1) males and (2) breaking up beneficial, parental genotypes (Maynard Smith, 1978). Not only do asexual populations increase at a rate that is twice that of sexual populations, but an asexual female will also pass her entire genome to her female offspring, thereby ensuring transmission of 100% of her alleles to future asexually producing generations. Producing male offspring only ensures the inheritance of 50% of a female parent's genome, while 50% of her resources are expended on producing a male offspring who cannot directly make his own offspring. When assuming that sexual and asexual females are equally fecund and their offspring have similar survival rates, sexual reproduction is demonstrably more costly in terms of resource expenditure and offspring production. However, it is well-known that sexual reproduction is ubiquitous in eukaryotic organisms. This paradox of sex gives way to theories examining the maintenance of sex in nature. Theory postulates that sexual reproduction is beneficial, such that recombination allows for the introduction of novel or rare genotypes on which selection can act (Radwan *et al*., 2016). On the other hand, the disadvantages of asexual reproduction are cited for the maintenance of sexual reproduction, such as Muller's ratchet in which asexual populations accumulate deleterious mutations (Muller, 1932). The Red Queen hypothesis seeks to explain the maintenance of biparental sexual reproduction through antagonistic fluctuating selection between biological enemies (Bell, 1982). This leads to an "arms race dynamic" between hosts and parasites, wherein both are constantly evolving to get ahead of the other. Essentially, hosts are constantly evolving resistance to evade parasites through the generation of rare resistance genotypes, while parasites are simultaneously evolving to continuously infect the host. The Red Queen hypothesis postulates that sex is maintained if the costs of parasites outweigh the benefits of asexual reproduction.

The costs of sex, however, are understudied relative to the benefits of sex (Gibson *et al*., 2017). One such cost is the energy and time investment required for mate selection. Females are often choosier in selecting mates than males, as females often produce fewer, larger gametes that are limited in quantity. Females reap higher fitness benefits by fertilizing their eggs with higher quality sperm from males who are more fit. This forces males to compete for access to females and mate with multiple females in order to optimize their reproductive fitness. Such differential reproductive success results from the ability to obtain mates and fertilized oocytes, and forms the basis of sexual selection (Darwin, 1859; Luck and Joly, 2005). Sexual selection involves mate choice and male competition. In mate choice, males must attract females so that female choice dictates mating. In male competition, the ability of males to compete with each other ultimately allows successful males to obtain access to females and reproduce. Male competition may materialize in a variety of ways: (1) the evolution of weapons to fight for access to females (Areja-Gavina, Torres, and Gamilla *et al*., 2021), (2) engaging in sneaking behavior to evade aggressive males and gain access to females (Newman and Higham, 2019), (3) sperm competition to evolve faster or larger spermatocytes to better inseminate oocytes (Vielle *et al*., 2016; LaMunyon and Ward, 2002), and (4) mate guarding in which males restrict access to their female partners (Elias *et al*., 2014). Previous investigations exploring male competition in *C. elegans* primarily concentrate on the phenotypic consequences of the competition on males, such as larger sperm sizes (Vielle *et al*., 2016; Ward and Carrel, 1979), while others have found evidence of copulatory plugs within the vulva of females after mating (Woodruff *et al*., 2014).

We are especially interested in the interaction between sexual selection and selection for resistance to a parasite in hosts that were coevolved on parasites to better understand the intersection of selective forces on populations. The Hamilton-Zuk hypothesis postulates that females use males' secondary sexual characteristics to evaluate their resistance to parasites, signaling to females their vigor and genetic resistance (Hamilton and Zuk, 1982). This hypothesis assumes that hosts are continuously coevolving with parasites so that resistance to parasites is heritable (Hamilton and Zuk, 1982). Females will, therefore, choose the most ornate males—their secondary sexual traits do not pose a handicap to their fitness and survival—and thus pass on strong secondary sexual traits and resistance genes for their offspring. For instance, the genes involved in adaptive and innate immunity were found to regulate the feather patterning responsible for ornate tail plumages in male peacocks (Jaiswal *et al*., 2018). Such tail feathers are selected for and preferred by female peacocks, causing heritable secondary sexual traits to become associated with immunity (Jaiswal *et al*., 2018). Past studies have examined the hypotheses contributing to parasite-mediated sexual selection, namely by observing that (1) females avoid infected males with directly transmissible parasites, (2) females choose less-parasitized males who provide better parental care, (3) females choose males with lower parasite loads to pass on parasite resistance genes to offspring (Møller *et al*., 1999). A metaanalysis of studies investigating the relationship between secondary sexual characteristics, parasitic abundance, and host immune function revealed that secondary sexual displays and parasite intensity varied across host-parasite systems, but host immune defenses were generally more strongly associated with secondary sexual characters than parasite loads (Møller *et al*., 1999).

This means that parasites play a large role in the sexual selection of hosts, as secondary sexual characters seem to reflect stronger immune defenses and functions. Noticeably, these experiments focus on female mate choice rather than male competition. There is a lack of literature and experimentation exploring the interaction between male competition and selection for resistance to parasites. One of the few studies on the topic recently found that male competition resulted in precopulatory sexual selection against parasite load (Gómez-Llano, 2020). This enforces sexual selection against males with a higher parasite load, and reinforces the Hamilton-Zuk hypothesis wherein females seek healthier males with genetic resistance.

In an effort to expand upon the key questions of sexual selection and address the gap in the intersection of sexual conflict and selection for parasite resistance, we turn to experimental evolution using the model system *Caenorhabditis elegans* to test the role of selective pressures imposed by coevolving parasites in the evolution of male competition. The coevolution of *C. elegans* with the virulent pathogen *Serratia marcescens* involves an evolutionary arms race between the nematodes and the pathogen to adapt to a constantly changing ecological landscape: *C. elegans* evolve survival mechanisms and resistance to *S. marcescens* while *S. marcescens* evolve the means to infect *C. elegans* (Morran *et al*., 2011). Such an interaction is consistent with the Red Queen hypothesis in which selection for biparental sex in a constantly evolving, virulent environment of parasites was imposed on the nematodes (Morran *et al*., 2011). Although this experiment found evidence of increased outcrossing rates in *C. elegans* when exposed to the parasite *S. marcescens, there has not been a comprehensive characterization of the mating ability of male C. elegans that have evolved resistance to parasites, especially since the presence of males drives outcrossing*. Such an increase in outcrossing rates could significantly increase male competition since males maximize their reproductive success by mating with as many females as possible, thereby heightening the competition between males to mate and spread their genes. Males are generated primarily through outcrossing in the previous generation. Since half of the progeny of one male will be male, more males will be present in the population. This results in more outcrossing and a higher number of males that must compete to sire offspring in the next generation. Therefore, this investigation seeks to elucidate the dynamics between male competition and the selective pressures imposed by coevolving, pathogenic evolutionary history. The extent to which sexual selection facilitates or hinders the evolution of resistance may occur in three possible ways: (1) sexual selection and selection for resistance work in the same direction, facilitating each other, with sexual selection speeding up the evolution of resistance, (2) there is no interaction between sexual selection and selection for resistance, or (3) sexual selection and selection for resistance counteract each other and work in opposite directions. Refer to **Figure 1**.

Figure 1. Schematic representing the possible interactions between sexual selection and selection for resistance.

An experimental evolution approach was taken to parse through the relative contribution of the different selective forces that interact in the evolution of resistance to a parasite. Experimental evolution allows for the isolation of variables that are particularly helpful in exploring the strength of different types of selection on a single system, removing the influence of uncontrolled variables. In order to evaluate evolutionary forces and observe measurable changes within a short timescale, we performed the experiment within the laboratory using the model system *Caenorhabditis elegans*. *C. elegans* are ideal for this experimental evolution investigation due to their short generation times (~3 days), our extensive knowledge of the nematodes' genome, and the ability to cryogenically preserve nematodes for sexual selection assays that have previously evolved resistance to parasites (Frézal and Félix 2015).

To determine the effects of parasite exposure and host-parasite coevolution on male competition, we competed obligately outcrossing *C. elegans* males that were coevolved on *S. marcescens*, evolved on *S. marcescens*, evolved on heat-killed *S. marcescens* (control), and ancestral populations against red fluorescent protein (RFP)-labeled tester male *C. elegans*. Given the rapid adaptation to the pathogen exhibited by the coevolved *C. elegans* host populations (Morran *et al*., 2011; Morran *et al*., 2013; Penley, Ha, and Morran, 2017), we predicted that the coevolving males will be the most competitive in sexual conflicts against the RFP-labeled tester males as male competition helps drive outcrossing, which generates novel genotypes and allows for resistance to evolve. We subsequently predicted that control males evolved on heat-killed *S. marcescens* will be the least competitive in male competition against the tdTomato CB4856 RFP-labeled tester males as a consequence of their lack of exposure to parasites, resulting in less of a selective force to evade the pathogen and compete with other males. Either way, any change measured in the competitiveness of the experimental males promises interesting implications and insights into the complex interaction between evolutionary history in a pathogenic environment and sexual selection.

Methods

C. elegans maintain three modes of reproduction that can be exploited for specific experimental uses, namely obligately selfing, obligately outcrossing, and a mixed-mating system. In this experiment, we utilize obligately outcrossing populations which require the fertilization of hermaphrodite oocytes with male sperm in order to produce the subsequent generation. For additional information about the mating system and genetic manipulations employed in previous experiments, see the **Addendum**.

Here, we competed males with differing evolutionary histories (coevolved, evolved, and evolved on heat-killed parasite *S. marcescens*) against RFP-labeled tester males to reveal any changes in the ability of males to successfully compete for mates and reproduce. RFP-labeled tester males functioned as competitors to experimental males to deduce the identity of the father when counting offspring. The relative proportion of RFP-labeled or experimental offspring indicated the competitive ability of the tester and treatment males. To test the aforementioned hypothesis, we used obligately outcrossing populations of *C. elegan*s (loss of function *fog-2* alleles, see **Addendum**) from populations F1, F2, F3, F4, and F5—hereinafter referred to as F(1-5)—that were coevolved on *Serratia marcescens* (CoF1-5), evolved on *S. marcescens* (EF1-5), and evolved on heat-killed *S. marcescens* (CF1-5) (non-virulent parasite), which was the control (Morran *et al.*, 2011). The three separately evolving treatment populations from the ancestor are illustrated in **Figure 2** below, with the ancestor strain shown at the left and the various evolutionary conditions on the right. These treatment populations were competed against RFP-labeled tdTomato CB4856 *C. elegans*. The ancestral population was initially mutagenized through exposure to ethyl methanesulfonate to create novel genetic variation within the populations (Morran *et al.*, 2011). The mutagenized ancestral population was then distributed across the treatments (coevolved, evolved, evolved on heat-killed parasite). To clarify, each of the five populations have the same genetic background regardless of treatment; therefore, the ancestral, coevolved, evolved, and evolved on heat-killed parasite *C. elegans* from population 1 are the same population. Each population is genetically unique: population 1 is genetically distinct from population 2. Mutagenesis introduced novel alleles to each of the populations; therefore, each population has different alleles in their initial gene pool.

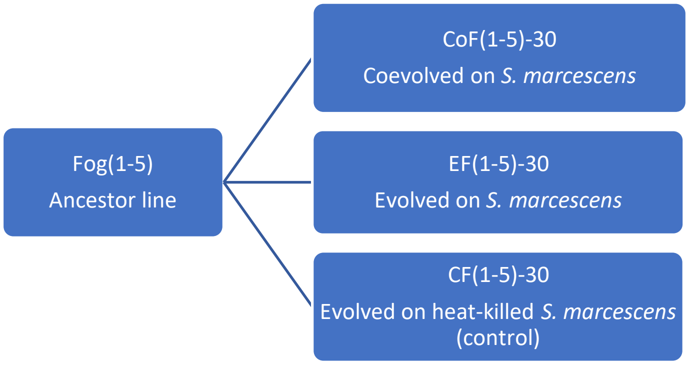

Figure 2. Schematic representing the relationship between the ancestral, coevolved, evolved and control treatments used for the experiment. Each population was comprised of the ancestor, coevolved, evolved, and evolved on heat-killed *S. marcescens* treatment groups. There were five populations comprised of the same four treatment groups. Each population (1, 2, 3, 4, 5) of four treatment groups had the same genetic background, but each individual population was genetically distinct from all other populations.

From each of the four populations, including the ancestor, coevolved, evolved, and evolved on heat-killed parasite treatments, we transferred one individual L4 female (which is a functional female, having lost the ability to self) and one adult, sexually developed male onto 35 x 10 mm mating plates that contained aseptically poured NGM with a small, 5µL circle of OP50 *E. coli* (*C. elegans*' food) at the center of the plate to help the males better locate the female. We then added three RFP-labeled tester male *C. elegans* onto the same 35 x 10 mm mating plate with the experimental female and male. For each treatment (ancestral, control, evolved, and coevolved) in each of the five populations [F-(1-5)], five mating competition replicates were performed, wherein each replicate included one experimental male and female with three RFP-labeled tester males. For example, one coevolved male and one coevolved female were plated with three RFP-labeled tester males on five 35mm plates in population 1. The process was repeated for all treatments within each of the five populations. Mating competition replicates were performed by treatment, one population at a time.

Subsequently, all mating competition replicates from one population were incubated on 35 mm diameter mating plates for 48-72 hours in a 20ºC incubator, which provided sufficient time for the nematodes to mate and the eggs to hatch. After 48-72 hours, we transferred the nematodes from the 35 mm diameter mating plates onto 100 mm diameter petri plates containing aseptically poured NGM with an OP50 *E. coli* lawn covering the entire plate to provide plenty of *E. coli* for the growth of the F(1-5) progeny. These plates were then incubated at 20ºC for 24-48 hours. After 24-48 hours, we counted the experimental and RFP-labeled tester strain progeny using a fluorescent microscope on the RFP (588 nm) setting to calculate the proportions of RFP-labeled and experimental offspring. Additionally, the total offspring count by treatment from all populations were summed and analyzed to test for a treatment-level effect. Since the RFP-labeled offspring contain a fluorophore that fluoresces red-orange when excited under the 588 nm RFP setting, the progeny were counted by examining each of the hatched nematodes for red-orange fluorophores. Discerning between RFP-labeled progeny and experimental offspring entailed transitioning between the compound microscope setting and the RFP filter (588 nm) to locate each nematode and detect the presence of red-orange fluorophores.

All *C. elegans* populations in this experiment, including the RFP-labeled and experimental nematodes, were derived from the same CB4856 strain. This means that all experimental worms, whether they were exposed to a treatment or RFP-labeled, were equally attracted to each other, thereby mitigating mate preference that results from attempting to mate *C. elegans* from disparate backgrounds. Most RFP labels interfere with the fecundity and mating ability of the *C. elegans*, warranting the addition of three RFP-labeled tester males onto the mating plate to compensate for their decreased sexual competitiveness. Biological markers such as RFP and GFP interfere with the fecundity of *C. elegans*; however, since such protein tags are passed on to progeny, they are useful morphological markers indicating which of the competing males sired the most offspring through visualization with a fluorescent microscope. Not only does this streamline data collection, but the data allows for a direct comparison between the proportion of RFP-labeled offspring and male competition. The purpose of the mating competition assays were to compare males from different evolutionary histories (ancestral, evolved, coevolved, and evolved on heat-killed parasite) to each other. RFP-labeled tester males served as a standard competitor, or baseline, so that the competitive ability of the males from each treatment could be evaluated against each other. In order to adequately provide this baseline for comparison between treatments, three RFP-labeled tester males were added.

The differential proportions of RFP-labeled and experimental offspring indicates the competitive success of the RFP-labeled or experimental sire, as the RFP-labeled and experimental males are competing to mate with the experimental female. Whether or not the offspring displays the RFP-tag indicates the identity of the father, and thus suggests which of the males (RFP-labeled or experimental) was more competitive and reproductively successful, allowing us to determine the impact of evolutionary history on male competition between treatments (ancestral, evolved, coevolved, and evolved on heat-killed *S. marcescens*) and lineages F(1-5). The more competitive male will have sired the most offspring, which can be documented based on the presence or absence of an RFP tag. For instance, if the experimental males were more competitive, a greater proportion of offspring would lack the RFP tag, and vice versa if the RFP-labeled tester male was more competitive and reproductively successful.

Statistical Analysis Methods

To determine the significance of both the number of offspring by treatment (ancestral, control, evolved, and coevolved) and the percent change of RFP-labeled offspring from the ancestor, we performed a generalized linear model fitted with a normal distribution and identify link function. In the model analyzing the percent change from the ancestor, the fixed factors included the treatment, population, and treatment x population. Overdispersion was not detected using a Pearson test. The fixed factors for the generalized linear model assessing the number of offspring by treatment included the ancestral, coevolved, evolved, and control treatments.

Results

Since siring offspring through the one experimental female is the only way for the males to successfully reproduce, the competition to mate is especially steep. This competition is heightened according to the Hamilton-Zuk hypothesis, as the experimental female is theoretically more attracted to the experimental male since he should be more resistant to parasites. Interestingly, different lineages of *C. elegans* have specific release and responses to intralineage pheromones (Bose *et al*., 2014; McGrath & Ruvinsky, 2019; Falcke *et al*., 2018). Due to this, the experimental male and female should theoretically prefer to mate with each other. The male competition assays were carried out by plating one experimental female, one experimental male, and three RFP-labeled tester males on 35 mm diameter mating plates. Following this, the proportions of RFP-labeled and experimental progeny were counted to track the siring success of the father. Whether the offspring fluoresces red-orange is indicative of which of the males (RFP-labeled or experimental) fathered the offspring. Theory posits that the presence of four males and one sexual-reproducing female will lead to significant male competition; the resulting offspring count should demonstrate the relative competitiveness and sexual virility of the experimental male. We predicted that the coevolved population will be the most competitive and the control males, evolved on heat-killed *S. marcescens*, will be the least competitive in sexual conflicts against the RFP-labeled tester males as the population rapidly adapted to the parasite and male competition drives the evolution of such resistance.

In analyzing the results for the total offspring counts—including both RFP-labeled and experimental offspring—by treatment, no statistically significant difference between treatments (ancestral, control, coevolved, evolved) was found (**Fig. 3**; df = 3, 2=4.9373, p-value = 0.1764). This means that there was likely no difference in male competition and reproductive success across all the treatments (ancestral, control, evolved, and coevolved). Each male was as competitive and reproductively successful as males from other treatments.

To explore how male competitive ability changed over the course of the experiment, we sought to determine how the proportion of RFP-labeled offspring differed according to population and treatment. In comparing the percent change of RFP-labeled offspring from the ancestor to the evolved, coevolved, and control treatments, the statistical analysis yielded no significant difference between treatments (df=2, 2=5.8463, p-value = 0.0538).

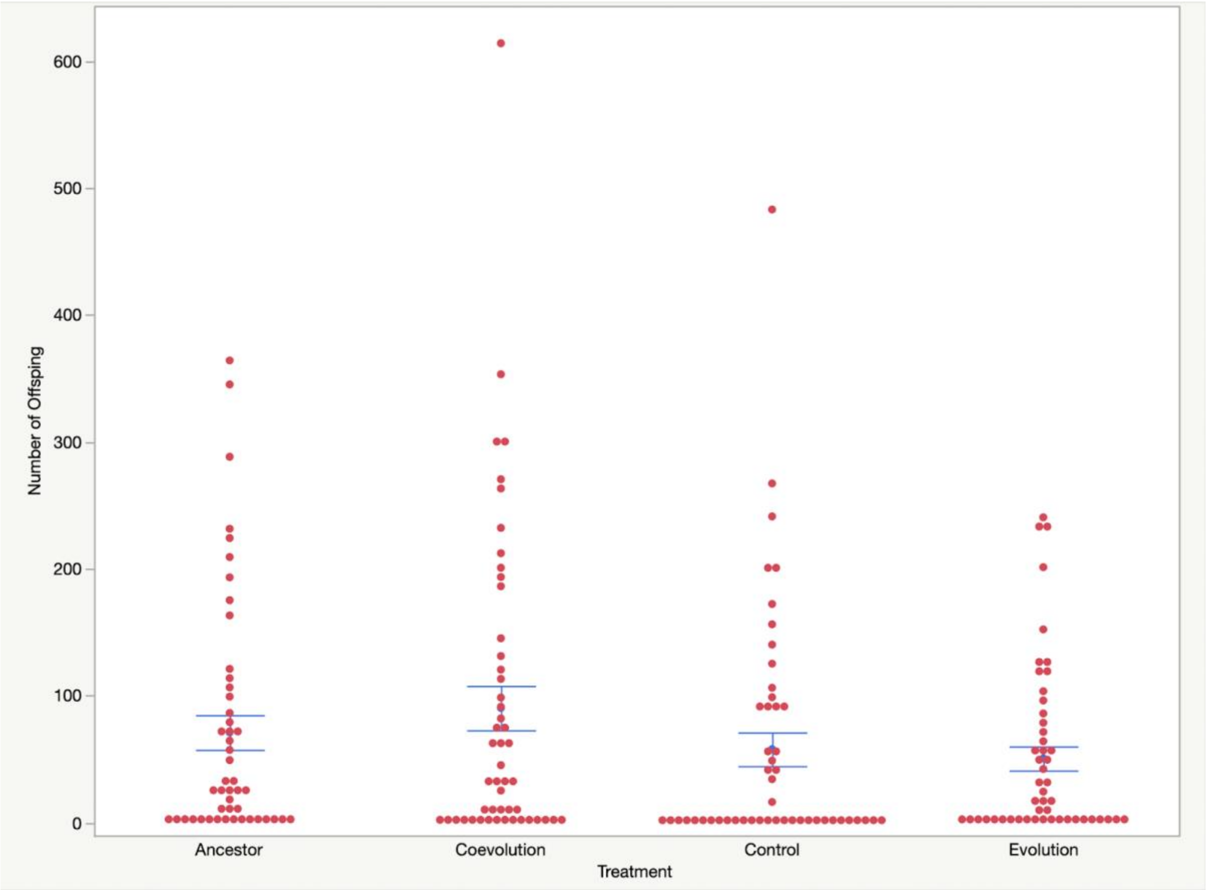

Figure 3. Total offspring counts by treatment (ancestral, coevolution, control, evolution). The total number of offspring count of experimental progeny, grouped by treatment, across all five populations. This analysis was used to compare the number of offspring between treatments to see if one treatment was associated with male competitive ability. No significance difference was observed between treatments.

However, we observed significant differences when testing for a population level effect (df=4, 2= 66.9209, p-value < 0.0001) and a population x treatment effect (**Fig. 4**; df = 8, 2= 19.4234, p-value = 0.0128). As shown in **Figure 4,** experimental males are, on the whole, less competitive than RFP-labeled tester males in populations 1 and 5, as evidenced by a more positive percent change of RFP-labeled offspring from the ancestor. In each treatment (control, evolved, and coevolved) in populations 1 and 5, the RFP-labeled tester males consistently outcompeted the experimental males. In populations 2, 3, and 4, the experimental males were generally found to be more reproductively successful than the RFP-labeled tester males as there was a negative percent change of RFP-labeled progeny from the ancestor, meaning that there was a higher proportion of experimental offspring across all treatments. This may indicate that variation in male competition is largely driven by mutagenesis rather than evolutionary history.

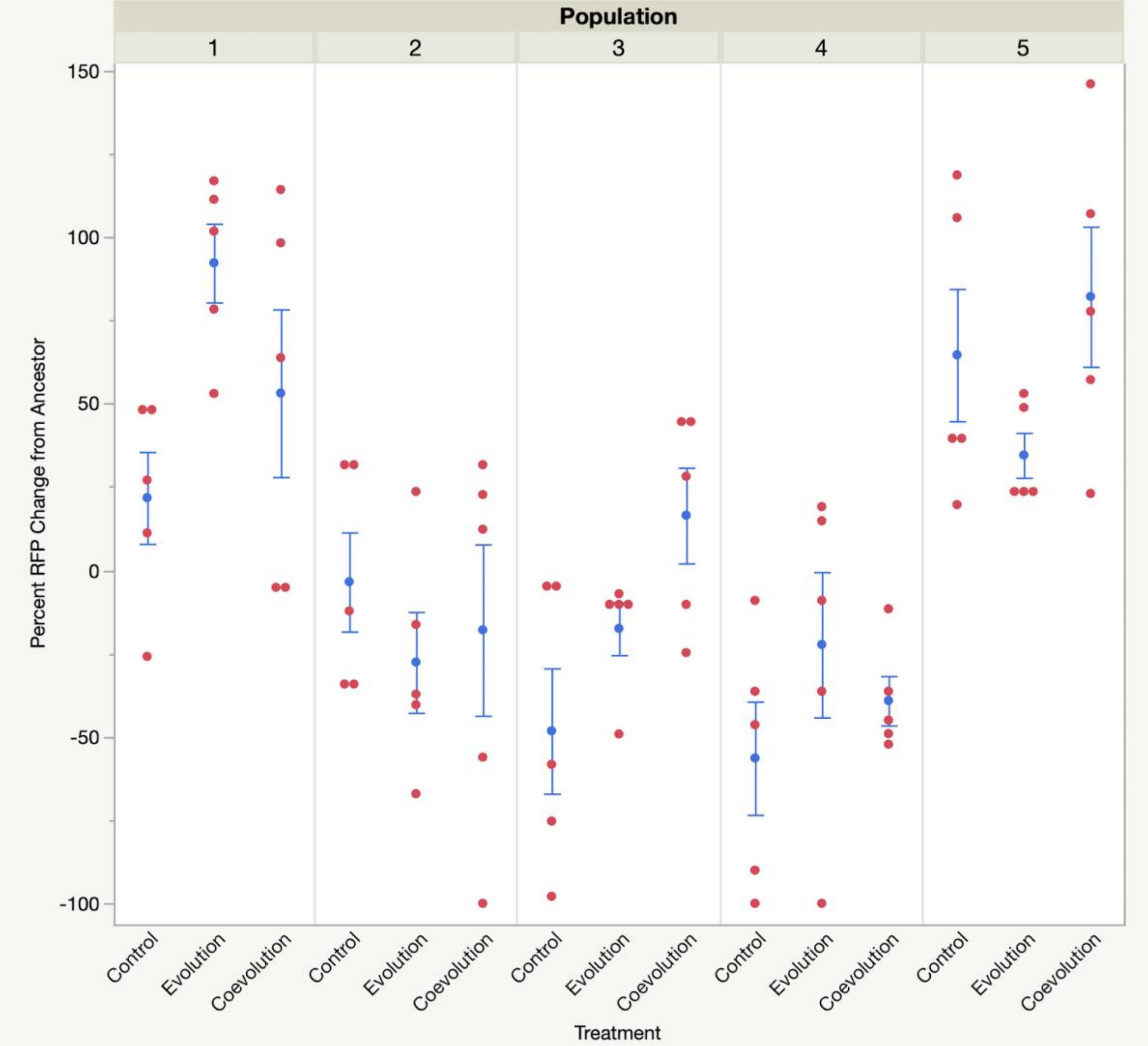

Figure 4. **Percent RFP-labeled offspring change from the ancestor, across treatment groups (control, evolution, and coevolution) and across five populations**. A *positive percent change* in RFP-labeled offspring from the ancestor indicated that experimental males were *less* competitive within the population, while a *negative percent change* in RFP-labeled offspring from the ancestor indicated that experimental males were *more* competition within the population. A population effect and population x treatment effect was observed.

Discussion

Here, we predicted that control males would be the least competitive and the coevolved males would be the most competitive in sexual conflicts, thereby assuming that there would be a treatment effect on males across all populations. This was not the case as no significant difference was observed between the total offspring counts by treatment, meaning that none of the experimental males significantly produced more progeny than males in other treatments. Treatment effects across the populations were not consistent, as RFP-labeled tester males outcompeted experimental males in some populations (1 and 5) while experimental males were more competitive than RFP-labeled tester males in other populations (2, 3, and 4). On a population level, we observed more experimental male competitiveness in sexual conflicts against the RFP-labeled tester males on the whole, rather than singling out any one treatment group as the most and least competitive. We were unable to discern the most competitive experimental male in populations 2 and 4 as there were no consistent differences in reproductive success among the experimental males. Each population's measure of male competitiveness when comparing treatments (coevolved, evolved, evolved on heat-killed parasite) was independent; this was potentially a consequence of mutagenesis introducing differing levels of beneficial alleles in the initial gene pool. This potentially emphasizes the importance of initial genetic variation within a population in determining the trajectory of male competition over the course of experimental treatments. The treatment, therefore, affected the populations, but was possibly mediated by the initial diversity of the gene pool.

These analyses suggest that population was a greater predictor of the change in male competitive ability than treatment. Both sexual selection and selection for parasite resistance were operating differentially on each population due to the initial variance in their genetic pool.

This resulted in divergent evolutionary trajectories, especially when comparing the reduction in male competition in populations 1 and 5 to the increase in male competition in populations 2, 3, and 4. In ascertaining a population effect and population x treatment effect, we were able to speculate that the underlying, initial genetic variation affects how males perform across treatments, over the course of the experiment. We speculate that the underlying genetic variation of the initially mutagenized populations was driving the change in male competitiveness and virility; yet, differences between the populations may be entirely stochastic. More analysis and sequencing are needed to determine the initial pool of novel and rare genotypes generated within each population to better conceptualize this hypothesis. Here, we found no evidence that different evolutionary histories with a parasite affected the total number of offspring (RFP-labeled and treatment). However, a population and population x treatment effect was observed in the percent change of RFP-labeled offspring from the ancestral population. Each of the five populations started with a unique pool of genetic variation, and the populations showed different responses to the treatments. This suggests that the diversity found in the initial gene pool—brought about by mutagenizing the populations—might have influenced the competitive ability of experimental males. Generating variance in the initial population may affect the extent to which experimental evolution assays can measure host-parasite coevolution and male competition.

This does not mean that male competition is the only facet of sexual selection at play in this experiment: female preference, also involved in mate choice, may play a potential role in the dynamics of the observed sexual selection. However, we did observe an increase in male competition in some populations. While this could potentially reflect an overall increase in fitness for all individuals or an increase in male-specific mating ability, the results demonstrate a strong test of the connection between male mating ability and the evolution of increased parasite resistance. This experiment is not a comprehensive investigation of sexual selection within *C. elegans* or within the broader context of mate selection; it merely seeks to initially understand the intersection between male competition and parasite resistance through the lens of the Red Queen and Hamilton-Zuk hypotheses. Much more experimentation is needed to look for the patterns observed in this study, in both *C. elegans* populations and other systems.

C. elegans males are an understudied minority of the species since their appearances are rare in nature, and they are chiefly used as a means to generate genetic diversity through genetic recombination within the lab (Cutter *et al.*, 2019). The presence of males within *C. elegans* populations was previously thought to be a conserved mutation that is selected against, since outcrossing is detrimental to hermaphrodite fitness (Anderson *et al*., 2010). This investigation, therefore, sheds light on the function of male *C. elegans* in populations by observing the presence of male competition in light of evolutionary histories with a parasite. Males in a population are maintained by the selection for resistance and sexual selection. Such forces may be understood against the backdrop of overcoming the cost of males and contribute to our understanding of how males may be maintained through the confluence of different types of selective forces. The male competition and its varied population-level effects helps us better parse through the question: “Why is male function maintained within *C. elegans*”? Some of the proposed answers are: (1) selfing is a recent evolutionary development, so male function has not had time to decay (Loewe and Cutter, 2008; Cutter *et al.*, 2008) and (2) males and male function are under direct selection (Anderson, Morran, and Phillips, 2010). Males and outcrossing appear to be directly selected for in the presence of parasites to create more male offspring. Outcrossing can generate recombinant genomes that allow the population to better adapt to parasites. Male competition further provides evidence against the claim that males are a meiotic mistake (Chasnov & Chow, 2002; response by Cutter & Payseur, 2003). These results provide a richer, more nuanced view of the mosaic of male behaviors in *C. elegans*. Such competition demonstrates their active role in maintaining diversity and immune defenses against parasites. By understanding the evolutionary role of males in *C. elegan*s, we may likewise contextualize and better explore the evolution of mating systems.

Observationally, it seemed as though tail fan sizes of the *C. elegans* in populations 1 and 5 appeared to be less prominent and less defined than the average male *C. elegans*, while the tail fan sizes of populations 3 and 4 were very prominent and easily recognizable. When these phenotypic observations are interpreted in tandem with the results in **Fig. 2**, it appears that larger tail-fan sizes may be associated with more competitive males in sexual conflicts. This investigation was conducted under a limited time frame so that the tail fan sizes and prominence were not quantified. Testing this potential association would be beneficial: future experiments could directly measure the tail fan sizes in each population, and subsequently test whether the genes involved in tail fan development are linked to immune response. Alternatively, future investigations may directly test for tradeoffs between tail fan development and immune response in the ancestral population to document whether the traits are present and track how selection works on such traits in the presence of parasites. This will allow researchers to better understand how secondary sexual characters arise and become associated with better immune defenses in host-parasite coevolution. Such an investigation is imperative, as there is a lacuna in the literature exploring the relationship between the coevolution of male tail morphology traits and characters associated with sexual selection and sexual conflict, namely male competition or male mating vigor (Cutter, Morran, and Phillips, 2019). A study found that mutations that interfere with tail tip morphogenesis may be involved in sex-determination and heterochronic regulatory pathways, where evolutionary forces can engender morphological diversity in the tip of the tail fan (Nguyen *et al.,* 1999). These results bring us one step closer to understanding the developmental and genetic networks involved in the evolution of sexual dimorphism. Interestingly, male sex mesoblasts migrate posteriorly during the late L3 developmental stage to create 42 daughter cells, which comprise male-specific muscles in the tail with some cells controlling the movement of spicules (Sulston and Horvitz, 1977). Part of the tail is also formed by male ventral cord hypodermal cells (Sulston and Horvitz, 1977). Another investigation found evidence that sexual attraction and chemotaxis behaviors require both male-specific and core sensory neurons (White *et al*., 2007). The neural pathway connecting these two structures and associated behaviors has yet to be explored. These results would provide the essential connection between male tail morphology and male sexual behavior, and perhaps bring the field closer to analyzing the behavioral and physiological basis of parasite resistance and male competition.

Experimentation exploring the effects of parasite exposure and host-parasite coevolution on male competition will contribute to a better understanding of the interaction between the Red Queen and Hamilton-Zuk hypotheses. Through the intersection of the two theories, we can further explore how sex is sustained within populations and examine the role of males in maintaining outcrossing and propagating resistance genes. There is strong empirical support for the Red Queen hypothesis, that hosts must constantly evolve to evade parasites in coevolutionary antagonistic relationships (Bell, 1982; Morran et al., 2011; Soper et al., 2014; Vergara, Jokela, & Lively, 2014; Neiman et al., 2018; King et al., 2011). As delineated by Hamilton and Zuk, females in such situations use secondary sexual characteristics to evaluate the vigor and parasite load of males (Hamilton and Zuk, 1982). Yet, the effects of antagonistic coevolution and parasite exposure on male competition are rarely examined. This study provides a glimpse into the effects of intraspecies conflicts (male competition) against the backdrop of interspecies conflicts (host-parasite interactions) on a population level. The results of this experiment may be important to help counter the effects of repeated parasite infection in nature, as the Red Queen hypothesis predicts that constant exposure to parasites increases the rate of mating and the number of mates. Being able to understand male mating behavior in parasite-mediated selection for sexual reproduction will allow conservation efforts to be tailored to each species. This assists us in protecting endangered species involved in antagonistic host-parasite coevolution, as previous investigations have found that having multiple mates could increase genetic diversity among offspring (Soper et al., 2014). An increase in the rate of mating and having multiple mates begets an analysis of parasite-mediated sexual selection, and demonstrates the necessity of exploring the intersection between the Red Queen and Hamilton-Zuk hypotheses to better conceive of how genetic diversity is generated and maintained in nature.

The lack of investigation into the role of male *C. elegans* has greatly limited our knowledge of male behaviors, such as male competition, in evolutionary contexts. Specifically, host-parasite mediated sexual selection appears to be dominated by literature on female choice, rather than exploring the mechanisms of host-parasite coevolution and male competition that engender secondary sexual characters. Red Queen dynamics established a constantly changing environment vis-a-vis antagonistic fluctuating selection which heightens male competition, as host-parasite coevolution generates heritable immune defenses (resistance genes) that become increasingly associated with secondary sexual characters (Hamilton and Zuk, 1982). More research is necessary to better conceptualize the underlying genetic interactions and physiological pathways involved in regulating the intersection between the Red Queen and

Hamilton-Zuk hypotheses. In finding variations in male reproductive competitiveness relative to the ancestor by population and population x treatment, this experiment illustrates the role of male *C. elegans* within the context of evolving resistance to parasites and establishing Red Queen dynamics through mate competition. The establishment of such competition within populations of *C. elegans* assists in tackling the ever-present question regarding the evolutionary role and function of male *C. elegans*, through the lenses of the Red Queen and Hamilton-Zuk hypothesis.

Addendum

Mating systems and genetic manipulations

In nature, *Caenorhabditis elegans* are predominantly hermaphrodites that produce their own sperm and oocytes which combine in a process known as self-fertilization (Cutter *et al.* 2019). There are occasional males in the population allowing for genetic crosses and recombination to take place (Cutter *et al.* 2019). Obligately selfing populations are composed of hermaphrodites that mainly reproduce through self-fertilization, using their own sperm and oocytes. Obligately outcrossing populations require the fertilization of hermaphrodite oocytes with male sperm in order to produce the subsequent generation. A mixed-mating system refers to wildtype mating habits in which selfing predominates with very low levels of outcrossing. *C. elegans* are an especially well-suited model system to test theoretical questions concerning sexual selection and sexual conflict—which is the main focus of this thesis—as the nematodes may be genetically manipulated to become a dioecious (male-female) population by mutating the *fog-2* F-box proteins (Cutter *et al.* 2019). *Fog-2* alleles allow hermaphrodites to develop gonads; by inducing a loss-of-function of the *fog-2* alleles, researchers may eliminate hermaphroditic sperm production and effectively create a dioecious population of “females” (hermaphrodites that are functional females) and males (Cutter *et al.* 2019). This is typically carried out by engineering individuals to have a nonsense mutation of G → A in the *fog-2(lf)q71* allele which creates a nonfunctional gene relative to the wild type (Katju *et al*., 2008). The absence of the wildtype *fog-2* allele generates an obligately outcrossing population with more genetic diversity, made possible through sexual recombination between female oocytes and male sperm. Luckily, engineering a loss-of-function of only the *fog-2* alleles does not impact the development of maternal traits, alter male behavior, or any other peripheral traits in *C. elegans*, as the *fog-2* allele is necessary only for spermatogenesis in hermaphrodites but not in males (Schedl & Kimble, 1988).

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