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Manraj Singh Dua April 9, 2019

Mapping the Loci Underlying *Caenorhabditis elegans* Resistance to the Bacterial Parasite *Serratia Marcescens*

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Abstract

Mapping the Loci Underlying *Caenorhabditis elegans* Resistance to the Bacterial Parasite *Serratia Marcescens*

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Understanding mechanisms of host defense is critical because parasites are widespread and capable of imposing fitness costs on hosts. Here, we look at the nematode host *Caenorhabditis elegans* and the bacterial parasite *Serratia marcescens* (SM2170), where most strains of *C. elegans* die after exposure to the parasite. We see that the N2 strain has a survival percentage of 0.72%, while Hawaiian CB4856 has a much higher survival percentage of 35.68%. From previous studies, it is known that Chromosome V of *C. elegans* is where many loci responsible for mediating interactions with parasites reside. In this study, our goal was to determine if and where the loci underlying higher CB4856 survival resided on Chromosome V. By utilizing Chromosome V introgression strains of CB4856 with an overall N2 background, we isolated the EWIR 68 strain that despite having only a portion of CB4856 Chromosome V, showed an even higher survival than its CB4856 parent. Thus, the portion of CB4856 Chromosome V present in EWIR 68 underlies increased survival in the presence of *S. marcescens* (SM2170). Next, we set out to determine the mechanism of defense employed by CB4856 and EWIR 68. Given *C. elegans* innate preference for *S. marcescens*, we tested for a role of parasite avoidance in host defense. We found that CB4856, EWIR 68 and N2, all displayed similar preference towards *S. marcescens*, meaning that parasite resistance rather than avoidance accounted for increased survival in the CB4856 and EWIR 68 hosts. Knowing this, we set to determine the specific loci underlying resistance on chromosome V. We mated EWIR 68 with N2 males, generating 44 new introgression strains that further split the portion of CB4856 Chromosome V in EWIR 68. Running survival assays on these 44 strains, only two strains, 25 and 30, expressed high survival. These results confirmed that the gene/genes underlying *C. elegans* resistance against *S.* marcescens resides on Chromosome V as depicted by EWIR 68. In the future, genotyping strains 25 and 30 will give us better a resolution on a specific Chromosome V region underlying *C. elegans* resistance.

Mapping the Loci Underlying *Caenorhabditis elegans* Resistance to the Bacterial Parasite *Serratia Marcescens*

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Introduction

The genetic makeup of *Caenorhabditis elegans* is known to be complex, with multiple loci interacting with other loci to produce many different phenotypes (Spieth et al., 2005). There are 20,152 protein-coding regions in *C. elegans*, and while most coding regions are relatively small at around 3kbp, there are some larger regions with over 7kbp (Spieth et al., 2005). Together these 20,152 genes give rise to important phenotypes in *C. elegans* such as behavior, fecundity, and many other traits that are vital for the evolutionary persistence of the species. In particular, the specific genes giving rise to the traits that confer *C. elegans* host defense against parasites are of critical importance (Couillault & Ewbank, 2002).

Parasites are widespread in nature and capable of imposing substantial fitness costs on hosts. Parasitism is a relationship between species, where the parasite lives inside the host and harms the host by diverting resources needed for growth, reproduction, and survival (Sorci & Garnier, 2008). In addition to decreased survival and fecundity, fitness costs can also include decreased host locomotor movement via impacts on morphology and physiology (Binning et al., 2017). Due to this high fitness cost, hosts are expected to evolve defense mechanisms in order to lessen this negative effect (Sorci & Garnier, 2008). These defense mechanisms consist mainly of: 1) avoiding the parasite and 2) resisting or tolerating the infection by clearing or mitigating its damage after infection (Kutzer & Armitage, 2016).

C. elegans live in bacteria rich environments, where they are able to feed on bacteria (Shtonda & Avery, 2006). *C. elegans*, in nature, can survive by eating various types of bacteria, with one of the main types being *Escherichia coli* (Brenner, 1974). In nature, it is important that *C. elegans* are able to distinguish between nutritious and parasitic bacterial food sources (Samuel et al., 2016). While *E. coli* is a nutritious type of bacterial food source, there are also many parasitic bacterial food sources such as *Serratia marcescens* (Schulenburg & Ewbank, 2004). *C. elegans* lack a dedicated adaptive immune system, but they still have some defenses to fight infection by parasites (Ermolaeva & Schumacher, 2014).

Previous research has shown some strains of *C. elegans* actively avoid the parasitic bacteria *S. marcescens*. This avoidance of *S. marcescens* is a learned behavior rather than an innate behavior. After *C. elegans* are exposed to *S. marcescens* and then evolved, they can avoid the bacteria (Penley & Morran, 2018). One way *C. elegans* can avoid certain strains of *S. marcescens* is by using their chemosensary neurons (Pradel et al., 2007). After *S. marcescens* produces a cyclic lipodepsipentapeptide serrawettin W2, *C. elegans* can then use these chemosensory neurons to modify their olfactory preferences and avoid that pathogenic bacteria in the future (Zhang et al., 2005). Another way *C. elegans* can avoid *S. marcescens* is by triggering innate immune effectors when it senses a pathogen has disrupted its core cellular activities (Melo & Ruvkun, 2012). Inactivation of these essential cellular processes stimulates an aversion phenotype, thus letting the *C. elegans* avoid any pathogen that caused this inactivation in the beginning (Melo & Ruvkun, 2012).

Beyond avoidance, *C. elegans* also have cellular defenses that allow them to resist infection by *S. marcescens*. Unlike avoidance, resisting an infection means that *C. elegans* do get infected, but they are able to clear out this infection before substantial negative fitness costs. This innate immunity in *C. elegans* comprises of the secretion and action of antimicrobial molecules such as lysozymes, lectins, and antibacterial factors (Marsh & May, 2012). This immunity is regulated by multiple signaling pathways, of which the most significant include the extracellular signal-regulated kinase mitogen-activated protein kinases (MAPK, ERK), the insulin signaling/DAF-2, and the transforming growth factor β (TGF-β) / DBL-1 pathway. While some of these responses to parasites are general, *C. elegans* can also trigger specific responses depending on what parasite is causing the infection (Wong et al., 2007). Therefore, there may be a specific gene that confers *C. elegans* defense against *S. marcescens.*

C. elegans live in diverse microbial environments and thus different strains have adapted to their local microbiota (Samuel et al., 2016). Due to this, different strains of *C. elegans* have natural genetic variation in their interaction with bacteria (Gerbaba et al., 2017). Speculating off this, we have seen from previous research that the Hawaiian *C. elegans* strain CB4856 exhibits greater survival than the laboratory N2 strain when exposed to the parasitic bacteria *S. marcescens* SM2170 (Zhang et al.). By using QTL mapping with recombinant inbred lines between CB4856 and N2, we have mapped CB4856's increased survival to chromosome V (Zhang et al.). In this study, we wanted to determine the nature of CB4856 host defense, which allows it to exhibit a much higher survival relative to N2. We assessed both the genotypic and

phenotypic nature of this host defense. To determine the genotype, we will use CB4856 Chromosome V introgression strains with an overall N2 background to determine a specific Chromosome V region underlying CB4856's increased survival relative to N2 (Doroszuk et al., 2009). Then, we will split this region even further to eventually determine the specific loci underlying higher survival in CB4856. To determine the phenotype, we also used these same introgression strains to determine if parasite avoidance or resistance/tolerance accounted for increased survival in CB4856.

Methods

1. Host populations

Host population consisted of the laboratory *C. elegans* N2 strain and the *C. elegans* Hawaiian CB4856 strain. 10 EWIR introgression strains of chromosome V were also derived from strain CB4856 with an overall N2 background. These 10 introgression strains were acquired from Dr. Kammenga (Doroszuk et al., 2009). The genetic map of these 10 EWIR introgression strains is shown in Figure 1.1. In addition, 44 introgression strains of chromosome V were derived from EWIR strain 68 with an overall N2 background. Figure 1.2 shows the cross between EWIR 68 and N2 resulting in the 44 introgression strains.

Introgression strains were created by crossing the strain of interest, in this case CB4856 or EWIR 68, with the baseline strain, in this case N2. By mating these strains, the F1 offspring received one chromosome from each parent strain. In F2 offspring, crossing over occurred between the

2 different parent chromosomes. In the subsequent generations, the worms were inbred to get a homogenous genetic region. This allowed us to study a single defined genetic region while keeping the N2 genetic background constant.

Chromosome V (Mb)	590,471	1,773,465	2,878,209	3,606,323	4,550,758	5,814,403	6,612,376	7,690,531	8,567,608	9,405,441	10,368,660	10,912,994	11,796,050	13,033,974	13,951,861	15,158,759	16,008,404	17,377,158	18,574,593	19,525,561	20,758,352
Strain name																					
N2	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
CB4856	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
CSSV	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
ewIR64	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ewIR65	H	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ewIR66	B	H	H	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ewIR67	B	B	B	B	H	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ewIR68	B	B	B	B	H	H	H	H	H	H	H	H	H	H	B	B	B	B	B	B	B
ewIR69	B	B	B	B	B	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
ewIR70	B	B	B	B	B	B	B	B	B	B	B	H	H	H	H	H	H	H	H	H	H
ewIR71	B	B	B	B	B	B	B	B	B	B	B	B	H	H	B	B	B	B	B	B	B
ewIR72	B	B	B	B	B	B	B	B	B	B	B	B	B	H	B	B	B	B	B	B	B

Figure 1.1 Genetic map of 10 EWIR introgression strains derived from CB4856 with an overall N2 background. B represents N2; H represents CB4856.

Figure derived from (Glater et al., 2014)

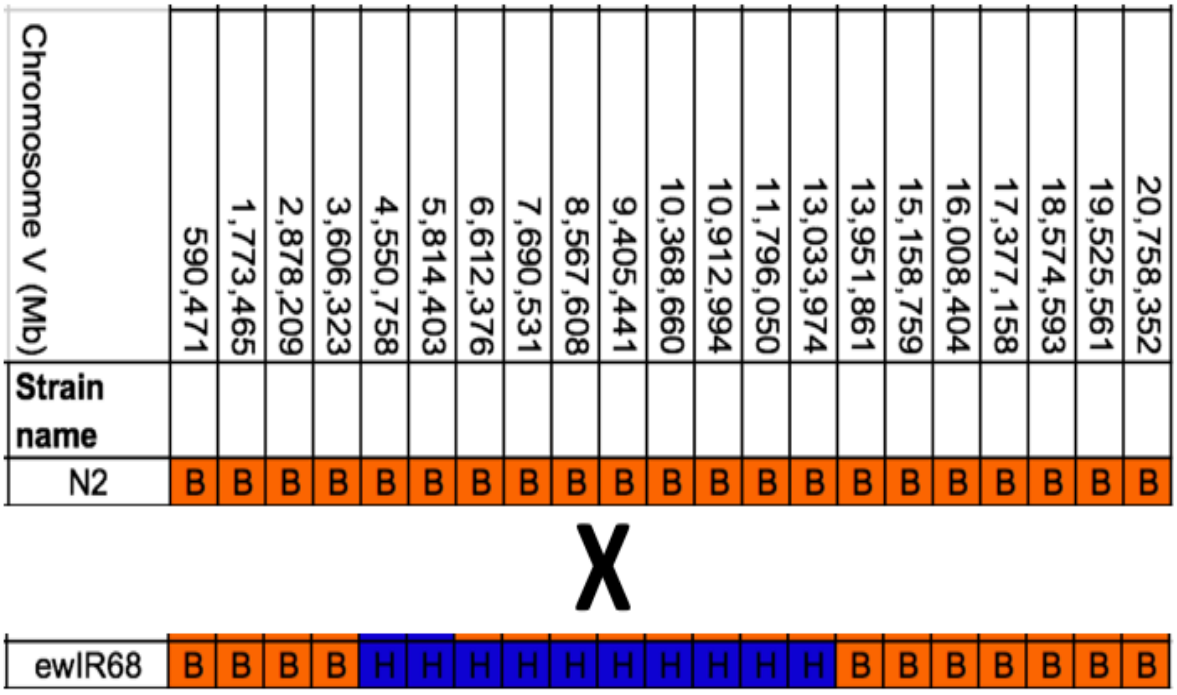

Figure 1.2 Cross between EWIR 68 and N2. This resulted in 44 introgression strains derived from EWIR 68 with an overall N2 background. B represents N2; H represents CB4856

Figure derived from (Glater et al., 2014)

2. Bacterial populations

Bacterial populations consisted of the SM2170 strain of *Serretia marcescens. S. marcescens* are naturally occurring gram negative bacterium and are virulent to *C. elegans* causing about 80% mortality within 24 hours of consumption (Kurz et al., 2003). The SM2170 strain was obtained from Dr. Sue Katz in 2006.

3. Survival assay

The protocol for setting up a survival assay has been detailed in Morran et al. 2011. Survival assays were set up in 100mm petri dishes containing 30 mL of autoclaved NGM lite (US Biological, Swampscott, MA). For survival assay #1, host populations consisted of *C. elegans* strains N2, CB4856, and the 10 introgression strains (Figure 1.1). For survival assay #2, host populations consisted of 44 introgression strains resulting from the cross between EWIR 68 and N2 (Figure 1.2). Two types of bacteria, *E. Coli* OP50 and *S. marcescens* SM2170, were used for the survival assay. Prior to use on the survival assay, these bacteria were first incubated for 24 hours at 28 degrees Celsius in test tubes containing Lysogeny broth. Survival assay plates were plated with 25uL of *E. coli* OP50 and *S. marcescens* SM2170 on opposite sides and then allowed to incubate at room temperature for 5 hours. After incubation, 40 ul of ampicillin (400 mg/mL) was lined down the middle of the plate to prevent the spread of SM2170. To run the survival assay, host populations were bleach synchronized and 200 L4 worms in M9 buffer were plated directly on SM2170. The M9 buffer was allowed to evaporate for 30 minutes, at which point the plates were then transferred to the 20C incubator. After 48 hours, the number of live worms were counted in the middle of the plate and on the OP50 side. Survival was calculated based on this formula:

Survival = (# hosts in OP50 + # hosts in middle) / Total # hosts plated

One-way ANOVA was performed on the survival assay results. Where the results were significant, student's t-test was performed to compare different strains.

4. Choice index/avoidance assay

The protocol for setting up a bacterial choice assay has been modified from Zhang et al. 2005. Bacterial choice assays were set up in 100mm petri dishes containing 30 mL of autoclaved NGM lite (US Biological, Swampscott, MA). Host populations consisted of *C. elegan* strains N2, CB4856, EWIR 68, and CSSV. Two types of bacteria, *E. Coli* OP50 and *S.* marcescens SM2170, were used for the choice index assay. Prior to use on the choice index assay, these bacteria were first incubated for 24 hours at 28 degrees Celsius in test tubes containing Lysogeny broth. Choice index assay plates were plated with 25uL of *E. coli* OP50 and *S. marcescens* SM2170 on opposite sides and then allowed to incubate at room temperature for 5 hours. To run the choice index assay, host populations were bleach synchronized and 200 L4 worms in M9 buffer were plated in the middle of the plate. The M9 buffer was allowed to evaporate for 30 minutes, at which point the plates were then transferred to the 20C incubator. After 1 hour, the number of worms were counted in each bacteria spot, the middle of the plate, and the rest of the plate. Bacterial choice index was calculated based on this formula:

Bacterial Choice Index = (# hosts in SM2170 - # hosts in OP50) / Total # hosts plated

One-way ANOVA was performed on the choice index assay results.

5. Ramping up male N2 population

In order to cross EWIR 68 and N2, N2 males were needed first. However, most *C. elegans* exist as hermaphrodites, and males are very rare. To find that rare N2 male, 10-15 N2 plates were set up. Among those plates, once a N2 male was found, it was transferred onto a small *E. coli* plate. Along with the N2 male, 4-5 N2 hermaphrodites were also transferred onto the small

plate. The N2 male and the hermaphrodites were allowed to reproduce, resulting in more males. The whole cycle was repeated several times until there was a considerable size of male N2 population.

6. Cross between EWIR 68 and N2

In several small *E. coli* plates, one N2 male and 4-5 EWIR 68 hermaphrodites were transferred. In each plate, the N2 and EWIR 68 were allowed to reproduce. After 2 days, the number of males and number of hermaphrodites were counted in each plate. The ideal plate consisted of 50% males and 50% hermaphrodites, meaning that the N2 male and EWIR 68 hermaphrodites had actually crossed with each other rather than the EWIR 68 hermaphrodites self-reproducing. Finding that ideal plate, 44 L3 hermaphrodites from that plate were transferred onto their own individual small *E. coli* plates. These 44 hermaphrodites were allowed to self-reproduce. Then 1 L3 hermaphrodite from each of the 44 plates was transferred onto their own plates. This process was repeated 9 times. At the end, we had 44 homogenous introgression strains resulting from the cross between EWIR 68 and N2.

Results

1. Survival assay of introgression strains derived from CB4856 with an overall N2 background

C. elegans survival against *S. marcescens* was evaluated using survival assays where the worms were allowed to migrate from *S. marcescens* to *E. coli.* For each strain, the greater number of worms that were able to leave *S. marcescens*, the greater the mean survival. Normally, *S. marcescens* acts as a pathogen for *C. elegans* and causes high mortality rates. Generally, we see

high rates of mortality within 24 hours of consumption (Kurz et al., 2003). This was seen in the case of N2 worms, with a significantly low mean survival of only 0.007 (Figure 2 and Table 1; $P<0.001$; Student's t-test N2 vs all, $P<0.05$). Confirming previous research, when we evaluated the CB4856 strain, we saw significantly high mean survival of 0.36, indicating that *S. marcescens* didn't have as big of an impact on CB4856 compared to N2 (Figure 2 and Table 1; $P<0.001$; Student's t-test CB4856 vs all, $P<0.05$).

Next, we performed survival assays on CB4856 x N2 introgression strains to determine the portion(s) of chromosome V conferring greater host survival in CB4856. For the survival assays conducted on the CB4856 Chromosome V introgression strains, most strains displayed lower mean survival relative to their C4856 parent. However, there was one introgression strain, EWIR 68, that displayed a mean survival even higher than that of its CB4856 parent. While CB4856 had a mean survival of 0.36, EWIR 68 had a mean survival of 0.5 (Figure 2 and Table 1; $P<0.001$; Student's t-test EWIR 68 vs all, $P<0.05$). These results confirmed the fact that Chromosome V was indeed playing a role in higher survival for CB4856 and in turn EWIR 68. In addition, we could conclude that this portion of CB4856 Chromosome V represented by EWIR 68 is responsible for greater mean survival against SM2170.

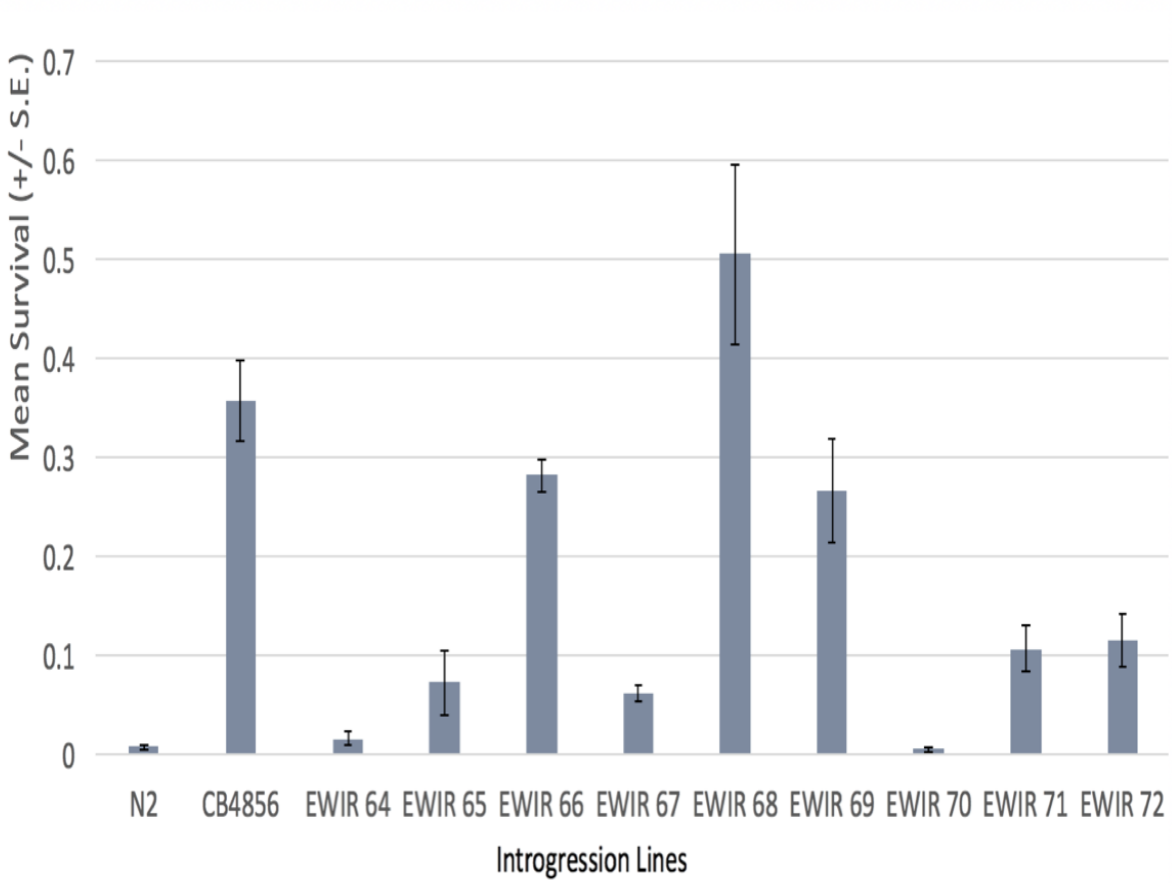

Figure 2. Mean survival of *C. elegans* CB4856 introgression strains exposed to *S. marcescens* strain 2170 (+/- one standard error). Four to ten replicates of approximately 200 worms were assayed against SM2170. As expected, N2 had a really low mean survival while CB4856 had a high mean survival. EWIR 68, despite only having a portion of CB4856 genome, still displayed high mean survival.

Source	Sum of Squares	df	Mean Square	F	P
Model	1.897	10	0.190	40.47	<0.0001
Error	0.225	48	0.005		
Total	2.122	58			

Table 1. One-way ANOVA for survival assay on introgression strains of CB4856.

2. Survival assay of introgression strains derived from EWIR 68 with an overall N2 background

Knowing that the portion of CB4856 Chromosome V represented by EWIR 68 was responsible for greater survival against SM2170, we set out to determine the specific loci underlying higher survival. Due to the portion of CB4856 Chromosome V represented by EWIR 68 being so big, we couldn't use that large region to determine the specific loci. We needed to make this region smaller to pinpoint a specific locus or several loci. To do this, we crossed EWIR 68 and N2, generating 44 new introgression strains that further split the portion of CB4856 Chromosome V in EWIR 68 (Figure 1.2). Running survival assays on these 44 EWIR 68 x N2 introgression strains, we found that most of the strains performed significantly worse than the parent EWIR 68 with the exception of 2 introgression strains: strain 25 and strain 30. Both strains 25 and 30 had a mean survival of 0.61 (Figure 3 and Table 2; $P<0.001$; Student's t-test 25, 30 vs all, $P<0.05$). This was especially interesting since the mean survival expressed by these new strains was even higher than the mean survival of its parent EWIR 68 of 0.5 (Figure 2).

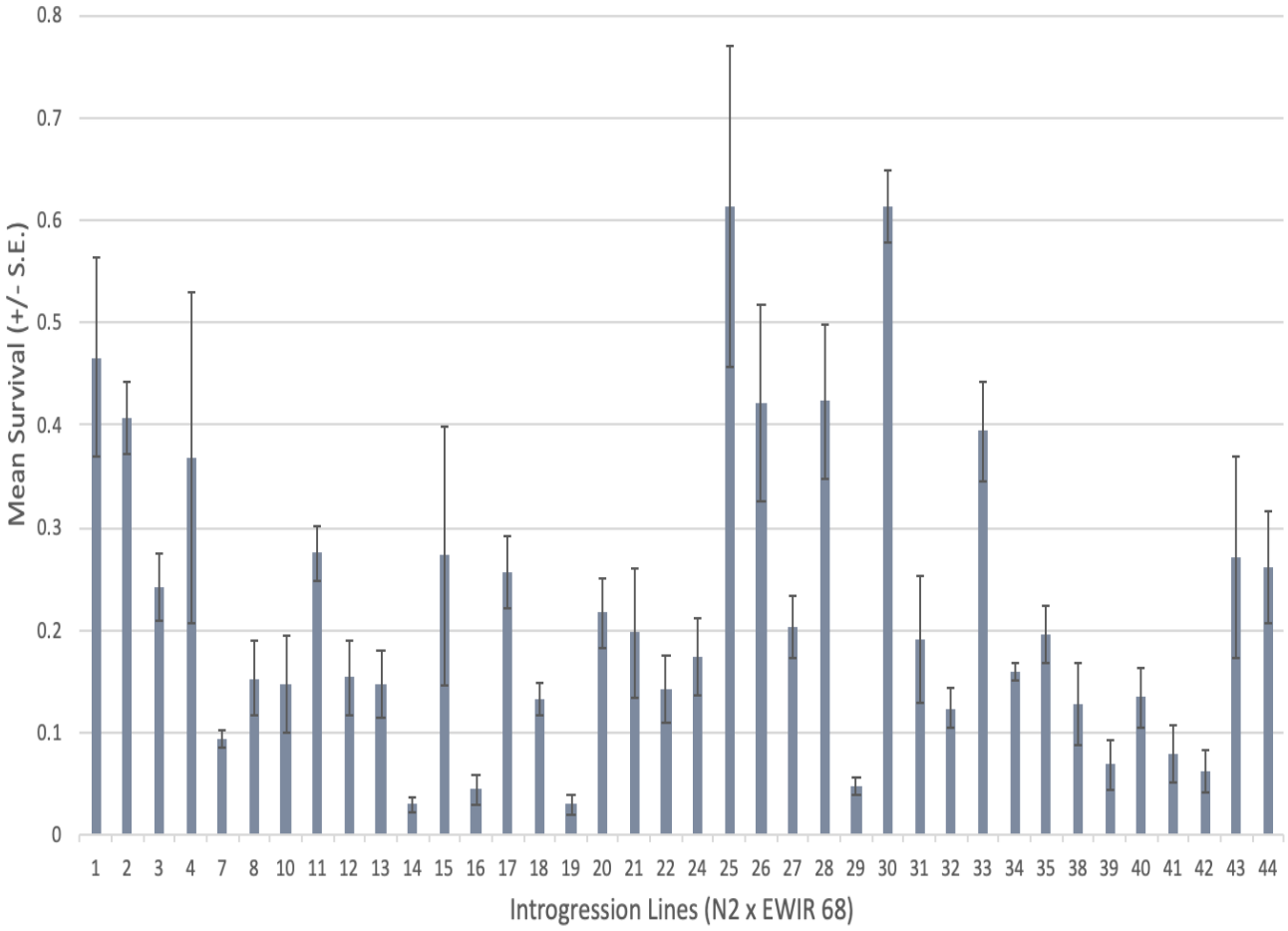

Figure 3. Mean survival of *C. elegans* EWIR 68 introgression strains exposed to *S. marcescens* strain 2170 (+/- one standard error). Three to five replicates of approximately 200 worms were assayed against SM2170. While most introgression strains had a low mean survival, introgression strains 25 and 30 displayed a high mean survival.

Source	Sum of Squares	df	Mean Square	F	P
Model	3.56	37	0.096	5.97	<0.0001
Error	2.16	134	0.016		
Total	5.72	171			

Table 2. One-way ANOVA for survival assay on introgression strains of EWIR 68.

3. Survival assay of introgression strains 1, 25, and 30

Along with introgression strains 25 and 30 being of interest due to their high mean survival, strain 1 was also of interest. While strain 1 didn't express as high a mean survival as strains 25 and 30, strain 1 did have high variation among replicates. Between its replicates, the mean survival was as high as 0.74 and as low as 0.28. Due to this, we decided to run the survival assay again for strain 1. Along with strain 1, strains 25 and 30 were also assayed again to see if we could replicate the high mean survival.

Looking at the new survival data, we saw that strain 1 and N2 expressed a significantly low mean survival of 0.08 (Figure 4 and Table 3; $P<0.001$; Student's t-test 1, N2 vs all, $P<0.05$). This meant that the high mean survival we saw in the previous survival assay replicate was just an anomaly. In reality, strain 1 had a similar phenotype to N2. In addition, while strains 25 and 30 displayed a lower mean survival in this assay compared to the previous one (Figure 3), their mean survival was still relatively high, letting us conclude that strains 25 and 30 performed similarly to their parent EWIR 68 (Figure 4 and Table 3; $P<0.001$; Student's t-test 25, 30, EWIR 68 vs all, $P<0.05$).

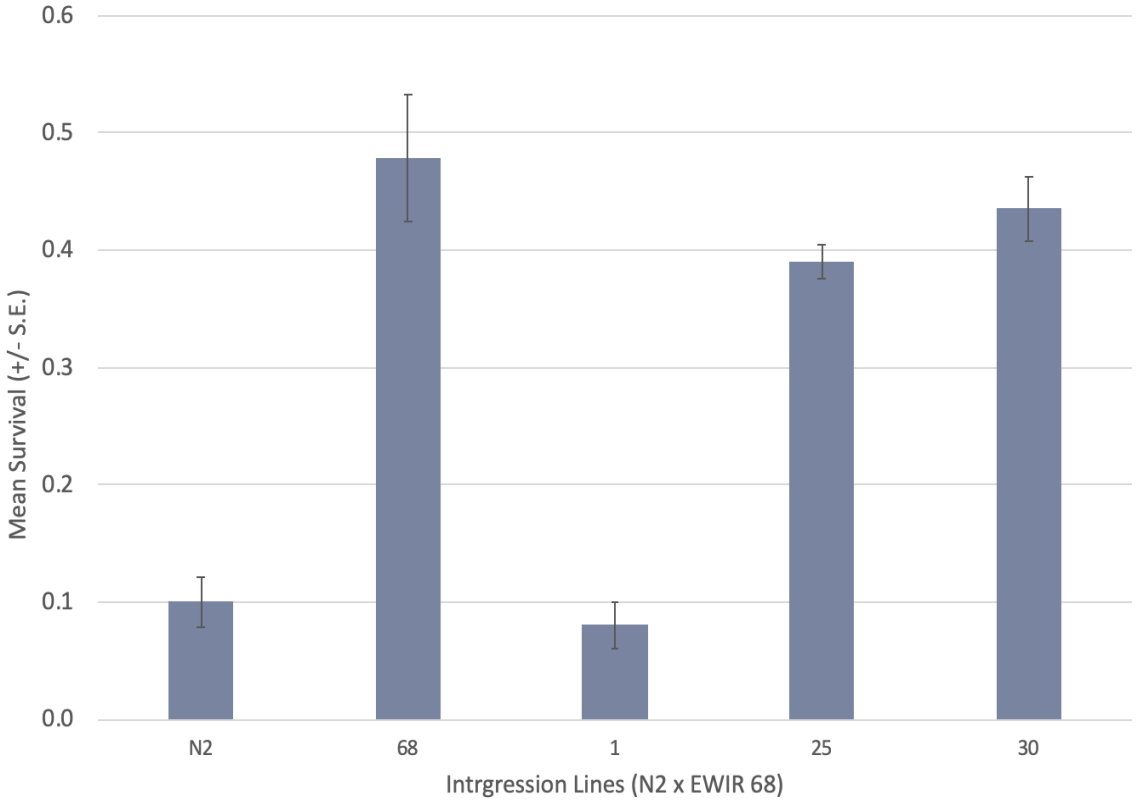

Figure 4. Mean survival of *C. elegans* EWIR 68 introgression strains exposed to *S. marcescens* strain 2170 (+/- one standard error). Four replicates of approximately 200 worms were assayed against SM2170. While strains 25 and 30 displayed high mean survival, strain 1 displayed low mean survival.

Source	Sum of Squares	df	Mean Square	F	P
Model	0.0587	4	0.147	35.52	<0.0001
Error	0.056	15	0.004		
Total	0.643	19			

Table 3. One-way ANOVA for survival assay on strains N2, EWIR 68, 1, 25, and 30.

4. Choice index assay between S. marcescens and E. coli

C. elegans bacterial preference of *S. marcescens* versus *E. coli* was evaluated using a choice index assay in which the worms migrated to one of the two bacterial spots. The host

populations used in this assay were N2, CB4856, and EWIR 68. Despite CB4856 and EWIR 68 having a much higher mean survival compared to N2, all the populations showed similar preference for *S. marcescens* (Figure 5 and Table 4; P=0.992). N2, CB4856, and EWIR 68 had bacterial mean choice index of 0.33, 0.34, and 0.36, respectively (Figure 5 and Table 4; P=0.992). A positive mean choice index for all strains meant all were attracted towards *S. marcescens.*

C. elegans defense mechanisms consist mainly of: 1) avoiding the parasite and 2) resisting the infection by clearing it from inside. Since CB4856 and EWIR 68 displayed high mean survival (Figure 2) and yet still were attracted towards *S. marcescens* (Figure 5), it meant that the host defense mechanism for these strains was resistance to *S. marcescens* rather than avoidance.

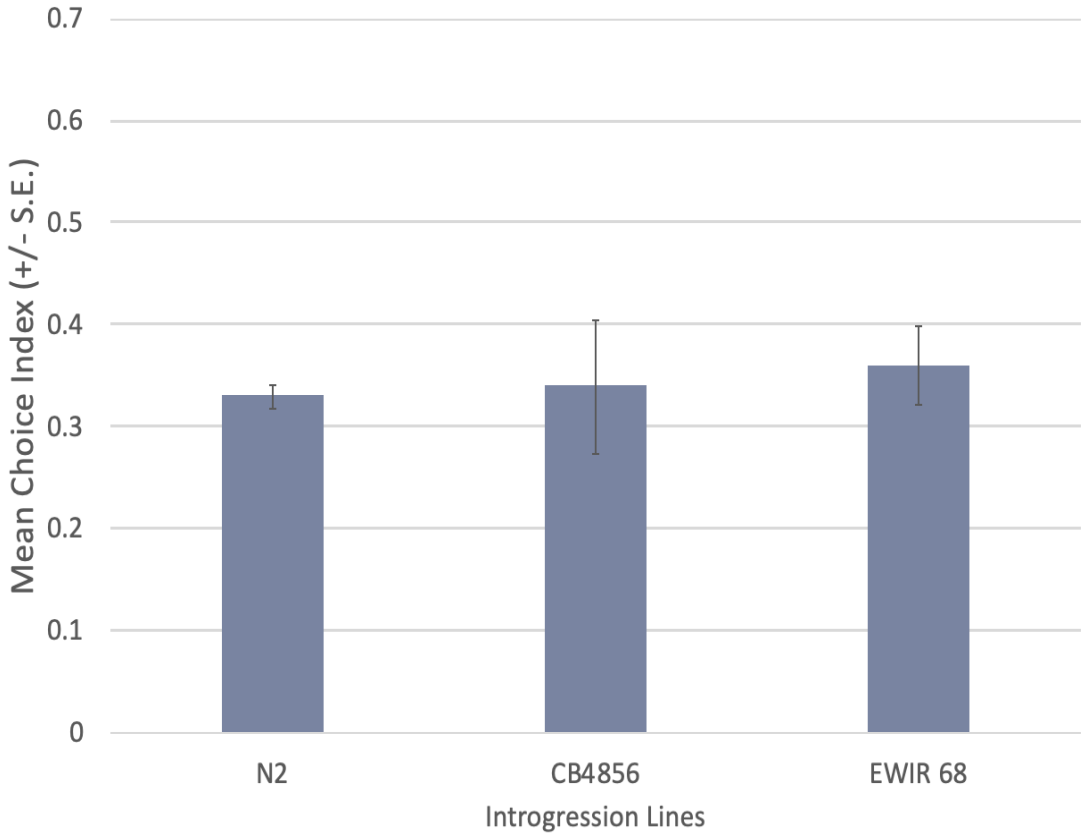

Figure 5. Mean choice index of *C. elegans* N2, CB4856, and EWIR 68 between *E. coli* OP50 and *S. marcescens* 2170 (+/- one standard error). Four replicates of approximately 200 worms were assayed. A positive mean choice index indicates that the *C. elegans* prefer to move towards *S. marcescens* 2170 while a negative mean choice index indicates preference to move towards *E. Coli* OP50. Despite CB4856 and EWIR 68 having much higher mean survival compared to N2, all strains show similar preference for *S. marcescens* 2170.

Source	Sum of Squares	df	Mean Square	F	P
Model	0.001	2	0.0006	0.08	0.922
Error	0.071	9	0.0079		
Total	0.072	11			

Table 4. One-way ANOVA for choice index assay on strains N2, CB4856, and EWIR 68.

Discussion

In this study, we looked at the relationship between the host *C. elegans* and the parasite *S. marcescens* Sm2170. Specifically, we looked at the defense mechanisms, both at the genotypic and phenotypic level, that *C. elegans* utilized to lessen the fitness costs imposed from *S. marcescens*. Confirming previous research, we saw in our initial survival assay that the Hawaiian *C. elegans* CB4856 strain displayed a high mean survival while the *C. elegans* N2 strain displayed a low mean survival (Figure 2, Zhang et al.). Running survival assays on introgression strains of CB4856 with an overall N2 background (Figure 1), we found that strain EWIR 68 had an even higher mean survival than that of its CB4856 parent (Figure 2). EWIR 68 only contains a portion of CB4856 Chromosome V (Figure 1) and yet still showed high mean survival (Figure 2). This meant that the genes underlying the elevated host defense of CB4856 against *S. marcescens* are located somewhere in the portion of CB4856 Chromosome V represented by EWIR 68. Thus, we generated 44 new introgression strains of EWIR 68 to break the region further (Figure 1.2) as a means to determine the host defense loci. Of these strains, 25 and 30 displayed high mean survival similar to their parent EWIR 68 (Figure 3).

To determine the phenotypic nature of this host defense, we ran a choice index assay on strains N2, CB4856, and EWIR 68, where the worms were allowed to choose between *S. marcescens* 2170 and *E. coli* OP50. Despite EWIR 68 and CB4856 having high mean survival compared to N2 (Figure 2), all strains showed a positive preference to move towards *S. marcescens* (Figure 5). This meant that resistance, rather than avoidance, played a role in *C. elegans* defense mechanism.

In the absence of the DNA sequence for strains 25 and 30, we can conclude one of two things. The first option is that we were successful in further breaking up the EWIR 68 region in strains 25 and 30. If this is true and the Chromosome V regions are small enough in strains 25 and 30, we may be a step closer to pinpointing a specific locus or loci responsible for the defense mechanism in CB4856 against *S. marcescens.* The other option is that no crossing over occurred in the CB4856 region of chromosome V in strains 25 and 30 relative to EWIR 68, meaning strains 25 and 30 are essentially copies of EWIR 68. If this is the case, we will have to create additional EWIR 68 introgression strains to find a strain that has a smaller EWIR 68 region and yet still shows high mean survival. Further, there may be multiple loci along the EWIR 68 section of chromosome V that contribute to host defense, such that it cannot be broken without reducing host defense. In this case, we need to identify different introgression strains that possess reduced host defense but can produce offspring with restored defense. To see which of these options is correct, we plan to run PCR along chromosome V of strains 25 and 30 to see if they are different or identical to EWIR 68.

For many years, it has been believed that parasites play an essential role in the evolution of hosts. In 1949, J. B. S. Haldane published a paper explaining how infectious disease from pathogens has potential potency as an agent of natural selection in hosts (Haldane, 1949). We saw in our results that a particular strain of *C. elegans* had developed resistance against the parasite *S. marcescens,* most likely due to selective pressures in accordance with Haldane. Knowing the loci under selection will furthermore allow us to study how *C. elegans* evolved

host defense against *S. marcescens*. From this, we can not only determine the genetic changes at these loci but also monitor the patterns of evolutionary change in *C. elegans* in the presence of *S. marcescens.* More specifically, we can study the patterns of coevolution between *C. elegans* and *S. marcescens.* Coevolution is defined as reciprocal, adaptive genetic changes between interacting species (Woolhouse et al., 2002) and is highly prevalent in host-parasite interactions due to strong selective pressure each agent exerts on the other (Thompson, 1994). With this concept of coevolution and evolutionary change, we can specifically look at not just how *C. elegans* has evolved greater host defense in the presence of *S. marcescens* but also how *S. marcescens* has evolved itself to counteract this resistance.

While resistance to a parasite is often seen as beneficial due to it increasing host fitness, there are substantial costs to resistance in the absence of infection. These costs include reduced survival (Yan et al., 1997), reduced fertility (Webster & Woolhouse, 1999), and increased susceptibility to non-communicable diseases (Searle & Blackwell, 1999). However, despite these costs to resistance, studies have shown that some organisms maintain resistance even in the absence of infection (Besier et al., 2005, Bjorkman et al., 2000). This is mainly due to secondary site mutations that compensate for fitness costs of resistance (Wiesch et al., 2010). By determining the loci in *C.* elegans conferring higher host defense against *S. marcescens*, we can study how the resistance phenotype has evolved and changed over time. Particularly, we can see whether resistance, in the evolutionary history of *C. elegans*, has ever had negative fitness costs and if there are other mutation in the genome compensating for these costs.

Overall, the host-parasite relationship is not just limited to *C. elegans* and *S. marcescens*. We see this relationship all throughout nature, where hosts have responded to selection from parasites. In agriculture, we see crops developing resistance in the presence of parasites (Rispail et al., 2007). In addition, farmers have been known to specifically breed certain strains of crops to maximize resistance among the population. The host-parasite relationship is also seen in human beings. The human genome has evolved over many years to fight off and develop resistance against certain pathogens (Karlsson et al., 2014). For example, in parts of Africa where malaria has high fitness costs, the people there have developed high frequencies of the heterozygous sickle cell allele which confers partial resistance against malaria (Aidoo et al., 2002). Since this host-parasite relationship is so important in nature, it is crucial we obtain a better understanding of it. Working with model organisms such as *C. elegans*, we can study the evolution of this relationship, particularly how hosts have developed resistance in the presence of selection from parasites.

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