Assessment of competition between the *dpy-5* and N_2 strains of *Caenorhabditis elegans*

Akashdeep Aujla, Chengyue Niu, and Charl Stafleu

Abstract

Two strains of *Caenorhabditis elegans*, the N_2 wild type and the *dpy-5* dumpy mutant, were studied to determine whether the wild type has a competitive advantage over the mutant. Three treatments were set up: a mutant control, a wild type control, and an experimental group (mutant and wild type present at a 1:1 ratio). For each treatment, initial populations of four nematodes were plated on 100 mm Petri dishes containing *Escherichia coli*, and subsequently incubated at 17 °C. From the fifth to the ninth day after the initial plating, the total number of adult individuals on each treatment was recorded. This data was analyzed for significant differences in the total number of adults per original number of adults, growth rate, and the ratio of number of mutants to number of wild type. On the eighth day, the wild type control group saw a significant increase in population, and had significantly more individuals than the mutant control group. However, on the ninth day, the mutant control group had drastically increased in population, such that there was no significant difference between the control groups; the experimental groups, however, did not demonstrate this trend. These results were supported by the observed daily growth rates. In the experimental treatments, the ratio of wild types to mutants did not ever deviate significantly from the original 1:1 ratio. The results do not provide any support for a competitive advantage in the wild type over the *dpy-5* mutant. Lack of a limiting resource, excessive biological variation, and poor representation of natural habitat are discussed as possible issues affecting the results of this study.

Introduction

Caenorhbditis elegans is a free-living soil nematode of the Rhabditidae family, widely distributed in Europe, Madeira, North Africa, Asia, North America, Hawaii, and Australia (Kiontke 2006). These nematodes proliferate in bacteria-rich environments, such as decomposing vegetal matter or decaying invertebrates. Their natural environment is shared with a myriad of other species, including arthropods, molluscs, other nematodes and sometimes other *Caenorhabditis* species. Therefore, intra- and inter-specific competition is an important factor that affects their population growth and survival in their natural habitat (Braendel *et al.* 2007). *C. elegans* are usually described as good colonizers or “enrichment opportunists” in literature. They are often observed as being the first species to conquer new habitats when new food sources become available, establishing large

populations of offspring in short periods of time. This, in turn, can lead to the depletion of food sources (Strange 2008). Previous studies on the effects of temperature on population growth rate have reported that at 20 °C, among several species of bacterial-feeding nematodes, *C. elegans* has the largest population growth rate average (Venette and Ferris 1997). The same authors also found that, of the various bacteria-feeding nematodes, *C. elegans'* population growth rate was the most sensitive to changes in food density (Venette and Ferris 1998). Although many *C. elegans* population studies have been conducted, few have analysed population growth in the context of competition.

Our objective was to conduct an experiment in which the N_2 wild type and the *dpy-5* mutant would be observed in a competitive environment, and investigate whether one strain has a competitive advantage over the other.

Our alternate hypothesis (H_a) states that the wild type is competitively superior to the mutant, demonstrated by greater population growth after a period of nine days. Our null hypothesis (H_o) states that there is no observable competitive advantage for either strain, or the *dpy-5* mutant is the better competitor.

We believed that the wild type *C. elegans* would be the better competitor for a variety of reasons. The *dpy-5* worms are, on average, half the length of the N_2 worms, with a "chubbier" body and slower movements. They also have a smaller brood size, and their development from L1 (the first larvae stage) to adulthood is delayed by as much as 12 hours (Thacker *et al.* 2006).

Methods

1. Set up

Before the procedure was undertaken, our equipment and workstation were sterilized to minimize contamination. Afterwards, we obtained 15 100 mm Petri dishes filled with nematode growth medium. The medium consisted of agar and 100 µL of the bacteria *Escherichia coli*, strain OP50. The 15 Petri dishes were separated equally into three groups: a dpy-5 mutant control group, a wild-type control group, and an experimental group. This allowed us to obtain five replicates for each of our treatments.

2. *C. elegans* transfer

We obtained mutant *dpy-5* and wild-type stock. From each stock, 30 healthy and viable adults were transferred into two separate transfer dishes using a worm pick and a dissecting microscope. This was done to allow for the separation of adults from larvae and eggs. Further, this helped us to ensure that only healthy and active adults were subsequently plated on our replicates.

3. *C. elegans* plating

Four adult mutants were transferred by worm pick under dissecting microscope from the mutant transfer dishes to each of the five mutant control 100 mm Petri dishes. Similarly, four wild-type adults were transferred by worm pick from the wild-type transfer dishes to each of the five 100 mm wild-type control Petri dishes. Two mutant and two wild-type adults were transferred to each of our five experimental replicate plates. After plating was completed, the nematodes were checked for vitality, and the Petri dishes were then sealed with parafilm. All worms in our replicates were placed in the center of the plate to ensure similar starting conditions for all individuals. Figure 1 below depicts steps 2 and 3 of the procedure.

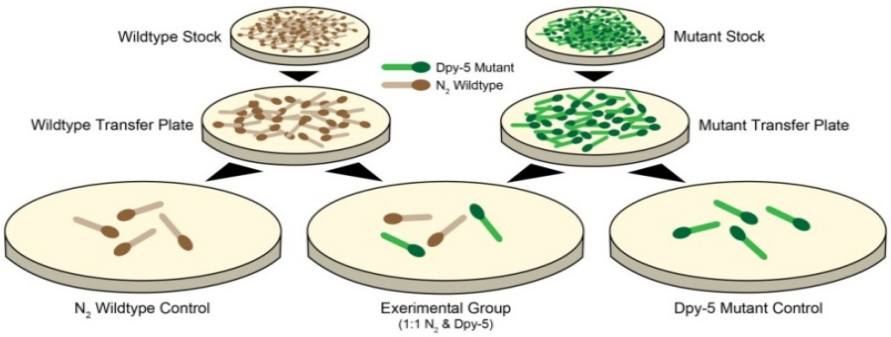

Figure 1: Procedural steps 2 and 3. First the *C. elegans* adults were transferred to a transfer plate. Subsequently, they were transferred to the control and experimental replicates. Both control groups held four individuals of either mutant or wild-type adults. The experimental replicates held 2 mutant and 2 wild-type adults. This schematic represents 1 of the 5 replicates for each of our treatments.

4. Incubation and Counts

After plating was completed, we incubated the Petri dishes for five days at 17 °C. On the fifth day, we removed the Petri dishes and started our daily counts. After each day's count, the plates were returned to the 17 °C incubation chamber.

Counts were started on the fifth day of the experiment and carried forth daily until the ninth day. Count was kept of how many adult mutant and wild-type *C. elegans* were present on the experimental replicates. See Figure 2 for the different morphologies of mutant and wild-type adults.

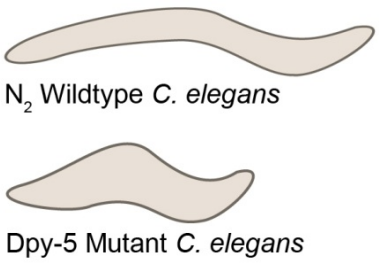

Figure 2: The difference in morphology of *dpy-5* adults and wild type adults. *Dpy-5* adults are thicker and shorter than the wild-type adults.

On the mutant and wild-type control replicates, count was kept of how many adults were present. Only adults were counted to ensure accuracy in our experimental counts. Larval *dpy-5* mutant and wild-type adults are nearly impossible to distinguish. To ensure that counts were accurate among group members, scores were crosschecked numerous times. If any plates were found to be contaminated in any manner, they were discarded from the study.

6. Other factors measured

Qualitative data, such as the prominence of tracks in agar, movement of individuals, and spread of individual worms, was recorded. The temperatures in the lab room and under the microscope during counts were recorded to ensure no thermal fluctuations were occurring.

7. Data analysis

To account for the differential starting numbers of wild-type and mutant adults between the control and experimental replicates, counts of adult worms were divided by the number of adults originally plated. Each group was assessed for statistical significance using 95% confidence intervals. The daily ratio of mutant to wild-type adults on each experimental replicate was calculated and averaged per day. Furthermore, 95 % confidence intervals were calculated to demonstrate if this ratio changed significantly as the days progressed. Growth rate was calculated from the previous day for Days 6-9. The growth rate 95 % confidence intervals were calculated and assessed for significance. For sample calculations, please consult Table 1 in the results section.

Results

Qualitative observations

The first striking result observed was the variability in worm tracks on the different treatment

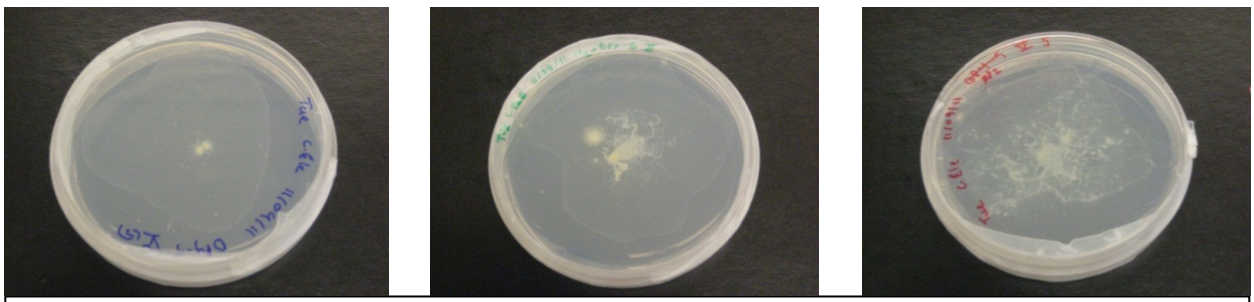

Figure 3: The typical differences in *C. elegans* tracks of the different treatments. The left plate is a mutant control plate. The middle plate is an experimental plate. The right plate is a wild-type control plate.

plates. This was indicative of the differences in the ability of the mutant and the wild type to locomote (Figure 3). The mutant control plates showed the least extensive tracks, whereas the wild-type control plates showed the most. The experimental treatment showed average worm movement to be between the two control treatments. Additionally, the mutant *dpy-5* produced noticeably fewer larvae than the wild type.

Quantitative Results

Sample calculations for data transformations are provided below in Table 1.

Sample Calculations

Adults per original adults

Day	Treatment	Number of adults	Number of adults per original adults
0	Wild-type Control replica	4	NA
5	Wild-type Control replicate	35	35/4=8.75
0	Experimental treatment	2 mutants	NA
5	Experimental treatment	7 mutants	7/2=3.5

Growth rate since Day 5

Day	Treatment	Number of adults	Growth rate
5	Mutant control replicate	17	NA
6	Mutant control replicate	48	(48-17)/17=1.83

Mutant to Wild type ratio

Day	Number of mutant adults	Number of wild-type adults	Ratio
5	7	30	7/30=0.23

Table 1: Sample calculations for data transformations: Adults per original adults, growth rate and mutant to wild type ratio.

Of note, two experimental replicates had to be removed from calculations: one due to fungal contamination, and one due to the sudden death of all worms. Concerning counts, the control groups both appeared to have achieved a day of maximum growth (Figure 4a). On the eighth day of the experiment, the wild-type control group demonstrated a statistically significant increase in the number of adults per original number of adults (38.5 ± 12.0 adults) compared to the previous day (15.2 ± 8.3). As well, on the eighth day, there were significantly more wild-type adults per original number of adults (38.5 ± 12.0 adults) than mutant *dpy-5* adults (13.3 ± 4.3). On the ninth day, the mutant *dpy-5* control group saw a statistically significant increase in numbers from the previous day, and there was once again no significant difference between the two control groups. In the experimental treatments, the number of *dpy-5* and wild-type adults per original adults at no point differed significantly from each other (Figure 4b). In addition, the experimental treatments did not

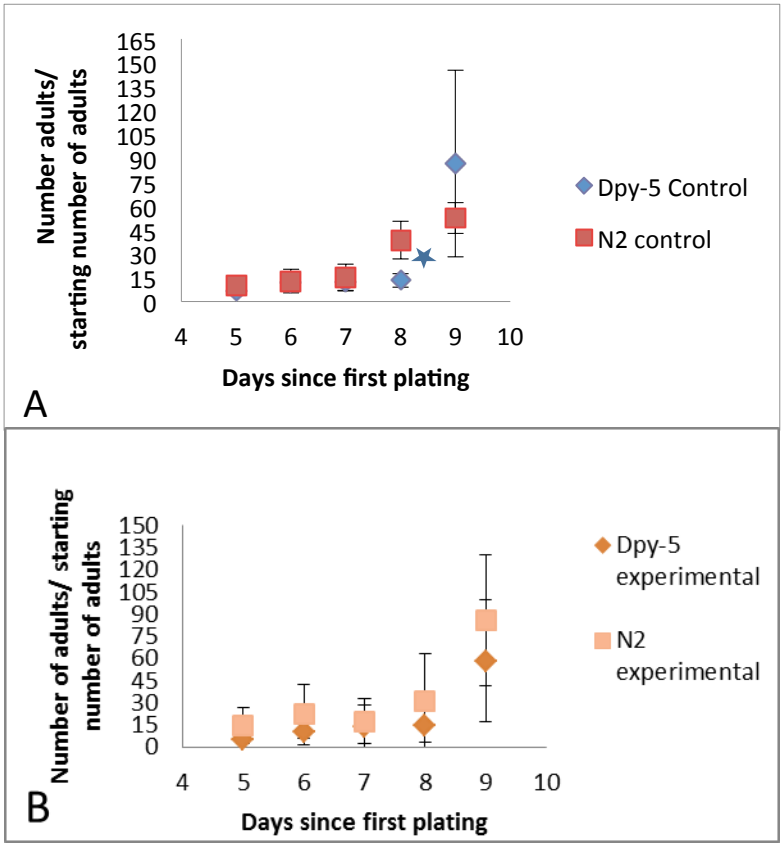

Figure 4: Number of adults per starting adults for Days 5-9. A) The control groups. B) The experimental treatment. The blue star indicates values of significance. Error bars indicate 95 % confidence intervals, N=5 for Figure 4a and N=3 for Figure 4b.

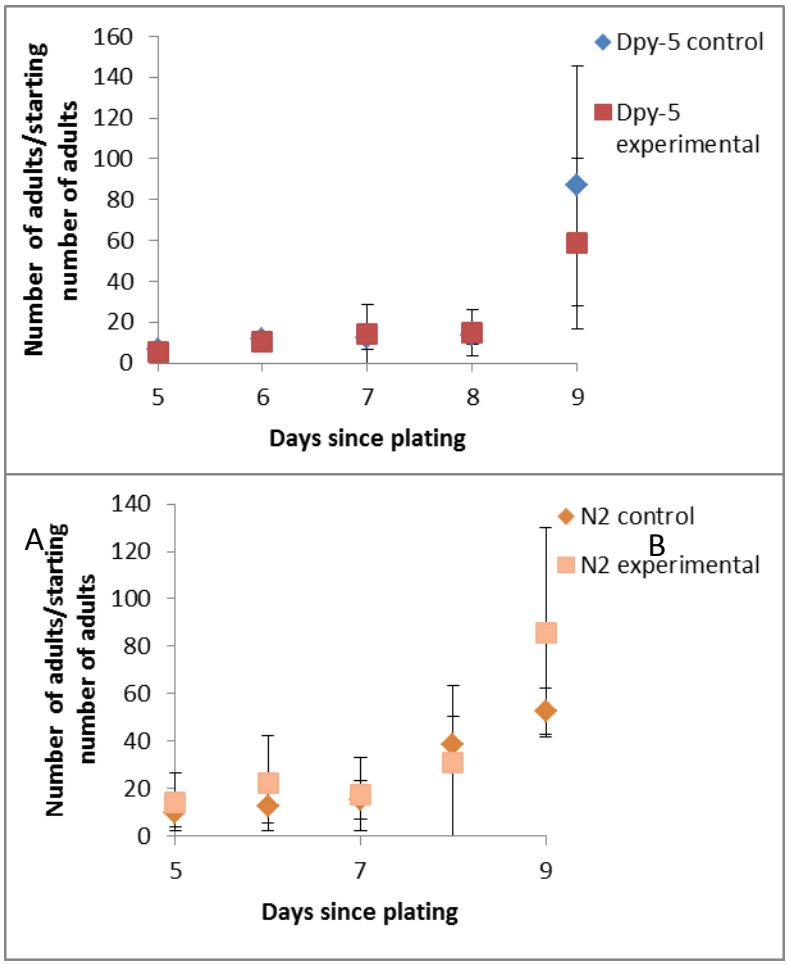

Figure 5: Number of adults per starting adults for Days 5-9. A) Comparison of the number of mutants in the experimental and control treatments. B) Comparison of the number of wild-type adults in the experimental and control treatments. Error bars indicate 95% confidence intervals. N=5 for control treatment; N=3 for experimental treatment.

demonstrate any significant differences on any day from the control treatments (Figure 5). Growth rates for Days 6-9 from Day 5 once again demonstrate that, on Day 8, there was significant growth for the wild-type control group. On the ninth day, this growth subsided. The mutant control group demonstrated a significant increase in growth rate on the ninth day (Figure 6a), congruent with the data in the adult counts (Figure 4a). On any given day, the wild type or mutant never demonstrated significantly more growth than the other in the experimental treatment (Figure 6b). Once again, there was no significant difference between the growth rates of the experimental groups and their controls (Figure 6c and d). The amount of population growth for any given day was highly variable for both the wild type and mutant in the experimental treatments. The ratio of *dpy-5* mutant adults to wild-type adults in the experimental treatment never significantly differed from the original 1:1 ratio, and was also highly variable, with some plates containing more wild-type adults and others more mutants (Figure 7).

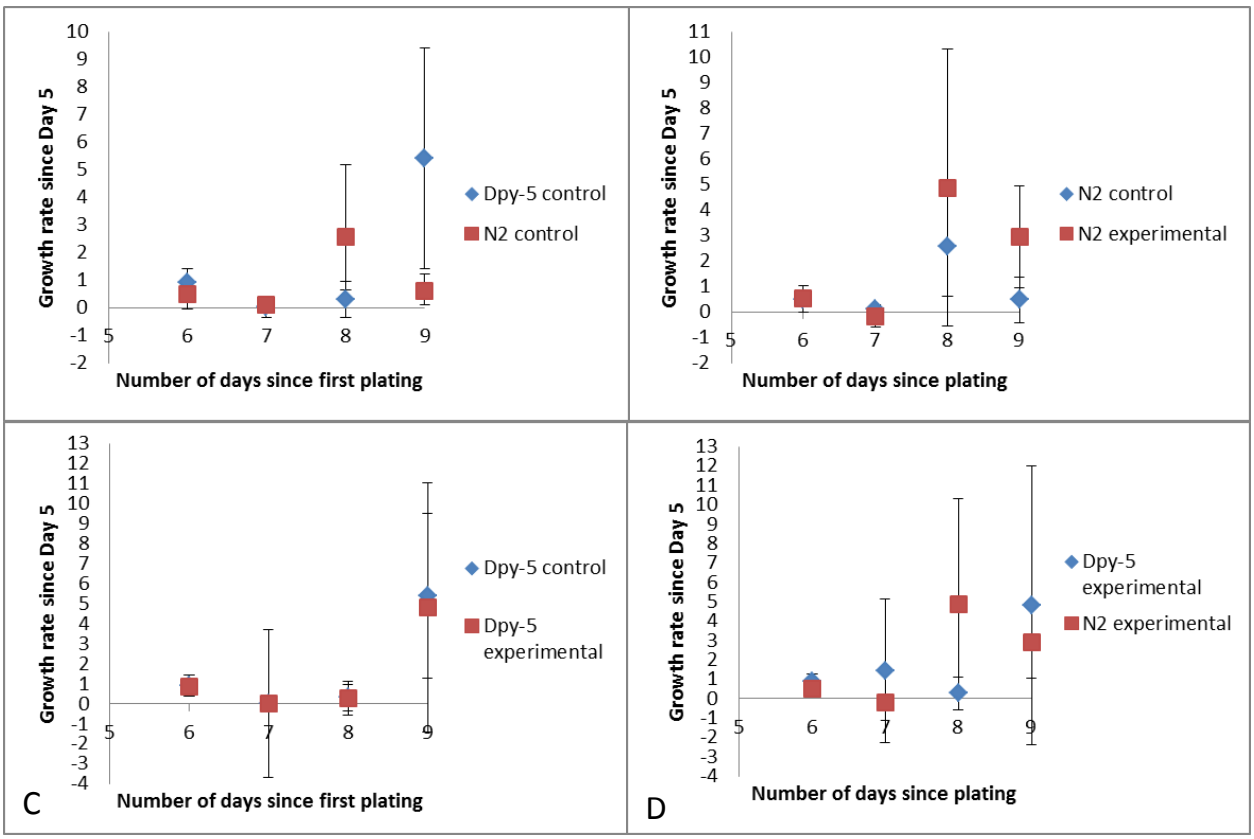

Figure 6: Comparison of growth rates since Day 5 for Days 6-9. A) Growth rate in the wild-type and mutant control groups. B) Growth rate in the wild-type control and experimental groups. C) Growth rate in the mutant control and experimental groups. D) Growth rate in the experimental group for the wild type and mutant type. Error bars indicate 95 % confidence intervals. N=5 for control treatment; N=3 for experimental treatment.

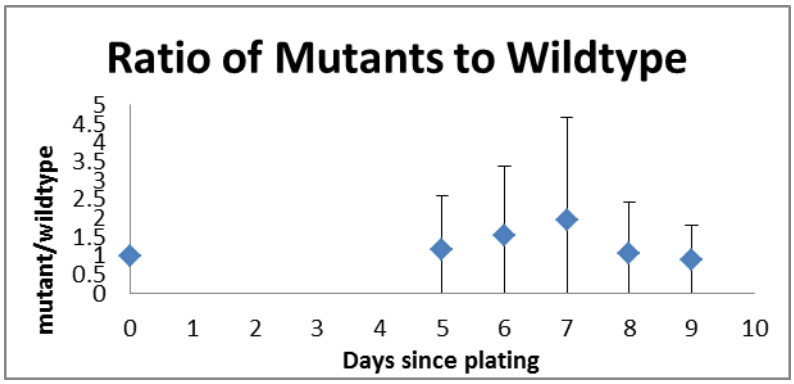

Figure 7: The ratio of mutant *dpy-5* adults to wild-type adults. The original ratio started at 1:1, as indicated on Day 0. Error bars indicate 95 % confidence intervals, N=3.

Discussion

The experimental groups yielded results that were not significantly different than the control groups, indicating that the N_2 wild-type and *dpy-5* mutant strains of *C. elegans* do not significantly affect

each other's growth and proliferation. The lack of an established relationship between one strain's growth and the presence or absence of the other suggested that competition does not occur between the wild-type and mutant strains to any significant degree. These results failed to support the alternative hypothesis (H_a), and failed to reject the null hypothesis (H_o); the study did not reveal the wild type to have a significant competitive advantage over the short and dumpy *dpy-5* mutant.

Despite the inability of this study to support the alternative hypothesis, several key observations were made that suggest that the N_2 wild type may be competitively superior to the *dpy-5* mutant.

More larvae and eggs were observed on the wild-type control plates than on the mutant control plates. Thacker *et al.* (2006) found that the *dpy-5* mutation results in a reduced brood size, which should serve as a competitive disadvantage for the mutants, since producing fewer progeny decreases species fitness.

The wild type left a greater number of tracks in the agar than the mutant. These tracks covered a greater area than those of the mutant, indicating that the wild type is the more motile strain. The decreased motility of the mutant is likely a consequence of its short and dumpy body form; the longer length of the wild type allows greater flexure, which enables greater movement (Hodgkin 1983).

Population spikes were observed to occur at different times for the wild-type and mutant strains, with the wild type showcasing a marked increase in population a day before the mutant (Figure 4). This offset has been determined to be due to a delay in the development of the mutant; the

maturation that occurs from the L1 larval stage to the adult molt can be delayed by up to 12 hours (Thacker *et al.* 2006). This means that the mutant takes longer to reach reproductive maturity.

Considering these observations, we expected that the wild type would demonstrate competitive superiority over the mutant. Since this was not the case, we postulated that other factors might have affected the results.

By Day 9, the wild-type and mutant controls and the experimental groups were showing signs of exponential growth (Figure 4). The exponential growth curves were only just beginning to form at this time, with no data suggesting that growth may be subsiding anytime soon. This indicates that our plates were likely well below their carrying capacities by the end of this study, meaning that resources were not yet limiting. *C. elegans* has been shown to require about 10^6 cells of bacteria per day per individual in order to support unconstrained growth; based on our results, it is likely that our treatments were able to provide this daily nutritional requirement throughout the duration of our study (Venette and Ferris 1998). Without a limiting resource, any potential indirect competition would not have been observed.

In addition, our study may not have been an accurate representation of the natural environment of *C. elegans*. Experimental manipulation can often result in changes to the studied system that prevent results from being applied outside of the study (Bergtold *et al.* 2005). As a result, even though our research did not detect any competition between the N_2 and *dpy-5* strains, the perceived advantages of the wild type may have been more important in *C. elegans'* natural environment.

An excessive amount of biological variation may have been another factor in the discrepancy between our results and our observations. Our organism counts varied widely, with disparities even between data from the same treatments. This resulted in very high standard deviations, which reduced the reliability of our results. Szeto *et al.* (2011) demonstrated that biological variation is very significant in *C. elegans*, with variation being represented by relative standard deviations of 29-39 %.

There are a number of possible sources of biological variation. A variable number of offspring was thought to have been one such source; although, it should be noted that Van Voorhies and War (1999) determined this to not be a significant issue. Another possible problem is that the worms that were initially plated were likely of slightly different ages; some may have even been L4-stage larvae. Signs of cross-fertilization were not observed, but if such fertilization did occur, the populations of the affected replicates would have swelled in size. Cross-fertilization has been found to occur less frequently in the *dpy-5* strain, which accentuates the potential impact that it could have had on the results (Hodgkin 1983). Certain worms may have been more able than others to handle the environmental stresses associated with this study; variable capacity to handle stress in the initially plated worms would have had direct effects on subsequent population growth.

A number of errors may have affected the results of our study. There may have been variability in counting technique; we attempted to mitigate the error of such variability by crosschecking each other's counts. The misidentification of worms is another possible source of error; larvae and adults were sometimes difficult to distinguish, and the mutant phenotype is usually not expressed until

adulthood (Brenner 1974). Many worms were grazing within bacterial growth, which made it difficult to accurately identify them. Fungus was found on Experimental Plate 2, meaning that contamination was a factor in this study. All worms on Experimental Plate 3 were dead by Day 7; it is possible that the *E. coli* used in this study began having a pathogenic effect on *C. elegans* (Darby 2005). Extra worms may have been transferred during the initial plating; having extra worms present at this early stage would have had a major effect on total population growth. Variability in plating technique may have affected this study, where difference in technique would have resulted in differing levels of stress applied to the worms; each treatment was handled by all members of the study to reduce the effects of such variation.

There are a number of ways in which this study can be altered to better assess the level of competition between the N_2 and *dpy-5* strains. Closer attention should be paid to the amount of *E. coli* supplied to ensure that resources become limited within the length of study. In addition, it would be beneficial to limit the accessibility of the food supply; making resources harder to reach may accentuate the effect of variation in motility. Lastly, more time should be allowed to observe population growth.

Conclusion

There were no significant differences between the N_2 wild type and *dpy-5* mutant controls and their respective experimental populations; the two strains of *C. elegans* did not significantly affect each other's growth and proliferation. Therefore, we fail to reject H_o and cannot provide support for H_a. We conclude that competition between the two strains was not observed in this experiment.

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