Effect of Temperature on N_2 and *unc-2* strains of *Caenorhabditis elegans* Locomotion

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Abstract

Temperature sensitive receptors play a key role in the survival of *Caenorhabditis elegans*. To gain insight into the role these receptors play, we explored the effects of temperature on distance travelled in 5 minutes by N_2 wild type and *unc-2* mutant *C. elegans*. The movements of four random N_2 wild type and *unc-2* mutant *C. elegans* were observed and measured through the use of the DinoXcope and ImageJ programs at temperatures of 11°C, 17°C, and 25°C. The mean distances travelled by the wild type were 40.5 mm, 39.1 mm, and 11.4 mm, and we measured 9.05 mm, 3.01 mm, and 3.30 mm for the mutant at the respective temperatures. As predicted, the N_2 wild type worms were more motile than the *unc-2* mutant, and the use of t-tests confirmed that these differences were significant ($p = 0.04$ for 11°C, $p = 0.01$ for 17°C). 95% confidence intervals revealed a significant difference between 17°C and 25°C for N_2 wild type whereas for *unc-2* mutant, there was a significantly more movement at 11°C than 17°C. The results for the wild type suggest that *C. elegans*' locomotion increases as it approaches its optimal temperature of 17°C while *unc-2* mutants appear to have a much lower preferential temperature due to the knockout of the *unc-2* gene, which is needed for the proper function of ADF neurons for physical movement.

Introduction

Caenorhabditis elegans is a transparent free-living soil nematode that is about one mm in length. It has an elongated cylindrical body with no segmentations or appendages (Wood 1988). *C. elegans* are found abundantly in bacteria-rich environments where decomposed vegetation and vertebrates are found. One interesting feature of *C. elegans* is the temperature-sensitive receptors on the surface of their bodies that enable them to sense the temperature of their environment (Wixon 2000). These receptors play a key role in the species' survival as increasing temperature has a negative effect on the species' locomotion (Wixon 2000). *C. elegans* possess a temperature sensitive gene (*unc-2*), which allows the species to move adequately under temperature stress (Wood 1988). Increases in temperature activate the expression of the gene, making the species less vulnerable to high temperature. According to Wixon (2000), a functional *unc-2* gene is only present in the N_2 wild types.

Our objective in this experiment was to determine whether changes to temperature have any significant impact on the motility of *C. elegans*. This investigation is important because by studying this, we can gain a better understanding about temperature sensitive receptors that are present in other

organisms like the snail, *Lymnaea stagnalis* (Sidorov 2003). Furthermore, we compared the effect of temperature on the distance travelled by N_2 wild type to the mutant in order to determine the function and characteristics of *unc-2* gene in *C. elegans*. This study is also relevant to human health because the *unc-2* gene and *CACNA1A* gene (Calcium Channel, Voltage-dependent, P/Q Type, Alpha-1A) in humans originate from the same ancestral gene (Wixon 2000). Interestingly, although both genes share the same origins, their involvements in the organism they reside in differ immensely. In nematodes, the *unc-2* gene controls locomotion functions, while the *CACNA1A* gene in humans is involved with making calcium channels involved in the transport of ions across cell membranes, cell-mediated immunity, and muscle motility functions (Wixon 2000). Learning more about the *unc-2* gene may help to understand diseases and mutations related to the *CACNA1A* gene.

Our alternative hypothesis (H_{1a}) states that both N_2 wild type and *unc-2* mutant *Caenorhabditis elegans* will increase in locomotion as temperature approaches its optimal temperature at 17^{o}C. Our null hypothesis (H_{1o}) states that there will be no observable effect for either strains or that there will be a decrease in locomotion. For our second set of hypotheses, we believe, as an alternative hypothesis (H_{2a}), that the *unc-2* mutant strain would move less than the N_2 wild type at all temperature treatments. The null hypothesis to this (H_{2o}) is that there will be no difference between the locomotion of wild type and mutant *C. elegans* or that the mutant will have greater locomotion than the wild type.

Methods

1. Set up

The *C. elegans* samples used were N_2 wild type hermaphrodites and *unc-2* mutant hermaphrodites. During the first week, we conducted the experiment on only N_2 wild type individuals and tested them at three temperature treatments: 11°C, 17°C, and 25°C. In the following week, the *unc-2* mutant sample worms had the same three temperature treatments.

Approximately twenty-four hours prior to the experiment, 11°C and 17°C incubators acclimated the appropriate worms to the temperature that they would be subjected to the next day; nematodes that were in the 25°C treatment did not require any incubation because the room temperature was already 25°C. There were two 60 mm petri dishes plated with *Escherichia coli*, a food source, for each of the two strains of *C. elegans*.

2. Procedure

For the treatment at 25°C (room temperature), we used sterile technique to transfer four healthy adult wild type *C. elegans* from the master plate to a transfer plate without *E. coli* under the dissecting microscope. We avoided transferring *C. elegans* from the initial plate to the empty plates with *E. coli* to prevent transferring *C. elegans* at its egg or molt stage. Adult *C. elegans* worms are distinct from the last molt stage (L4) by their larger size (approximately 1 mm in length), denser body, and lack of white spots (Hart 2006). From the transfer plate, one *C. elegans* worm was randomly chosen to be transferred to an empty plate with *E. coli*. Once placed, we first checked for vitality then observed the distance travelled by *C. elegans* under the dissecting microscope and a DinoXcope ocular camera (Figure 1) for five minutes. Digital photographs taken during the trials were to be used in the computer analysis later. We took a photograph of a ruler (Figure 2) for each magnification used to calibrate the scale of the pictures.

Figure 1. Image of dissecting microscope with Dinoxcope ocular camera set up at 17°C.

Figure 2. Sample image of ruler calibration under the dissecting microscope.

We analyzed the distance travelled by *C. elegans* using the ImageJ program to trace out the paths the nematodes moved in the photographs taken. The picture of the ruler was used to calibrate the number of pixels in the picture that make up 1 mm. With this calibration, we measured the total distance that *C. elegans* travelled during the 5-minute interval. The means and variance from that data was used to construct 95% confidence intervals to determine any significant differences among the 11°C, 17°C, and 25°C samples. Furthermore, we conducted t-tests to compare the distance travelled by the mutant to that of the wild type at these three temperatures settings.

N_2 wild type treatments at 11°C and 17°C followed the same procedure except that these treatments were conducted in the incubation rooms to keep the treatment temperatures constant. The trials with *unc-2* mutant *C. elegans* followed the same procedure.

(a) (b)

Figure 3. (a) Incubation room for 11°C treatment. (b) Incubation room for 17°C treatment.

There were four replicates (n=4) for each wild type and mutant temperature treatments except for the wild type treatment at 11°C, which only had three complete replicates (n=3) due to time constraint. In total, there were 23 replicates: 11 replicates of N_2 wild type and 12 replicates of *unc-2* mutant. The 17°C treatment served as our control treatment since it was the temperature in which the *C. elegans* were originally cultivated in the lab.

(a) (b) (c) (d) (e)

Figure 4. (a) Master plate for N_2 wild type replicates. (b) Master plate for *unc-2* (coded as VC854 strain) mutant replicates. (c) Transfer plate used. (d) Sample replicate used for N_2 wild types. (e) Sample replicate used for *unc-2* mutants.

Results

Qualitative observations

We observed worm trails left in the *E. coli* culture by *C. elegans* (Figure 7) and used them to help make our measurements in ImageJ. However, wild type worms often wandered off the bacterial culture and their trails were no longer visible (Figure 5). Other complicating behaviours include how worms would sometimes back up a short distance or would move only their heads side to side. The latter was not a part of our distance measurements.

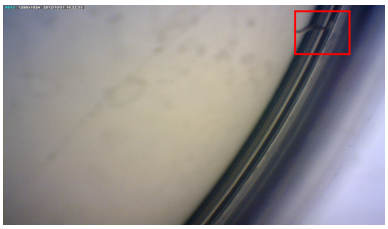

Figure 5. C.elegans off the bacterial culture

Quantitative observations

We first analyzed wild type and mutant data separately by making 95% confidence intervals (C.I.) for all the experimental and control conditions, and then we compared the two strains of *C. elegans* using a two-tailed t-test ($p = 0.05$). A sample C.I. calculation is in Table 1 and a sample t-test calculation is in Table 2.

Table 1. We obtained the 95% confidence intervals for our data by following the three steps outlined in this sample calculation for the wild type specimens at 25°C. These calculations were not done by hand but through spreadsheet functions in LibreOffice 3.6 Calc.

Replicate #	Distance travelled	Step 1) Find mean distance travelled $mean = \overline{X} = \frac{1}{n}\sum_{i}^{n} x_i$
1	10.290 mm	$\overline{X} = \frac{10.290 + 27.835 + 5.809 + 1.967}{4}$ = 11.475 mm
2	27.835 mm	
3	5.809 mm	
4	1.967 mm	
Step 2) Find standard deviation $\sigma = \frac{\sqrt{\sum_{i}^{n}(x_i - \overline{X})^2}}{N}$	$\sigma^2 = [(10.290 - 11.475)^2 + (27.835 - 11.475)^2 + (5.809 - 11.475)^2 + (1.967 - 11.475)^2]/4 = 130.520$ $\sigma = \sqrt{\sigma^2} = \sqrt{130.520} = 11.425$	
Step 3) Find 95% confidence intervals $C.I. = \overline{X} \pm 1.96\frac{\sigma}{\sqrt{n}}$	Upper Limit = $11.475 + 1.96\left(\frac{11.425}{\sqrt{4}}\right)$ = 22.671 mm	Lower Limit = $11.475 - 1.96\left(\frac{11.425}{\sqrt{4}}\right)$ = 0.279 mm

Table 2. Quantitative comparisons between wild type and mutant required using a two-tailed t-test, which uses calculations like that listed here for 25°C. These calculations were not done by hand but through spreadsheet functions in LibreOffice 3.6 Calc.

Step 1) Find the mean distance travelled for the wild type and mutant $mean = \overline{X} = \frac{1}{n}\sum_{i}^{n} x_i$	Wild type $\overline{X}_w = \frac{10.290 + 27.835 + 5.809 + 1.967}{4}$ = 11.475 mm	Mutant $\overline{X}_m = \frac{5.554 + 3.788 + 3.071 + 0.802}{4}$ = 3.304 mm
Step 2) Find variance $\sigma^2 = \frac{\sum_{i}^{n}(x_i - \overline{X})^2}{N}$	$\sigma_w^2 = [(10.290 - 11.475)^2 + (27.835 - 11.475)^2 + (5.809 - 11.475) + (1.967 - 11.475)^2]/4 = 130.520$	$\sigma_m^2 = [(5.554 - 3.304)^2 + (3.788 - 3.304)^2 + (3.071 - 3.304) + (0.802 - 3.304)^2]/4 = 3.870$

<table>
<tr><td>Step 3) Find the variance and standard deviation of the difference of the means</td><td colspan="2">$\sigma_d{}^2 = \frac{\sigma_w{}^2}{N_w} + \frac{\sigma_m{}^2}{N_m}$
$= \frac{130.520}{4} + \frac{3.870}{4}$
$= 33.598$
$\sigma_d = \sqrt{\sigma_d{}^2} = 5.796$</td></tr>
<tr><td>Step 4) Calculate the t-value
$t = \frac{\overline{X}_1 + \overline{X}_2}{\sigma_d}$</td><td colspan="2">$t = \frac{11.475 + 3.304}{5.796}$
$= 2.550$</td></tr>
<tr><td rowspan="2">Step 5) Find p using Student's t-distribution (two-tailed) and the correct degree of freedom</td><td>Degree of freedom = $n_1 - 1$
$= 4 - 1 = 3$</td><td>Degree of freedom = $n_1 - 1$
$= 4 - 1 = 3$</td></tr>
<tr><td colspan="2">Degree of freedom = 3 + 3 = 6
t = 2.550

t-distribution gives p = 0.249
(not significant)</td></tr>
</table>

The temperatures at which wild type (N_2) *C. elegans* moved the farthest are at 11°C (C.I. = 40.5 ± 12.8 mm) and 17°C (C.I. = 39.1 ± 12.7 mm), which are both significantly more than the 11.4 ± 11.2 mm mean distance travelled under the 25°C treatment. Unlike the wild type, the mutant *C. elegans* peaks in movement at only one temperature, 11°C (C.I. = 9.05 ± 0.94 mm), and this value is significantly higher than the 17°C (C.I. = 3.01 ± 0.89 mm) and 25°C treatments (C.I. = 3.01 ± 0.89 mm).

For all three temperatures, the wild type moved more than the mutant. As determined by two-tailed t-tests, differences between the two phenotypes are significant (p = 0.04 for 11°C, p = 0.01 for 17°C) except in the 25°C condition (p = 0.25), which has the greatest variation with respect to the size of its mean for both wild type and mutant travelled distances. The results are summarized in Figure 6.

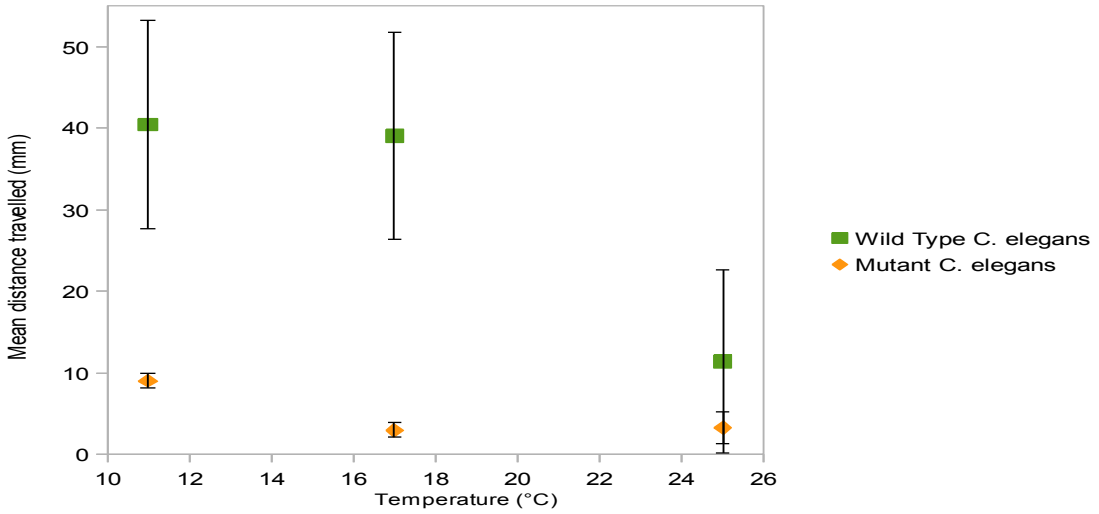

Figure 6. The mean distances travelled by wild type and mutant *C. elegans* in 5 minutes are shown with error bars representing 95% confidence intervals. N = 4 for all treatments except for the wild type 11°C condition where N = 3. Mean distances for the wild type 11°C and 17°C treatments were significantly greater than that of the wild type 25°C treatment. Mutant *C. elegans* moved significantly more at 11°C than the other two temperatures. At all temperatures observed, wild type worms showed greater average mobility than mutant type worms, but these differences are only significant at 11°C and 17°C.

Discussion

The experimental treatments showed some significant differences compared to the control group (17°C) for both the N_2 wild type and *unc-2* mutant strains. However, the results do not show a clear optimum temperature for *C. elegans*. As such, we failed to reject our H_{1o} to support our first alternate hypothesis ($H_{1a)}$ that 17°C is the optimum temperature for *C. elegans* locomotion.

The N_2 wild type *C. elegans* moved best at 11°C and 17°C and did not show a definite preference for one over the other. The 95% confidence interval (C.I.) for the 11°C treatment overlaps with the control at 17°C. This overlapping indicates that we cannot be 95% certain that the difference in means is not due to chance, and therefore, we failed to reject the null hypothesis (H_o). However, for the 25°C treatment, we see a relatively low mean movement that did not overlap in the confidence intervals with the control treatment. From this, we interpret that the optimum temperature is below 25°C and likely in the range of 11°C to 17°C. The observed differences were most likely due to the fact that the room temperature of 25°C was too close to their embryonic lethal temperature of 26°C which is characterized by random movements (Clark *et al.* 1997).

Conversely, the *unc-2* mutant had an evident peak in movement at 11°C. The 95% C.I. for the 11°C replicates is higher than and does not overlap with the C.I. of the 17°C or 25°C conditions, and thus, we conclude that the mutant's optimum temperature is about 11°C. This result is unexpected in that it is not in accordance to our hypothesized 17°C optimum. Instead, the recorded distances travelled by mutants were much less at 17°C versus 11°C. This difference suggests that the *unc-2* mutants have a much lower tolerance for temperatures greater than 11°C than the N_2 wild type, which did equally well at 17°C than at 11°C. The dysfunctional *unc-2* gene might have prevented the sample mutant nematodes from adjusting their motor or metabolic functions (Wixon 2000) to even moderate temperatures as high as 17°C.

Prior to the experiment, we also predicted in H_{2a} that the wild type *C.elegans* would show higher levels of locomotion, indicated by a greater distance travelled. As expected, our findings show that the wild types indeed travelled greater distances. However, the difference from the 25°C treatment is not significant so H_{2o} cannot be rejected. Again, this may be due to 25°C being too warm for nematodes to cope with (Clark *et al.* 1997).

Some qualitative observations noted were that the N_2 wild type specimens (Figure 7a) tend to move in a greater area and made more tracks in the agar compared to the *unc-2* mutant strains (Figure 7b). This is one indication of the wild type being the more motile strain. We also noticed that while transferring the wild type and mutant strains of *C. elegans* from the master plate to each of the transfer plates, there was a higher tendency to observe larvae and eggs on the wild type control plates (Figure 7c) than on the mutant control plates.

(a) (b) (c)

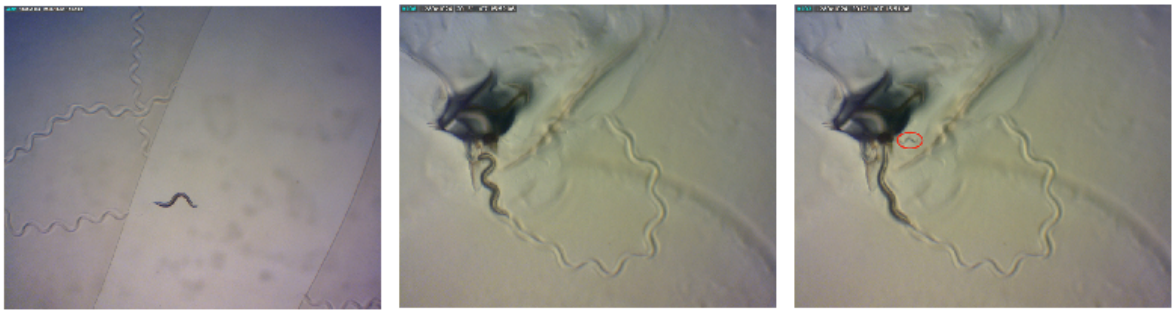

Figure 7. (a) Path made by N_2 wild type *Caenorhabditis elegans* replicate number 3 from the control treatment of 17^{o}C after 5 minutes. (b) Path made by *unc-2 mutant* replicate number 2 from the control treatment of 17^{o}C after 5 minutes. (c) *C. elegans* larvae circled in red for *unc-2* mutant control treatment replicate number 2 after 4 minutes.

Our recorded findings of there being higher levels of motility for the wild type strains versus the mutant strains were similar to the experimental results from Clark *et al.* (1997); they compared the wild type *C. elegans* to five mutant strains (*ky48,ky49, ky50, ky51 and ky52*) and found that the mutant strains exhibitted spastic movement at high temperatures (25°C) and subsequent recovery at lower temperatures. These perceived qualities of the tracks and of the progeny sizes could also serve as competitive advantages for the wild types.

Studies have found thermosensory neurons called AFN and AFD that are involved in regulating temperature-dependent behaviours such as the movements measured in this study (Prahlad *et al.* 2008; Beverly *et al*. 2011). The genes for stimulating these neurons are only expressed when under temperature stress, as in the 25°C experimental condition of our study, and help *C. elegans* to re-establish homeostasis. According to Prahlad and his team, this AFN regulatory signal pathway is only seen in wild type *C. elegans*. Building on this, Beverly and others' work (2011) suggests that this pathway helps *C. elegans* to behave in ways to climatize to new environments. Based on these previous findings, we would expect the average distances travelled by the wild type to be much greater than the mutant strain's at higher temperature settings. Our results showed that the wild type travelled an average distance of 11.5 mm while the *unc-2* mutant only travelled 3.3 mm under the 25°C treatment, thereby illustrating the impressive ability these nematodes have when adjusting to stress-inducing simulations.

In addition to the AFN and AFD thermosensory neurons, *C. elegans* also have ADF chemosensory neurons for motility. As illustrated in Figure 6, the ADF neuron is directly involved with the physical movement of the amphid, which are a pair of sensilla located in the head for directing movements (Herndon 2012). Specifically for our experiment, the *unc-2* mutants used had their *unc-2* function knocked out which is needed for the proper functioning of the ADF neuron and for maintaining normal serotonin levels (Sawin *et al.* 2000). Without a functional *unc-2* gene, we would expect decreased level of locomotion for the mutant replicates overall and less distance travelled at higher temperature treatments, which is what we observed in our results.

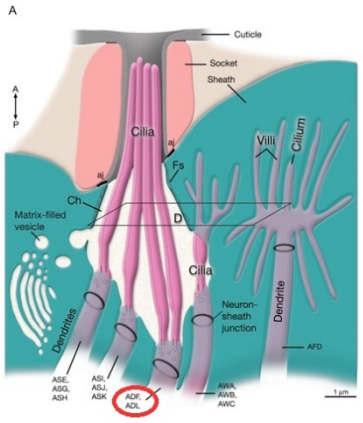

Figure 8. Anatomy of amphid sensillum of a wild type *C. elegans* with chemosensory ADF neuron circled in red. Image adapted from (Wormatlas 2012).

Overall, there were a few factors that could have contributed to the discrepancy in our predictions and results: biological sources, procedural sources, and personal biases.

Our nematode samples for all replicates were chosen randomly from a sample plate provided containing an excess of worms. The *C. elegans* candidates for our replicates were simply chosen randomly by various group members based on their visual sizes. This procedural step may have incorporated some personal biases along with biological variations as we could not verify whether the candidates chosen were truly mature.

Additionally, all the replicates cannot accurately represent the natural environment of *C. elegans*, since we manipulated both the abiotic factors and the biotic factors. In all the replicates, our samples were all given unused agar plates with *E.coli* as a food source and with all biotic competitors removed.

Conclusion

Our study failed to produce precise optimum temperatures, but was able to approximate the temperature ranges at which N_2 wild type and *unc-2* mutant *Caenorhabditis elegans* exhibit the greatest locomotion. The wild type genotype appears to move the most in the 11°C to 17°C range, and the mutant genotype is most motile around 11°C. These results fail to support the 17°C optimum temperature we hypothesized although the mutant's preference for low temperatures do imply that the unfunctional *unc-2* gene in the mutant leads to impairments in climatizing to higher temperatures. As further support for the wild type's superior fitness, the *unc-2* mutation has a profound deleterious effect on movement since there is a strong trend for the wild type *C. elegans* to have greater distances travelled than the mutant. The findings seem to suggest that the *unc-2* gene may be related to thermosensory neurons such as AFN and AFD. Further research on mutations of this gene can possibly expand our understanding of temperature sensitive receptors in other species and the evolutionarily close *CACNA1A* gene for calcium channels in humans.

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