Comparison of movement and speed in wild-type N2, VC854 *unc-2*(gk366) X and CB61 *dpy-5*(e61) I *Caenorhabditis elegans* strains

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Abstract

Caenorhabditis elegans have extensive molecular and physiological pathways that function to induce locomotion. In this experiment, the locomotion and speed of three different strains of *C. elegans*; wild-type N2, uncoordinated VC854 *unc-2*(gk366) X, and dumpy CB61 *dpy-5*(e61) I, were examined. From each strain, nine worms were randomly selected and their movement was recorded. Three sets of 30 second videos of were taken of each organism and these videos were analyzed using Wormlab software where the average speed of each strain was calculated. Using the average speed of each strain, we performed a one-way ANOVA using the *p*-value to determine significance. As a result, we found that the wild-type strain *C. elegans* moves the fastest (121.0 ± 21.1μm/s), the dumpy strain (69.2 ± 12.6 μm/s), and finally, the uncoordinated strain moves the slowest (57.8 ± 10.0 μm/s). To explain our findings, we have proposed locomotive pathways for each mutant. In summary, our findings will provide a better understanding of the movement and speed in the different strains of *C. elegans*.

Introduction

Caenorhabditis elegans are nematodes that are found all around the world. Their body is cylindrical in shape and measures approximately 1.2 mm in length. These simple organisms rely on highly developed chemosensory neurons to function normally (Bargmann 2006). The chemosensory neurons in *C. elegans* can be found in the amphid, phasmid and inner labial organs (Bargmann 2006). They contain a total of 32 chemosensory neurons that can sense different compounds in the environment (Bargmann 2006). For example, the ASE neurons, which are found in the amphid and phasmid, aid in the detection of water-soluble compounds such the attractant, salt (NaCl) (Komatsu *et al.* 1996; Ward 1973). Some synapses are triggered at neuromuscular junctions (NMJs) as the neurons sense compounds in the organism's surroundings (White *et al*. 1986). Ions, such as calcium (Ca^{2+}) are released at these junctions to induce muscle contraction, which essentially controls the movement of *C. elegans* (Figure 1).

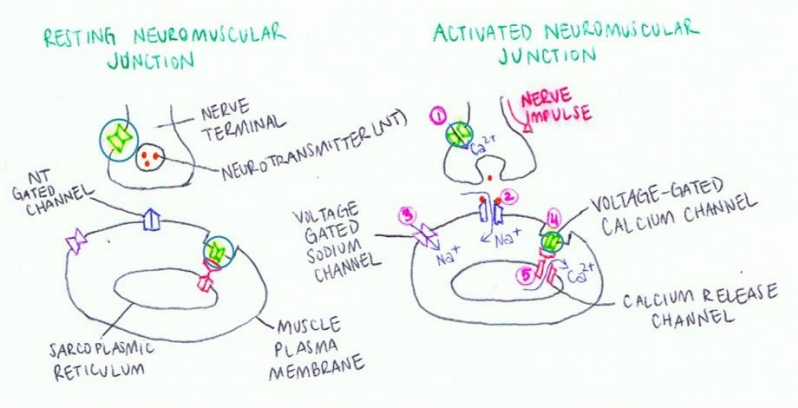

Figure 1. A diagram of a resting and activated neuromuscular junction showing action potential. When the neuromuscular junction is activated, Ca^{2+} ions flood into the nerve terminal via VGCC causing a release of neurotransmitters. The neurotransmitters bind to the gated channel causing the uptake of Na^{+} in muscle cells, which causes the VGCC on the muscle cell to bind the gated Ca^{2+} release channel releasing Ca^{2+} in the muscle resulting in muscle contraction .Image adapted from Molecular Biology of the Cell, 4th Edition.

In addition, *C. elegans* have a collagenous body wall, called the cuticle, that encapsulates the entire organism. Not only does the cuticle provide the organism protection, it is also necessary for movement, as muscles are attached to the cuticle (Thacker *et al.* 2006). When wild-type *C. elegans* sense different compounds in their surroundings, calcium is released at the NMJs. Calcium helps induce muscle contraction on both the ventral and dorsal side of the organism. The side that contracts causes the respective cuticle to compress (Camp 2003). As a result, the force created from the muscle contraction is transmitted to the opposite side of the organism and which causes the cuticle to stretch (Camp 2003). The continuous contraction of muscles on one side of the organism and alternatively, the stretching of muscles on the other side of the organism, ultimately allows the *C. elegans* to propel itself in its desired direction. The pathway for the normal movement of *C. elegans* is shown in Figure 2.

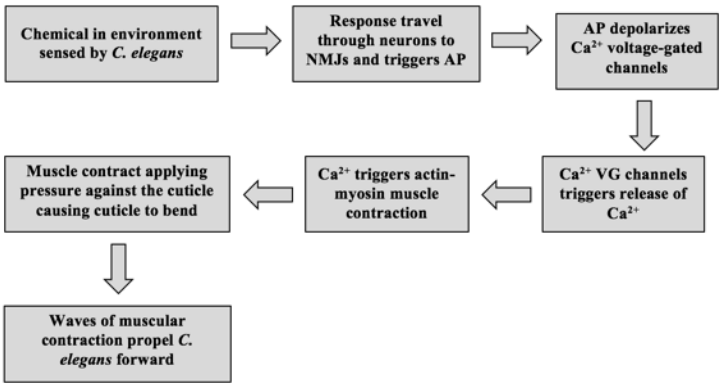

Figure 2. Pathway showing normal movement of wild-type *C. elegans* in response to compounds in its surroundings. First, compounds are sensed in the environment by the neurons in *C. elegans*. Chemical synapses are triggered at NMJs to release Ca^{2+}. Calcium then causes different muscles along the length of the organism to contract. This causes the cuticle to contract with the muscle. The contraction force is transmitted to the opposite side of the organism, and the cuticle on the opposite side stretches. Continuous contraction and stretching of the muscle and cuticle on opposite sides helps the organism move.

There are many mutations that can affect the speed and movement of *C. elegans.* Some of these mutations give rise to dumpy or uncoordinated phenotypes. Although we know that these mutants have impaired or slower movements than their wild-type counterparts, the actual mechanisms that explain their lethargy are not well understood. In this experiment, we compared the difference in speed among the wild type N2 (wild type), uncoordinated VC854 *unc-2*(gk366) X (Unc) and dumpy CB61 *dpy-5*(e61) I (Dpy) strains. The mutants, Unc and Dpy, were examined because they have impaired movement Hu *et al.* (2015) found that NaCl is sensed by *C. elegans* as a source of food; therefore, NaCl was added to the medium to stimulate the organisms to move. The null hypothesis states that there is no difference in speed among the Unc, Dpy, and N2 *C. elegans* strains. The alternate hypothesis states there is a difference in speed among the Unc, Dpy, and N2 *C. elegans* strains. We predicted that the N2 strain will move the fastest as it does not contain any mutations that would affect its movement and that the dumpy would travel faster than the uncoordinated *C. elegans*.

Methods

Strains

The three different strains that were used in this experiment were N2 (wild-type), CB61 *dpy-5*(e61) I (Dpy), and VC854 *unc-2*(gk366) X (Unc). Depending on the strain, between two to four adult stage worms, were transferred from an existing stock to 60-mm NGM agar plates streaked with 200-300 μL of OP50 *Escherichia coli*. These plates were then incubated for one day at 20°C and subsequently at 15°C for another six days as per standard growth protocol.

Pre-experimental Setup

Twenty-four hours prior to the experiment, we obtained 36 60-mm NGM transfer plates that contained only agar and no *E. coli*. *E. coli* was not added to the medium since it is a food source for *C. elegans* and could cause them to forage and interfere with their movement (Liu *et al.* 2012).

We marked the center of each plate with a dot, as shown in Figure 3 and added to it 16 μL of 0.1 M NaCl 24 hours before the experiment to allow adequate diffusion of 2 cm across the agar plate (Luo *et al.* 2010; Salt 2013). Next, we drew a dotted circle with a radius of 1 cm from the center. The worms were placed within this circle, so we could discern the position of each worm. We chose to place the worms 1 cm away from the source to ensure that all *C. elegans* were within the presence of the diffused NaCl. Following Matsuura *et al.* (2013), we chose to add NaCl, a water soluble substrate, to the medium of the agar plates to create a stimulus that would ensure the movement of the *C. elegans*.

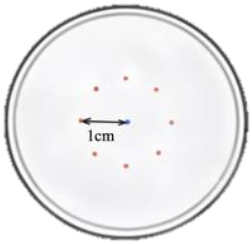

Figure 3. 60-mm NGM transfer plates with no *Escherichia coli*. 16 μL of 0.1 M NaCl solution was placed in the center of the plate, represented by the blue dot. The red dots represent the circle with a radius of 1 cm that marked where the individual *C. elegans* were placed.

Protocol Design and Techniques

Using sterile techniques we randomly selected a large, moving adult hermaphroditic *C. elegans* from each strain from the prepared 60-mm *E. coli* agar plate (n=12) and transferred it to the 60-mm marked, NaCl-diffused transfer plates. We placed the *C. elegans* within the circle with a radius of 1 cm to ensure uniformity. We selected the largest worms since adult hermaphroditic worms are characterized by the size of the worm (De Bono and Maricq 2005). Moreover, we selected adult *C. elegans* to minimize variation of age among the worms. We chose worms that showed movement to ensure that the worms were alive. Once successfully transferred to the plates, *C. elegans* were allotted 30 seconds to acclimate to the new medium. Then, using the DinoXcope attachment on the Kyowa dissecting microscope at 2.5X magnification, we recorded three sets of videos at 30 approximately seconds for each individual *C. elegans*. Videos were stopped if the *C. elegans* moved out of the field of view, therefore the length of some videos was less than 30 seconds. The average speed of each individual was calculated from the set of videos. Taking the average speed of an individual gave us a better representation of the true speed of each *C. elegans* and accounted for random changes in movement. This procedure was repeated for each individual in all three strains with four experimenters using four Kyowa dissecting microscopes.

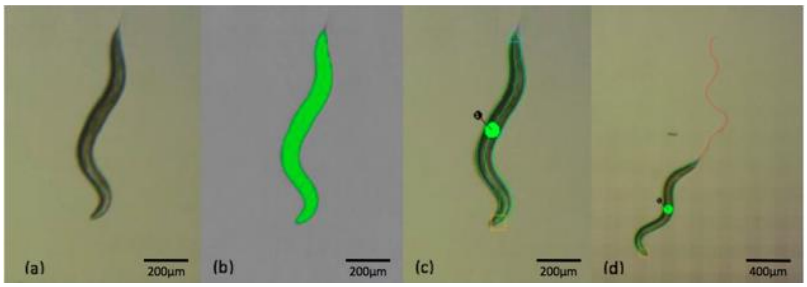

Figure 4. Pictures of N2 wild-type *C. elegans* analyzed by Wormlab. (a) Video of *C. elegans* before analysis. (b) Manual selection of optimal contrast level for *C. elegans* detection. (c) WormLab detection of *C. elegans*; yellow box marks the head, the green dot marks the middle of the worm, and the blue triangle marks the tail. (d) The detection of the *C. elegans'* movement and its tracks.

Data Analysis and Statistics

We used MFB Bioscience's WormLab software to track and analyze the recorded videos of the *C. elegans,* in order to determine the average speed of the 36 individual worms (n=12 per strain). Wormlab analyzed, frame by frame, the detected changes in movement to track the total length of forward and reverse movement in micrometers and the average speed of the worm in micrometers per second (µm/s).

Initially, we calibrated the Wormlab software with a picture of a ruler. Then, for each video, the image was manually adjusted so that there was an optimal contrast between the worm and the agar plate. This allowed for better detection of the worm (Figure 4b). Once the contrast was set correctly, Wormlab automatically detected the worms (Figure 4c). Following this, Wormlab began analyzing the motion of the detected worm (Figure 4d). The analyzed average speed of each video was then recorded in Excel. Each individual *C. elegans* had three different videos and so we obtained three different speeds for each worm. These three speeds were then averaged to determine the average speed of the respective individual. However, due to the limitations of Wormlab, a total of nine individuals (27 videos) were omitted because they were not able to be correctly tracked. With that deduction, we were left with a total of 27 individuals, nine individual replicates per strain (n=9 per strain). Examples of worms that Wormlab was not

able to track included some uncoordinated *C. elegans* that curled onto themselves (Figure 5a) and a few wild-type worms as seen in Figure 5b.

To determine the overall average speed of our wild-type, dumpy, and uncoordinated *C. elegans*, we took the average speed of the nine averaged individual speeds of each strain. Next, we performed a one-way analysis of variance (ANOVA) to determine whether there were significant differences among the average speeds of the different *C. elegans* strains. Finally, to determine which of the strains were likely to be significantly different from one another, we calculated and compared the 95% confidence intervals between the mean wild-type, dumpy, and uncoordinated *C. elegans* speed.

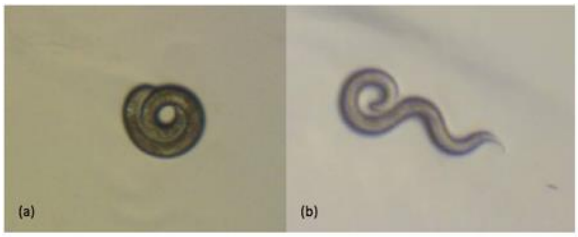

Figure 5. Screenshot images of particular *Caenorhabditis elegans* that were not detected properly with the Wormlab software. (a) An uncoordinated *C. elegans* curled up onto itself. (b) A wild-type *C. elegans* partially curled.

Results

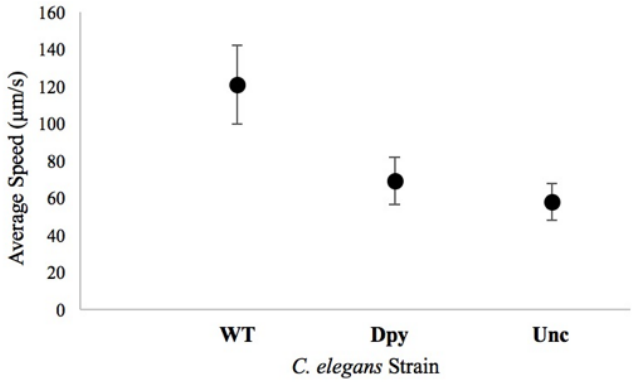

Figure 6. The average speed (µm/s) of wild-type (WT), dumpy (Dpy), and uncoordinated (Unc) *C. elegans* (n=9). Vertical lines represent the 95% confidence intervals. The *p*-value is 5.0×10^{-6}.

We observed that the wild-type (N2) worms moved in smooth, sinusoidal waves across the plate. Similarly, the dumpy mutants travelled in a sinusoidal motion. However, dumpy's

movement was more head-oriented, where the body followed a more rigid S-shaped or curved manner. On the other hand, the uncoordinated mutants moved in a slower, sinusoidal motion. In general, we observed that all strains moved both forward and backwards. We noticed that the uncoordinated mutants more often reversed and coiled into themselves (Figure 5a). Based on Figure 6, the results show the average speed of the wild-type, dumpy and uncoordinated *C. elegans* to be (121.0 ± 21.1) μm/s, (69.2 ± 12.6) μm/s, and (57.8 ± 10.0) μm/s, respectively. Wild-type *C. elegans* were approximately twice as fast as the mutants.

We used a one-way ANOVA test to determine if the average speed differences among strains were statistically significant. The calculated *p*-value was 5.0×10^{-6}. By comparing our calculated *p*-value with the significance threshold *p*-value of 0.05, there was a significant difference in the average speed for all three strains.

We also determined that the speed of the dumpy and uncoordinated *C. elegans* are likely not significantly different, since their 95% confidence intervals overlap (Figure 6). However, our results imply that the wild-type is significantly faster than both dumpy and uncoordinated *C. elegans* because its 95% confidence intervals do not overlap with the mutants (Figure 6).

Discussion

Based on the results of the one-way ANOVA ($p<0.05$), we reject the null hypothesis and provide support for our alternate hypothesis, which is that there is a difference in speed between the wild-type, uncoordinated and dumpy *C. elegans* strains. Even though the confidence intervals do not imply a difference between the two mutants' speed, we found that the dumpy *C. elegans* did move slightly faster than uncoordinated *C. elegans.* Therefore, the general trend is that wild-type moves the fastest, followed by dumpy and uncoordinated *C. elegans.* These results concur

with our original prediction that the wild type would be the fastest, as the wild-type *C. elegans* does not have a mutation that could possibly impair normal muscle movement.

Although minimal research has been conducted comparing the mechanisms of movement and speed in wild-type to uncoordinated and dumpy *C. elegans,* there is existing information about the genetics and physiology of nematodes. Using the known locomotive pathway of the wild-type *C. elegans,* as highlighted in Figure 2, and the information gathered about the mutations, we have tried to explain possible defects that could have occurred along the locomotive pathway resulting in the mutants' impaired locomotion.

Extensive knowledge about the wild-type shows that the organism has a covering known as the exoskeleton or cuticle (Bird 1971). The cuticle is attached to the body wall muscles by fibrous organelles (Petzold *et al.* 2011) and plays a role in protection, mobility and the morphology of the nematode (Page and Johnstone 2007). In addition, the internal body cavity of *C. elegans* contains a fluid filled cavity called the pseudocoelom (Coomans *et al.* 2003). Together the body muscles and the pseudocoelom allow the cuticle to function as a hydrostatic skeleton (Karbowski *et al.* 2006).

During locomotion, the muscles apply pressure to the cuticle (Karbowski *et al.* 2006). This pressure is consequently opposed by the high hydrostatic pressure of the pseudocoelom, which causes the cuticle to bend (Karbowski *et al.* 2006). Force must be transmitted from the body wall motors to the cuticle in order for muscle contraction to occur (Fritz and Behm 2009). Waves of muscular contractions and relaxations results in nematode propulsion and sinusoidal-like movement in *C. elegans.*

Dpy-5 encodes for the production of collagen in the cuticle, in conjunction with the gene *bli-4* (Thacker *et al.* 2006). However, the mutated *dpy-5* suppresses *bli-4*, resulting in a

phenotypic defect causing *C. elegans* to be short and dumpy (Thacker *et al.* 2006). The e61 allele in this mutation causes a nonsense substitution, halting the production of collagen in the cuticle (Thacker *et al.* 2006). Considering the main component of the cuticle is collagen and is required for normal body length and functions in cuticle production, mutations can cause abnormalities in its propulsion (Page and Johnstone 2007; Karbowski *et al.* 2006). This is seen in the dumpy *C. elegans.*

We propose, as seen in Figure 7, that this abnormality in the cuticle affects normal body wall muscle attachment to the cuticle, causing abnormal muscle contraction and insufficient power to thrust the *C. elegans* forward. Ultimately, this impedes movement and decreases speed. Our proposed pathway, as observed in Figure 7, can explain the results of our experiment, in that the dumpy *C. elegans* has a slower speed than wild-type.

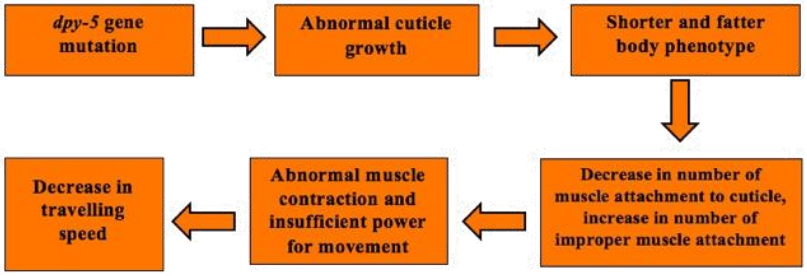

Figure 7. Predicted pathway affecting CB61 *dpy-5*(e61) I locomotion. A mutation in *dpy-5* causes abnormal cuticle growth resulting in the shorter and fatter body phenotype in dumpy *C. elegans.* Due to a shorter body, muscle attachment decreases in the cuticle and improper alignment of the muscle to cuticle increases. Abnormal contractions of the muscle decrease the contraction force that is transmitted. Therefore, insufficient power is generated for movement and dumpy *C. elegans* move slower.

Another probable explanation for the slower locomotion in the dumpy could be due to the biomechanics of the organism. The size or length of the *C. elegans* affects its speed (Boyle 2009). Longer *C. elegans* have a more body wall muscles, creating more power to make it move (Boyle 2009). Since the body length of dumpy *C. elegans* is shorter, this indicates that it will have less muscle tissue, causing it to have a decreased speed when compared to the wild-type.

Similar results were observed in the uncoordinated *C. elegans*, as it too, was slower in speed than the wild type. *Unc-2* codes for a voltage-gated calcium channel (VGCC) alpha subunit, which is required for normal flexion and speed of *C. elegans* (Mathews *et al.* 2003). The gene encodes an alpha subunit that is important in two places: in motor neurons at the muscle plasma membrane and the neuromuscular presynaptic terminal of neurons (Mathews *et al.* 2003; Caylor *et al.* 2013). At the neuromuscular presynaptic terminal, the alpha subunit regulates the entry of calcium ions, triggering the release of neurotransmitters shown in Figure 1. These neurotransmitters attach to the receptors on the muscle plasma membrane, triggering an action potential (AP). AP then triggers depolarization of VGCC, which is also expressed by the *unc-2* gene, which triggers the release of stored calcium ions. The flood of calcium ions in the cytoplasm then allows actin to bind to myosin, which ultimately leads to muscle contraction (Figure 1).

However, mutations in the *unc-2* gene will cause a reduction in the uptake of calcium ions from the presynaptic neuron, which will reduce the likelihood of an AP for muscle contraction to occur. This will ultimately reduce the locomotor ability of *C. elegans* (Caylor *et al.* 2013). Disrupting the function of the channel coded by *unc-2* affects all physiological processes that depend on the calcium channel. As a result, *unc-2* mutants suffer from poor coordination of the body wall muscles that are responsible for the control of movement (Tham 2005). Our specific uncoordinated mutant, VC854 *unc-2*(gk366) X, has an insertion mutation in the *unc-2* gene which results in a disruption in the formation of the VGCC alpha subunit. Our proposed pathway, as observed in Figure 8, can explain why uncoordinated *C. elegans* has a slower speed than wild type. Therefore, without this functioning *unc-2* gene, we would expect decreased locomotion, which is what we observed.

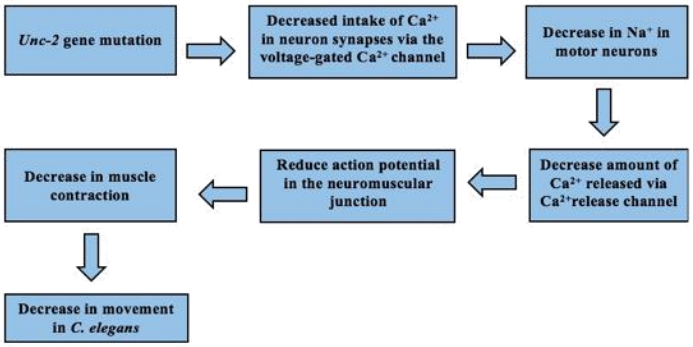

Figure 8. Proposed pathway for movement in VC854 *unc-2*(gk366) X. A mutation in *unc-2* disrupts the formation of the VGCC alpha subunit, which is essential for the proper release of Ca^{2+}. A decrease in Ca^{2+} concentration decreases the muscle contraction, since actin and myosin filaments in the muscle cannot bind at optimum levels. Therefore, movement in *C. elegans* will decrease as well.

Even though our results are statistically significant, potential errors still arose during the experimental procedure. One factor that could have affected our results is the age of each *C. elegans,* as older nematodes will move faster than younger nematodes (Collins *et al.* 2008). To minimize this error, the *C. elegans* were all transferred at the same time and when choosing each nematode, we selected the largest ones (refer to methods). Additional errors could have risen from discarding the videos of uncoordinated *C. elegans* that curled up onto itself. This would affect our results because the speed of those individuals would be 0 μm/s, causing a reduction in average speed of uncoordinated *C. elegans*. The Wormlab software could have also caused some errors. The program did not calculate the speed of *C. elegans* that moved out of the camera. A few wild-type *C. elegans* went out of frame and those videos could not be used in our data and this reduced our sample size.

Conclusion

In summary, the wild-type (N2) strain of *C. elegans* moves approximately twice as fast as the mutant strains, VC854 *unc-2*(gk366) X and CB61 *dpy-5*(e61) I. Based on these results, we

rejected our null hypothesis and have proposed two possible pathways to support our alternate hypothesis.

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Literature Cited

Bargmann, C.I. 2006. Chemosensation in *C. elegans*. WormBook: the online review of C. *elegans* biology.

Bird, A.F. 1971. The structure of nematodes. Academic Press, New York, NY.

Boyle, J.H. 2009. *C. elegans* locomotion: an integrated approach. Ph.D. Dissertation, University of Leeds, United Kingdom, England.

Camp, J. 2003. Nematodes [online]. Available from http://www.faculty.pnc.edu/jcamp/parasit/nematode.html [accessed 16 November 2015].

Caylor, R.C., Jin, Y., and Ackley, B.D. 2013. The *Caenorhabditis elegans* voltage-gated calcium channel subunits UNC-2 and UNC-36 and the calcium-dependent kinase UNC-43/CaMKII regulate neuromuscular junction morphology. Neural Development, **8**(1): 10.

Collins, J.J., Huang, C., Hughes, S., and Kornfeld, K. 2008. The measurement and analysis of age-related changes in *Caenorhabditis elegans*. WormBook: the online review of *C. elegans* biology.

Coomans, A., Claeys, M., Borgonie, G., and Link, C. 2003. Lysosomal and pseudocoelom routing protects *Caenorhabditis elegans* from ricin toxicity. Nematology, **5**(3): 339-350.

De Bono, M., and Maricq, A.V. 2005. Neuronal substrates of complex behaviors in *C. elegans*. Annual Review of Neuroscience, **28**: 451-501.

Fritz, J. and Behm, C. 2009. CUTI-1: a novel tetraspan protein involved in *C. elegans* cuticle formation and epithelial integrity. PLoS ONE, **4**(4): e5117.

Hu, L., Ye, J., Tan, H., Ge, A., Tang, L., Feng, X., Du, W., and Liu, B. 2015. Quantitative analysis of *Caenorhabditis elegans* chemotaxis using a microfluidic device. Analytica Chimica Acta, **887**(5): 155-162.

Karbowski, J., Cronin, C.J., Seah, A., Mendel, J.E., Cleary, D., and Sternberg, P.W. 2006. Conservation rules, their breakdown, and optimality in *Caenorhabditis* sinusoidal locomotion. Journal of Theoretical Biology, **242**(3): 652-669.

Komatsu, H., Mori, I., Rhee, J. S., Akaike, N., and Ohshima, Y. 1996. Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron, **17** (4): 707–718.

Liu, H., Wang, X., Wang, H., Wu, J., Ren, J., Meng, L., Wu, Q., Dong, H., Wu, J., Kao, T., Ge, Q., Wu, Z., Yuh, C., and Shan, G. 2012. *Escherichia coli* noncoding RNAs can affect gene expression and physiology of *Caenorhabditis elegans*. Nature Communications, **3**: 1073.

Luo, L., Greenwood, J., Soucy, E., Kim, D., and Samuel, A. 2010. Making linear chemical gradients in agar. The Worm Breeder's Gazette, **18**(3): 10-11.

Mathews, E.A., Garcia, E., Santi, C.M., Mullen, G.P., Thacker, C., Moerman, D.G., and Snutch, T.P. 2003. Critical residues of the *Caenorhabditis elegans* unc-2 voltage-gated calcium channel that affect behavioral and physiological properties. Journal of Neuroscience, **23**(16): 6537.

Matsuura, T., Izumi, J., Hioki, M., Nagaya, H., and Kobayashi, Y. 2013. Sensory interaction between attractant diacetyl and repellent 2-Nonanone in nematode *Caenorhabditis elegans*. Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, **319**(5): 285-295.

Page, A.P. and Johnstone, I.L. 2007. The cuticle. WormBook: the online review of *C. elegans* biology.

Petzold, B., Park, S., Ponce, P., Roozeboom, C., Powell, C., Goodman, M., and Pruitt, B. 2011. *Caenorhabditis elegans* body mechanics are regulated by body wall muscle tone. Biophysical Journal, **100**(8): 1977-1985.

Salt, A.N. 2013. Diffusion Coefficients [online]. Available from http://oto2.wustl.edu/cochlea/model/diffcoef.htm [accessed 12 November 2015].

Thacker, C., Sheps, J.A., and Rose, A.M. 2006. *Caenorhabditis elegans* dpy-5 is a cuticle procollagen processed by a proprotein convertase. Cellular and Molecular Life Sciences, **63**(10): 1193-1204.

Tham, K.L.D. 2005. Generation and analysis of suppressors of *Caenorhabditis elegans* unc-2 alleles. M. Sc. Thesis, Simon Fraser University, Vancouver, British Columbia.

Ward, S. 1973. Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. Proceedings of the National Academy of Sciences of the United States of America, **70**(3): 817-821.

White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, **314**(1165): 1-340.