Effect of Temperature on *Caenorhabditis elegans* Locomotion

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Abstract

Caenorhabditis elegans has a simple yet highly developed nervous system. Exploring the effects of temperature on locomotion can give insight on how more complex ectotherms may react to an increase in temperature. We focused on speed and wavelength as the components of locomotion to test the effects of temperature. The three temperature treatments used were 11°C, 17°C and 25°C. We placed a single *C.elegans* on a Petri dish and recorded the time it took to move a certain distance. Their track lengths and wavelengths were measured and analyzed using the programs DinoXcope and ImageJ. At temperatures of 11°C, 17°C, and 25°C, the speed of *C.elegans* was 0.017 mm/sec , 0.060 mm/sec , and 0.136 mm/sec respectively. The wavelengths were found to be 0.16 mm, 0.23 mm and 0.32 mm respectively. There was a significant difference between the 11°C and 25°C temperatures. Our results suggest that an increase in temperature increases the locomotion of the nematode *Caenorhabditis elegans.*

Introduction

Caenorhabditis elegans is a small transparent roundworm with a length of approximately one mm (Wood 1988). According to Wood (1988), *C. elegans* has a well described anatomy and sequenced genome that makes it an ideal organism for laboratory studies. These nematodes have nervous systems that allow them to sense temperature and react to various stimuli (Wixon 2000). Specific genes of *C. elegans* are seen to be temperature sensitive (Wood 1988). Upon a change in temperature, these genes may be altered to have an effect on the features and functions that are coded in the specific gene (Wood 1988). This characteristic of *C. elegans* is essential to the objective of this study: how different temperatures affect the locomotion of *C. elegans.* Locomotion is important because it is the primary method in which these nematodes interact with one another and their environment. Due to chemotaxis, they are able to sense a region that is nutrient rich and move towards it (Pierce-Shimomura *et al.*1999). The general trend of movement of *C.elegans* is sinusoidal and the variations to the motion depend on behavioural modes such as feeding and reproduction.

In order to determine how higher temperatures affect movement in ectotherms with complex nervous systems we examined the locomotion of *C. elegans* when exposed to three distinct temperatures. Studies in other ectotherms have documented an effect of temperature on locomotion. For example a study on the fresh-water-snail *Lymnaea stagnali* found that the locomotion and respiration increased with an increase in temperature (Sidorov 2003). This study suggested that this change may be a result of temperature dependent reactions in neurons (Sidorov 2003).

Ho: An increase in temperature will decrease, or have no effect on the locomotion of *Caenorhabditis elegans*.

Ha: An increase in temperature will increase the locomotion of *Caenorhabditis elegans*.

Methods

The *C. elegans* that we used were N2 wild-type hermaphrodites. This experiment had three temperature treatments (11°C, 17°C and 25°C). Prior to the addition of worms the petri dishes were incubated in each of the temperature treatments to prevent the nematodes from experiencing temperature shock. The length of worm incubation for each treatment was five hours. We also carried out the experiment in the respective controlled-temperature rooms.

There were six replicates (n=6) for the 11°C treatment, four replicates (n=4) for the 17°C treatment and three replicates (n=3) for the 25°C treatment. Two replicates were discarded for 17°C and 25°C treatments due to the complications during the initial run of the treatments (the tracks were too long to account for the distance). At 25°C, only three replicates (n=3) were considered because the *C. elegans* on one dish stopped for many extended periods of time and a continuous track could not be captured.

The following steps provide details as to how the nematodes were prepared for the administration of the experiment. A single *C. elegans* in either the last molt (L4) or adult stage was removed from the initial plate and placed in the centre of a new petri plate coated with *E. coli*. The last molt stage (L4) is recognized by a larger size similar to that of the adult stage, but is less dense and has

a white spot around the center of the body (Hart 2006). Once the *C. elegans* was placed onto the center of the plate, we began timing the movement. This portion of the experiment was undertaken inside each of the controlled-temperature rooms to maintain a constant temperature. The worms were recorded until they moved out of the field of vision from the dinoXcope's ocular camera. This allows one picture to be taken of the track and stored on a computer for analysis. A second picture was taken of the track at the same magnification but with a ruler focused in the field of vision. This second picture was used for data analysis in order to determine the track length.

We used the program ImageJ to trace the path made by the nematode in the picture frame. From this data we could calculate the speed of the worm by measuring the track made from the movement of *C. elegans*..A picture of ruler was used to standardized one mm on the ruler to the number of pixels in the picture. This ratio was used on the track picture to calculate the distance that the *C. elegans* travelled. Three individual wavelengths were chosen per worm to find an average wavelength for each track. The first wave selected for analysis was the tenth wave from the start of the nematode's motion and then every fifth wave was measured for its wavelength to a total of three wavelengths collected.

Figure 1. Setup of the microscope in the 17°C incubator

Results

The data collected on track length and time allowed us to determine the speed and wavelength of locomotion. The shape of the sinusoidal waves are seen to vary from temperature to temperature. At

11°C, the sine wave appears to be much closer, meaning shorter wavelength (Figure 2). As the temperatures increased, the sine waves increased in amplitude, the wavelengths also spread further apart covering more distance (Figures 3 and 4).

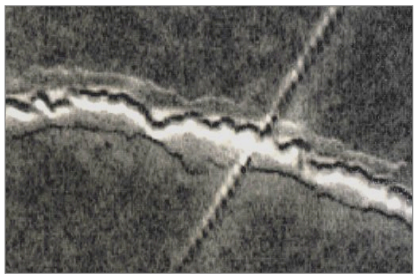

Figure 2. Image of *C. elegans* movement at 11°C; captured by by DinoXcope at magnification of 10X.

Figure 3. Image of *C. elegans* movement at 17 °C; captured by DinoXcope at a magnification of 7X.

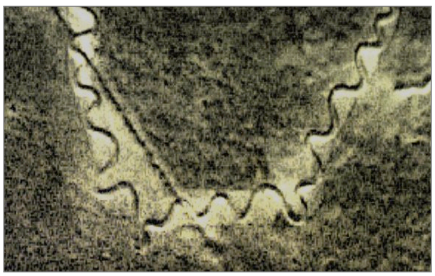

Figure 4. Movement of *C. elegans* at room temperature (25°C). Image taken by DinoXcope At a magnification <7X.

Figure 5 displays the results for the speed of motion. At 11°C the speed was found to be 0.017 +/- 0.016 mm/sec, at 17°C it was 0.060 +/- 0.027 mm/sec and 25°C the speed was 0.136 +/-0.013 mm/sec. Through calculating the 95% confidence intervals for each speed it was found there is statistical difference between 11°C and 25°C. Figure 6 displays the results of average wavelength and temperature. The average wavelength at 11°C is 0.16 +/-0.053 mm, at 17°C is 0.23 +/-0.060 mm and at 25 °C is 0.32 +/- 0.057 mm. Once again there is statistical difference seen between 11°C and 25°C.

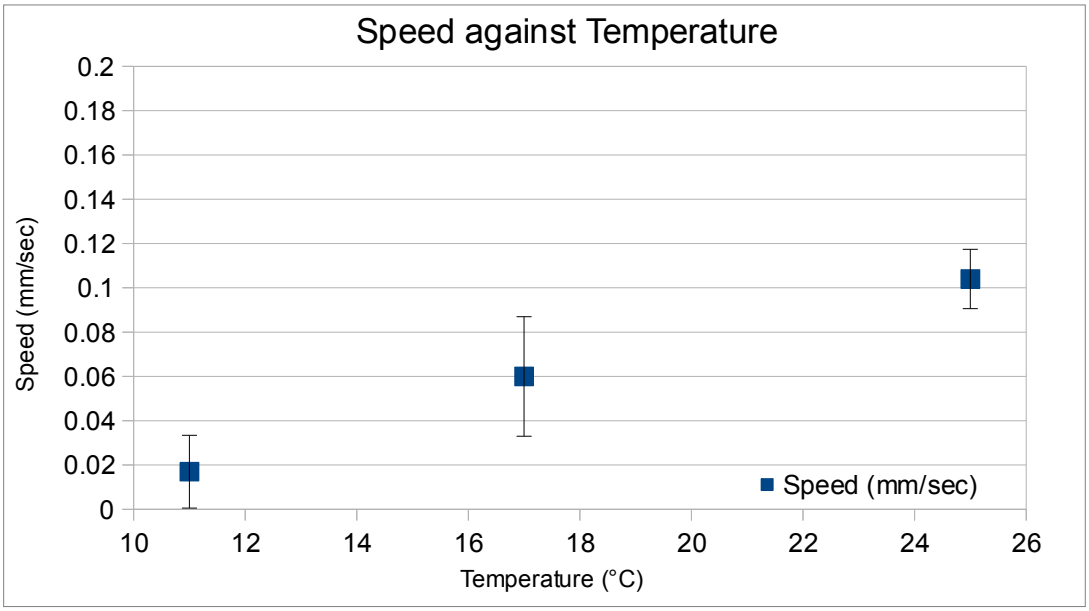

Figure 5 Speed (mm/sec) of *C. elegans* with respect to temperature (°C). At 11°C, 17°C, and 25°C, the average speed of *C. elegans* was 0.017 mm/sec , 0.060 mm/sec , and 0.136 mm/sec respectively.

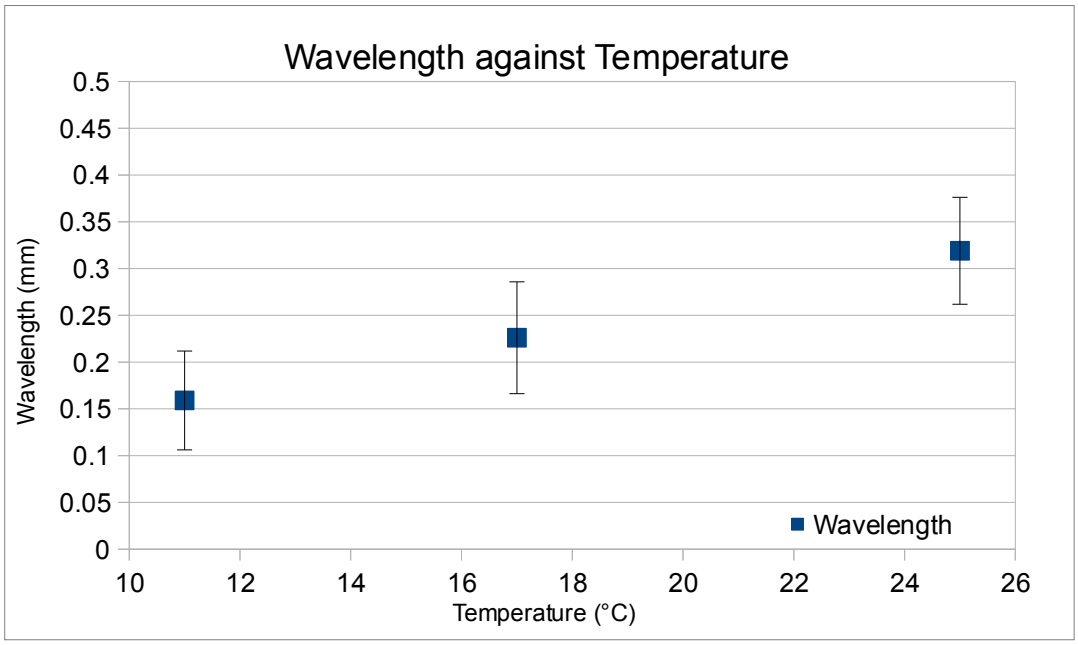

Figure 6. Average wavelength (mm) produced by the motion of *C. elegans* with respect to temperature (°C). The wavelengths are 0.16 mm, 0.23 mm and 0.32 mm at 11°C, 17°C and 25 °C, respectively.

Sample calculation for 17°C:

1) Finding the average (n=4) for the wavelengths in mm:

$$\frac{0.248+0.148+0.293+0.214}{4} = \mathbf{0.22567\ mm}$$

2) Finding Standard Deviation:

$$\sqrt{((0.248- 0.22567)^2 +(0.148-0.22567)^2+(0.293-0.22567)^2+(0.214-0.22567)^2)/4)}$$

=0.06099 mm

3) Calculating Confidence Intervals:

1.96*(Standard deviation/$\sqrt{}$ (number of replicates))

1.96*(0.06099/$\sqrt{}$ (4))

=0.05977 mm

Discussion

Locomotion of *C. elegans* was analyzed using two of its properties, speed and wavelength. From the results obtained in this experiment, we reject our null hypothesis(Ho) and support our alternate hypothesis (Ha). The 95% confidence interval determines the reliability of the statistical model and the interval in which there is sufficient statistical difference between samples. The confidence intervals for the means of the speeds of 11°C and 25°C do not overlap; therefore, it can be concluded that there is a significant difference in the mean speed between these two temperatures. With the analysis of the data, it can be stated that there is a correlation between speed and temperature: an increase in speed as temperature increases (Figure 5). Also, we observed that the wavelengths increase with an increase in temperature. The wavelengths on the *C. elegans* track had no overlap between the confidence intervals of 11°C and 25°C. Figure 6 shows that there is an increase between all the

temperatures even though at 17°C it is not statistically different. There is a significant difference between 11°C and 25°C in both locomotion components. These results correlate with the findings from the experiment performed on *Lymnaea stagnali,* an ectothermic freshwater snail (Sidorov 2003); in this study an increase in temperature resulted in an increase in locomotion.

The increase of locomotion with a temperature increase is a biological response by the cells of *C. elegans.* It can be correlated to responses seen in other ectothermic invertebrates. In a study done by Ramnanan and Storey (2006), the ATP turnover rate of the land-snail *Otala lacte* was studied via the Na+/K+-ATPase activity. It was found that the enzymes of active snails were found to increase in their affinity for ATP when temperature increased (Ramnanan and Storey 2006). Increased ATP affinity is important, as the function of ATP in the cell is to power the molecular motors that allow muscle cells to contract (Alberts *et al.* 2010). Therefore, if more ATP is being made in order to fulfill this high demand, more power is being supplied to these motor neurons to stimulate muscle cells. At increased temperatures, the stimulation of muscle cells is expected to occur more often if there is more ATP present to be used. This can explain why we observe the increase in locomotion with increased temperature.

As temperatures increased, the shape of the sinusoidal waves produced by *C. elegans* were observed to change. They were observed to be more pronounced over a greater distance when compared to the tracks at the colder experimental temperature. At 11°C, the tracks in Figure 2 appear closer together and indicate that the nematode is only moving by motions of the head; this is known as foraging. Foraging is a process where *C. elegans* feed on the *E. coli* surrounding them on a sampling plate. This movement involves a repeated side-to-side head motion; thus producing smaller tracks (Buckingham and Sattelle 2008). As the temperature increased to the temperature of 25 °C, the waves produced by *C. elegans* were more spaced out as seen in Figure 4; when this pattern is observed, *C. elegans* are no longer foraging. The larger wavelengths indicate that they are using their dorsal and ventral muscles to push themselves in either a forward or backward motion (Wood 1988). At this

higher temperature, there are elongated sinusoidal waves observed due to the muscles which work to push *C. elegans* forward.

There are responses by *C. elegans* to their environment which account for discrepancies seen in the tracks made. Plates were covered in *E. coli* because the tracks are immediately visible and in pre-experimental tests the nematodes appeared to move constantly through these plates. However, having a plate filled with food for the nematode will cause an environmental response. As found by Shtonda (2006), worms in food filled environments may move in reverse and slower compared to worms who have to search for food. This slower movement is referred to as dwelling (Shtonda 2006). Their amount of movement may also reflect whether they have been fed recently. The nematodes were all placed on similar *E. coli* rich plates but changes between roaming for food and dwelling may affect the overall locomotion.

Another source of variation in this experiment may be the result of the nematode transfer from the initial plate to the experimental Petri dish. If the *C. elegans* were injured from a worm pick, this would affect their movement. An abnormal movement would be visible on the tracks because the motions would become inconsistent and slow.

Conclusion

After observing the tracks of C. *elegans* at three temperatures (11°C, 17°C and 25°C), an increase in the locomotion components of speed and wavelength was found. Studying these organisms can help determine the relative temperature effect on locomotion of other larger and more complex ectotherms. We reject our null hypothesis(Ho) and support our alternate hypothesis (Ha).

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