

# The effect of temperature on the locomotion of *Caenorhabditis elegans*

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## Abstract

Many biological processes such as homeostasis and thermotaxis allow animals to thermoregulate, to stay within temperatures in which they can survive and reproduce. Although thermotaxis is an important principle for thermoregulation and animal survival, it is not known whether this principle also applies to higher-level biological processes such as animal behavior within a fixed incubation temperature. This investigation looks at mobility behavior of *C. elegans*, specifically speed of movement, at different incubation temperatures comparing the wild type and *unc-2* mutants. Twenty-four samples of *C. elegans* were incubated at three temperatures, 11°C, 15°C, and 20°C, video-taped and had their speed analyzed via the computer program, Wormlab). There were no significant differences in nematode speed at different incubation temperatures (p-value= 0.43). However, our analysis suggests that the effect of incubation temperature on *C. elegans* motility differs between the wild type and mutant (p-value= 0.01). This might be due to different behavioral strategies for each genotype across the incubation temperatures tested. We suggest further research for the effects of temperature on locomotion and animal behavior.

## Introduction

*Caenorhabditis elegans* (*C. elegans*) is a free living nonparasitic nematode found in both aquatic and terrestrial environments (Felix & Braendle, 2010). In a laboratory setting, they are grown on petri dishes with *Escherichia coli* (*E. coli*) for nutrition (Felix & Braendle, 2010) and grow to lengths of 1-2mm in their adult stage. The wild-type N2 *C. elegans* exhibits sinusoidal crawling movement in forward and reverse movements, and spends less time in the stationary phase than the mutant (Yemini, Jucikas, Grundy, Brown & Schafer, 2013). The mutant, *unc-2* *C. elegans* shows uncoordinated non-worm like movements and exhibits shallower sinusoidal movement than the wild type (Yemini et al, 2013). Furthermore, the mutant is more likely than the wild type to present coiled body movements and shapes (Yemini et al, 2013). In order to make turns, *C. elegans* makes omega shaped movements to change directions (Parida, Neogi, Padmanabhan, 2014). We studied the effects of temperature on locomotion, specifically speed, of the mutant and wild-type *C. elegans*. This study is important because there is a substantial

amount of research in the field of thermotaxis of *C. elegans* while there has not been a lot of research on the effects of fixed temperature on their locomotion.

The mutant studied in this experiment has a deletion in the *unc-2* gene, which codes for voltage-gated calcium channels (Caylor, Jin & Ackley, 2013). Voltage-gated calcium channels are used for the exocytosis of neurotransmitters in neuromuscular junctions (NMJ'S) (Caylor et al, 2013). The lack of these channels in the mutant decreases the number of threshold action potentials that lead to synaptic transmission which likely results in the lack of locomotion as seen in the mutant (Caylor et al, 2013). The mutation is known to affect the development of neuromuscular synapses as well as decrease the number of formed synaptic regions (Caylor et al, 2013). A decrease in successful NMJ synapses will decrease the amount of excitation-contraction coupling which occurs in muscle contraction (Mathews, Garcia, Santi, Mullen, Thacker, Moerman, Snutch, 2003). Overall, a decrease in voltage-gated calcium channels will lead to decreased muscle movement when triggered by stimuli.

*C. elegans* motility is affected by temperature. They are known to increase their speed linearly with the increase in temperature until they reach the lethal level of approximately 25°C (Anderson, Alebergotti, Proulx, Peden, Huey, & Phillips, 2007). We hypothesize that changes in temperature from the cultivation temperature (15°C) will have an effect on the speed of *C. elegans* in a laboratory setting. We predict that the wild-type *C. elegans* will exhibit a greater difference in speed than the mutant due to the decreased number of voltage-gated calcium channels in the neuromuscular pathways of the *unc-2* mutants.

Hypotheses:

1. H<sub>0</sub>: Changes in incubation temperatures have no effect on *C. elegans* speed.

H<sub>a</sub>: Changes in incubation temperatures have an effect on the speed of *C. elegans*.

2. H<sub>0</sub>: The effect of changes in incubation temperatures on *C. elegans* speed does not differ between the N2 wild type and *unc-2* mutant.

H<sub>a</sub>: The effect of changes in incubation temperatures on *C. elegans* speed differs between the N2 wild type and *unc-2* mutant.

## Methods

The *C. elegans* strains we used were N2 wild type and *unc-2* mutant. Hermaphrodites that were in the developmental stage of L4 (pre-adult) were selected for the measurement. The L4 were identified by their large body size of about 1mm in length. (Hall, Altun, and Herndon, date accessed 11 November 2016). We used incubation temperatures of 11°C, 15°C, and 20°C. The *C. elegans* were incubated for 30 minutes prior to measurement; the measurement was done using dissecting microscopes. The control groups were the nematodes in the petri dishes exposed to 15°C because all of the nematodes were previously cultivated at 15°C.

There were four petri dishes prepared for each strain of nematode for each temperature; each petri dish was a replicate for a temperature (n = 4). Each petri dish contained 4 pseudoreplicates that were analyzed. Pseudoreplicates associated with nonfunctional videos were eliminated. No fewer than three pseudoreplicates were analyzed for each temperature.

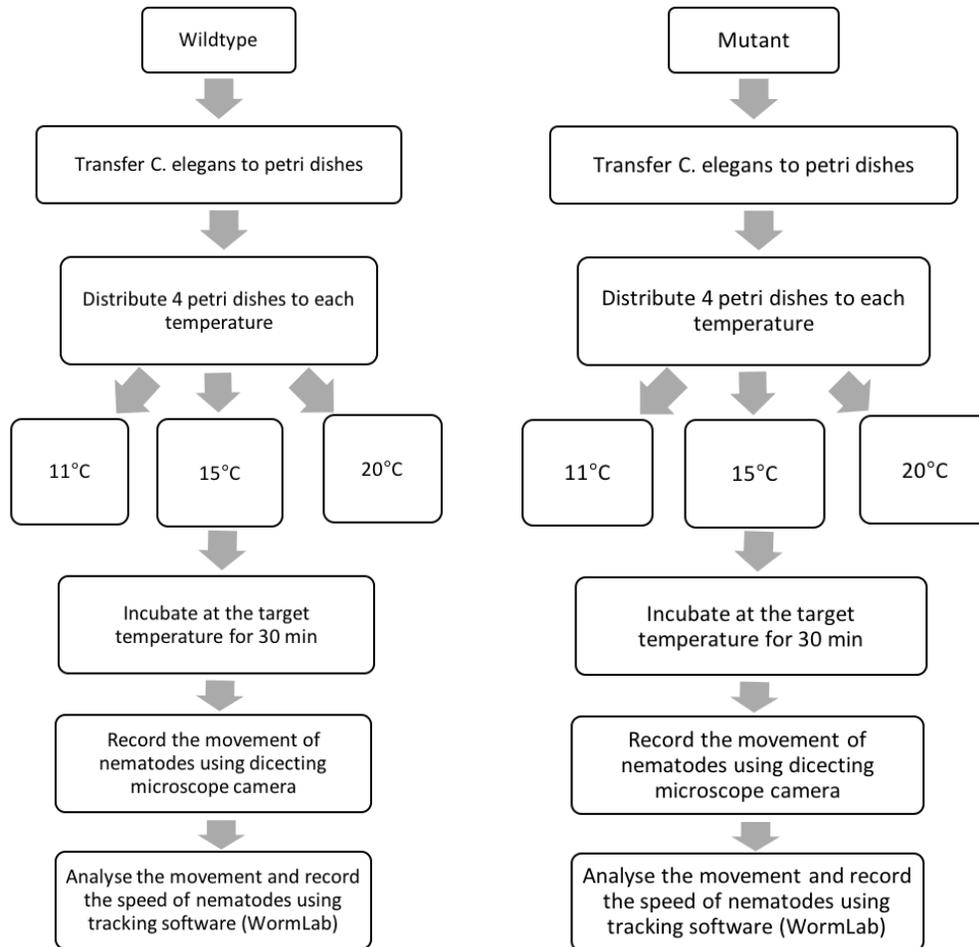
We used platinum picks and a sterile method to remove each *C. elegans* from a stock petri dish with *E. coli* as food. We used scooping technique to pick up the nematodes for wild type, and single-pick technique for mutant to pick up single nematodes. The *C. elegans* were grown to reach the L4 stage +1 day of age on the day of the measurement. We transferred each *C. elegans* from the stock dish to a fresh 60-mm petri dish with agar but without *E. coli*. Each petri dish was incubated for exactly 30 min in its designated temperature in incubators. One petri dish of wild type and one petri dish of mutant were incubated at the same time. After the 30 min

incubation, we removed the petri dishes and brought them back to the lab where all measurements were made. The time between removal from the incubator to the beginning of the measurement was kept under 2 min 30 sec to minimize any effect of exposure time to room temperature. Two dissecting microscopes, equipped with DinoCapture 2.0 were used for recording simultaneous measurements of one petri dish with N2 wild type and another petri dish containing *unc-2* mutant nematodes. The details of the procedure are depicted in a flowchart (Figure 1). Before we started taking measurements, a picture of a ruler was taken and used for scaling and field of view calculations. We placed individual *C. elegans* in the centre of the field of view of the microscope, and recorded their movements for two min. Figure 2 shows the experimental setup. Measurements of the other three individuals on the same petri dish were performed immediately after the previous measurement. All measurements were completed on one day.

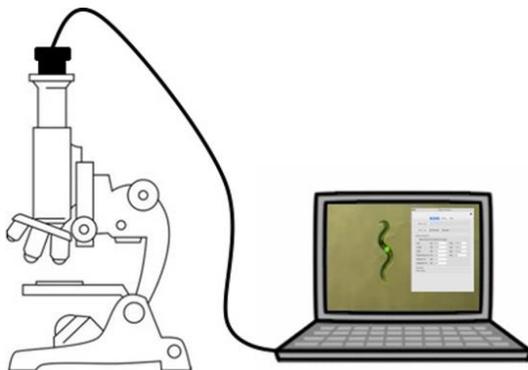
We analyzed the movements of nematodes using the WormLab program (MBF Bioscience 1988). We slowed the video to 10 frames per second and used a length of 150 frames, corresponding to 15 sec. This was done because some nematodes moved out of the field of view during the two-min recording; hence no analysis could be done for the full recording time for all nematodes. We used the part of the video where a nematode was easily distinguishable by the program from other nematodes, if present, for analysis. We then combined the mean speed of each nematode obtained using the program with all nematodes with the same genotype, wild type or mutant, to calculate the mean speed of the group for each temperature.

Analysis of the data was done using a two-way ANOVA statistical test. The three treatment groups, 11°C, 15°C, and 20°C, were compared within the same type of nematodes. Then we compared each same-temperature treatment for the wild type and mutant. The ANOVA

test required the data to have the same number of individuals in each replicate, so we excluded some samples to adjust the number of individuals in each sample. No more than 1 individual in a replicate was excluded for this adjustment.



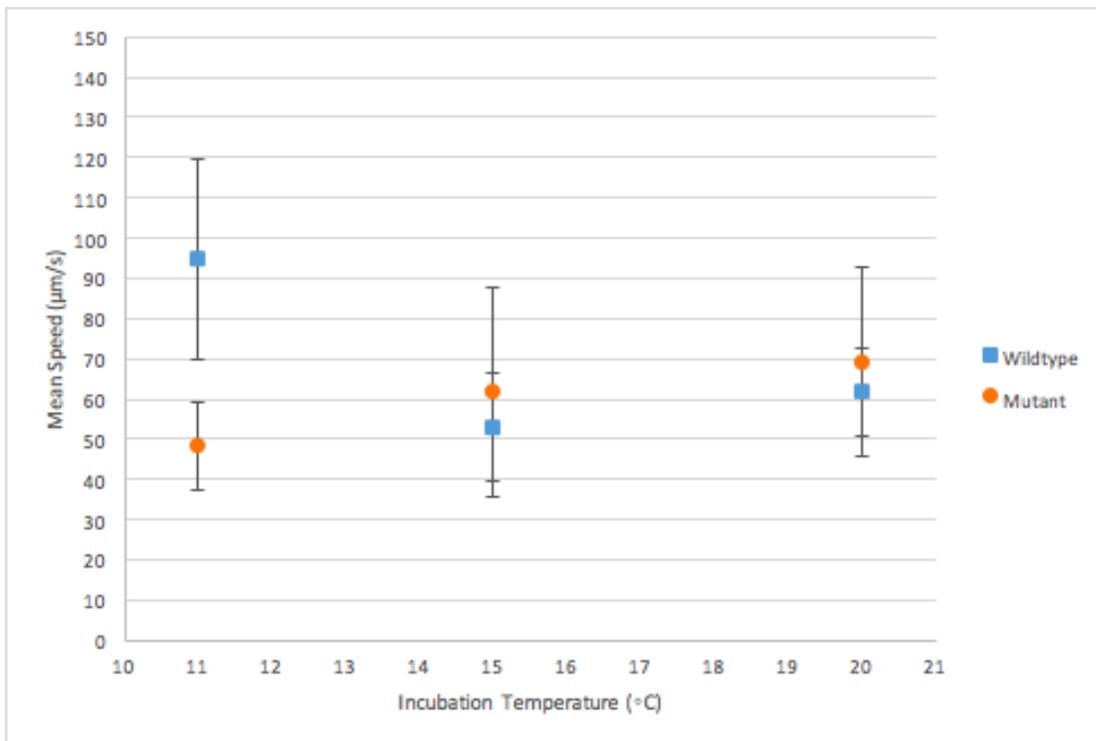
**Figure 1:** Flowchart illustrating the experimental procedure.



**Figure 2.** Experimental setup. Microscope is connected to a laptop with the recording program.

## Results

The results of our statistical analysis (two-way ANOVA) show that there is no significant difference in the *C. elegans* mean speed measured at different incubation temperatures (11°C, 15°C and 20°C), with p-value of 0.43. Further, the ANOVA analysis suggests that there is a significant difference between incubation temperature (11°C, 15°C and 20°C) and the *C. elegans* genotype, with a p-value of 0.01. Figure 3 shows that the wild-type nematodes generally move faster at 11°C compared to the other incubation temperatures, while on the other hand the mutant nematode were generally slower at 11°C compared to the other incubation temperatures 15°C and 20°C.



**Figure 3.** The mean speed of *C. elegans* after 30 minutes at 11°C, 15°C and 20°C. Error bars represent the 95% confidence intervals between replicates within the same treatment. n = number of individuals in the same treatment group = 4. p-value = 0.01

## Discussion

Based on our data analysis from the results, we fail to reject the first hypothesis. The results do not show any significant differences between the mean speeds of *C. elegans* at the

different incubation temperatures. However, our analysis shows that there is a significant effect of incubation temperature and *C. elegans* genotype suggesting that the different genotypes of *C. elegans* react differently to different incubation temperatures. Based on this result, we reject our second null hypothesis and provide support for the alternative hypothesis.

This significance may be because different genotypes of *C. elegans* adopt different survival strategies when it comes to thermoregulation. Neural pathways, which couple temperature-sensing neurons to motor and autonomic responses, allow animals to adjust metabolism rates and navigation direction in response to the temperature (Hobert et al, 1997). The voltage-gated calcium channels in *C. elegans*, used for the exocytosis of neurotransmitters in neuromuscular junctions, are inhibited in the *unc-2* mutant nematodes (Caylor et al, 2013). Therefore their response to stimuli is different and is generally slower than wild type nematodes (Caylor et al, 2013). Further, the lack of significant results for the first hypothesis was not expected. We expected to see an increase in speed when the incubation temperature was different than the cultivation temperature for both the wild type and mutant. *C. elegans* is known to show thermotaxis towards their cultivation temperature (Ito, Inada & Mori, 2006). Due to this behavior, we expected the nematodes to behave erratically in a fixed temperature situation due to this low affinity for temperatures below or above their cultivation temperature (Ito et al, 2006)

Sources of error could be a major factor that contributed to failing to support the first alternative hypothesis. Firstly, interactions between nematodes on each plate could have decreased the speed of the pseudoreplicates. The wild-type petri dishes contained more nematodes in many different life stages as well as eggs in close proximity to the measured nematode compared to the mutant due to the scooping technique used to transfer the nematodes. In populated areas, *C. elegans* release pheromones which attract other nematodes to aggregate in

one area (Jang & Bargmann, 2013). These interactions would have altered the nematodes behavior and speed of movement, leading to a lack of significant difference in speed between the nematodes at different incubation temperatures. It was expected that the wild type would have a higher mean speed in all treatments because of the presence of normal amounts of voltage-gated calcium channels compared to the mutant (Caylor et al, 2013). In addition, there were problems in detecting the exact movement of the nematode when using the WormLab program for the analysis, especially when the nematodes curved onto themselves or were interacting with other nematodes or eggs. This led to some inaccurate speed readings. This difference was also noted when the calculated speed did not match the qualitative observations made by the researchers. Additionally, data transcribed from the videos did not account for the whole length of videos; only 150 frames were analyzed because of the technical difficulty identifying nematodes of interest using the program. This short window of observation could result in either a decrease or increase in the measured speed of nematodes of both types leading to greater standard deviation.

## **Conclusion**

This investigation was not able to determine the effects of incubation temperature on *C. elegans*, however our data analysis showed a significant difference between the mean speed of N2 wild-type and *unc-2* mutant *C. elegans* at different incubation temperatures: 11°C, 15°C and 20°C. The failure to reject the first null hypothesis could be attributed to the inconsistent plate preparation method between N2 wild type and *unc-2* mutant and inaccurate data collection. Nevertheless, this kind of research is significant as it sheds light on animal thermoregulation and what role genetics play on biological processes and animal behavior. With improvements to the methodology and data collection accuracy, we could obtain more observations and possibly

significant results, as previous research shows that temperature does have an effect on the locomotion of *C. elegans* (Parida et al., 2014).

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