Effect of Temperature on the Growth Rate of *Caenorhabditis elegans*

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Abstract

The growth rate of N_2, wild-type, strain of *Caenorhabditis elegans* was studied at three different temperatures to determine if temperature significantly affects their growth rate. The setup consisted of three treatments, with four replicates each, which included incubation temperatures of 25˚C (the control), 17˚C, and 11˚C. Initially 5 adult hermaphrodites were placed on a transfer plate, and then transferred to 60 mm petri dishes containing *Escherichia coli* for each replicate, totaling 20 worms for each treatment. Incubated over a period of 9 days, the total number of adult *C. elegans* was estimated by using grids every 24 hours. The 95% confidence intervals of all treatments overlapped up to Day 4, and started to separate/not overlap for all treatments from Day 4 to Day 9. Thus, the total number of adults for each treatment was found to be significantly different. Plotting a linear relationship of the total number of adults versus time resulted in a correlation coefficient, *r*, of 0.9386, 0.9491, and 0.7572 for 25˚C, 17˚C, and 11˚C, respectively. The slope of these linear plots were found to be 150.87 ± 18.37, 61.05 ± 6.72, and 13.54 ± 3.87 with units of adults per day for temperatures of 25˚C, 17˚C, and 11˚C, respectively, which also do not overlap. Since the results are significantly different, these quantitative descriptions of the slopes from the linear plot are representations of the growth rates for the corresponding growth temperatures. Despite the significance of the results, there were some sources of error, including the overestimation in counting the adults, the damage done to the worms during pickup and transfer, genetic variation, and non-uniform observation period. We concluded that as the growth temperature decreases, the growth rate of *C. elegans* also decreases.

Introduction

Caenorhabditis elegans is a small species of roundworm, reaching lengths of 1-2 mm at the adult stage (Felix and Braendle 2010). *C. elegans* can be separated into two groups, hermaphrodites which are self fertilizing, and males which mate with hermaphrodites to reproduce (Stiernagle 1999). Popular in many different fields for its excellent qualities as a model organism, *C. elegans* has widely been maintained in laboratories for experimental use (Leung *et al*. 2008). *C. elegans* need only a suitable environment with ambient temperature ideally around 20-25°C, atmospheric oxygen and bacteria (as food) to grow and reproduce (Stiernagle 1999). Despite being known as a soil nematode, *C. elegans* can be grown in petri

dishes with nutrient agar with strains of *Escherichia coli* as food (Felix and Braendle 2010). *C. elegans* possess sensory, chemosensory and thermosensory neurons that enable them to perceive different environments (Lee and Kenyon 2009). When exposed to different stressors, such as heat, cold, toxins or pH changes, *C. elegans* are able to endure these environmental changes due to their complex stress response system (Felix and Braendle 2010). Focusing on temperature in particular, *C. elegans* have been noted to be able to survive in various temperatures from 16-25°C (Epstein 1995). Considering that changes in temperature affect all cellular processes (Prahlad *et al*. 2008), it is important to consider how temperature will affect population density of *C. elegans* as it is such an extensively used organism for many studies.

To study the effect of temperature on population, we exposed wild-type N_2 *C. elegans* to three different temperatures (11°C, 17°C, 25°C) with the objective to analyze population size within this temperature range. Since high temperature generally correlates to faster metabolism due to an increase in reaction rates (Lee and Kenyon 2009), we developed the following hypotheses.

H_0: A decrease in temperature will have no effect or increase the growth rate of wild-type *Caenorhabditis elegans.*

H_A: A decrease in temperature will decrease the growth rate of wild-type *Caenorhabditis elegans.*

Previous studies investigating temperature and its effect on *C. elegans* have concluded that 25°C is generally considered to be the optimal growth temperature, with growth rates slowing down as the temperature decreases (Stiernagle 1999). Although higher temperatures yielded greater populations, Felix and Braendle (2010) were able to distinguish upper and lower limits as they concluded that development completely stops below 8°C and the organisms become sterile at temperatures above 27°C.

Methods

Monday (Day 1)

Setup:

This experiment had three temperature treatments, which were 11°C, 17 °C and 25 °C (control). There were four replicates (A, B, C and D) for each treatment. We obtained three petri dishes of N_2 *C. elegans* wild type hermaphrodites, and transferred 20 *C. elegans* to each of the three transferring plates; this was to prevent the pickup of larvae, eggs, or anything that was not an adult. Then from the transferring plates, 5 *C. elegans* were transferred to each of the 60 mm replicate petri dishes A, B, C, and D with *Escherichia coli* as a food source, for all three temperature treatments. **Figure 1** below depicts the procedures described above.

Figure 1: The set up of the four replicate petri dishes for each temperature treatment.

Tuesday (Day 2), Wednesday (Day 3) and Friday (Day 5)

C. elegans Transfer:

On the second day of the experiment, we observed *C. elegans* larvae in all petri dishes for all temperature treatments. In order to make sure both the larvae and the initial hermaphrodites had

enough nutrients for the whole experiment, we decided to transfer all the hermaphrodites to a new set of 60 mm petri dishes covered with *E. coli*. **Figure 2** below shows the transferring process. For 11°C, we initially started with five hermaphrodites on Day 1, but only four were being transferred on Day 2, indicating that one hermaphrodite was no longer alive.

We carried out the transferring process only for our control treatment, 25 °C, on Day 5. This was due to the large number of hermaphrodites observed (as the numbers shown on **Figure 2** "633 and 190"); we decided to split them to two fresh *E. coli* 60 mm petri dishes.

Figure 2: A schematic diagram showing how we transferred the worms to new *E. coli* 60 mm petri dishes. The transferring process is similar for replicates B, C and D. The numbers in the circle represent the number of hermaphrodites being observed or transferred.

Thursday (Day 4), Monday (Day 8) and Tuesday (Day 9)

Counting Method:

Firstly, we placed a measuring grid (1x1cm) with the petri dish sitting on top of the grid under the dissecting microscope as shown in **Figure 3**. Next, we counted the number of hermaphrodites with the click counters in five boxes on the grid; where three of them were randomly chosen from the central boxes of the grid, and the other two were randomly chosen from the outer regions of the grid. Finally, we estimated the number of adult *C. elegans* by multiplying the average obtained by the total number of boxes (found to be 25) that the petri dish covered on the grid.

Figure 3: A photo showing how we put our petri dish on top of a grid for counting to measure the population density of adult *C. elegans* in all the 60 mm petri dishes.

Data Analysis:

In order to see whether there is a significant difference between the different temperature treatments and their corresponding growth rates, we summed the number of adult *C. elegans* found in all petri dishes associated to a certain replicate, on a specific day. Then, we calculated the averages of the number of adults in each day from the four replicates (A, B, C, and D) for all of the nine days. We also calculated the 95% confidence intervals associated with these means. Using Excel, we did a regression line to identify the slopes (growth rates) of each treatment. We calculated the 95% confidence intervals of each slope to see whether they overlapped. (Refer to the Results section.)

Other Factors Recorded:

We also recorded some qualitative data, such as the spread of the worms in the petri dishes. In addition, we were consistent with our counting method and we tried to record our results every 24 hours to keep our data collection consistent, thereby minimizing sources of error.

Results

During the nine day growth period in this experiment, significant differences were recorded in the growth rate of *C. elegans* under the specified temperatures. For the purpose of this study, we only considered the number of adult *C. elegans* in each treatment. In **Figure 4**, we observed that *C. elegans* cultured at 25^{o}C had the first mature population on Day 4, and 17^{o}C and 11^{o}C had mature populations on Day 5 and Day 8, respectively. Moreover, the first group of eggs were observed on Day 2 for cultured media at all temperature treatments.

Figure 4. A plot of the total number of adult *C. elegans* over time for all of the temperature treatments. The points indicate averages and the bars indicate the 95% confidence intervals.

The results indicate a significant difference between the population densities of the different temperature treatments. Based on **Figure 4**, the 95% confidence intervals of the treatments start to not overlap from Day 5 and onwards, indicating a significant difference between the treatments.

Figure 5. The plot from Figure 4 with best-fit lines for each temperature treatment.

From **Figure 5**, the corresponding correlation coefficients for 25˚C, 17˚C, and 11˚C were found to be 0.9386, 0.9491, and 0.7572, respectively. According to these values, a linear plot is a reasonable approximation in estimating the corresponding growth rates (the slopes of the lines) for each temperature treatment. Accordingly, the slopes for 25˚C, 17˚C, and 11˚C were found to be 150.87 ± 18.37, 61.05 ± 6.72, and 13.54 ± 3.87 with units of total number of adult *C. elegans*

per day, respectively, with the intervals representing the 95% confidence intervals. Since these numbers were calculated from results that were significantly different, they are representations of the growth rates for the temperature treatments (25˚C, 17˚C, and 11˚C).

Table 1. A table indicating the data for the 25˚C treatment, with mean and 95% confidence intervals for each day.

		Days						
	Replicates	1	2	3	4	5	8	9
Total Number of Adult *C. elegans*	A	5	6	6	406	828	1169	883
	B	5	4	2	188	864	1023	1166
	C	5	3	3	346	1005	742	885
	D	5	5	6	400	624	1070	1170
	Mean	5	4.5	4.25	335	830.25	1001	1026
	95% Confidence Interval	0	1.2909944487358 1	2.0615528128088 3	101.64644607658 4	157.290336638968	183.075576379447	163.977640752228

Sample Calculation:

For 25˚C

Notes: (1) SD_m represents std. dev. in slope. (2) σ represents the std. dev. (generally).

Discussion

According to the results obtained, we reject the null hypothesis (H_0), and support our alternate hypothesis (H_a). According to **Figure 4**, some 95% confidence intervals of the total number of adult *C. elegans* for 25˚C, 17˚C, and 11˚C in a certain day do not overlap (for example, Day 4 and Day 8 for 25˚C) –thus these means represent statistically different populations. Therefore, we conclude that there is a positive correlation between the growth rate of adult wild-type *C. elegans* and incubation temperature. In other words, the growth rate of adult wild-type *C. elegans* is significantly lower at low temperatures compared to higher temperatures. In **Figure 4**, we can see that the increase in total number of adult *C. elegans* starts relatively sooner and is sharper or steeper as the temperature increases from 11˚C to 17˚C, and then 17˚C to 25˚C. Furthermore, we determine the slopes to be 150.87 ± 18.37 adults per day, 61.05 ± 6.72 adults per day, and 13.54 ± 3.87 adults per day for temperatures of 25˚C, 17˚C, and 11˚C, respectively; the error in the slopes represent the 95% confidence intervals, which do not overlap. Since the results in **Figure 4** are significantly different for each temperature, it indicates that the slopes calculated are representations of the actual growth rates in *C. elegans*.

The results of our experiment agree with descriptions of the life cycle of *C. elegans*. According to Stiernagle (1999), the entire life cycle of *C. elegans* from eggs to adult is roughly 3 days at 25˚C, 3.5 days at 20˚C, and up to 6 days for 15˚C. Such a correlation between growth temperature and the time it takes for one life cycle to be completed may indicate why there is a delay of growth in the population density of *C. elegans* at different temperatures. Furthermore, according to Epstein (1995), the growth rate of *C. elegans* at all stages of their life cycle at 25˚C is observed to be 1.3 times faster than 20˚C, and 2.1 times faster than 16˚C. The differences in growth rate of *C. elegans* at varying temperatures, according to Epstein (1995), can explain why

our results indicate a relative increase in slope in the rising phase from 11˚C to 17˚C, and then 17˚C to 25˚C.

Many ectotherms including *C. elegans* have shorter life spans at high temperatures compared to lower ones. This is largely thought to be because of the effect that temperature has on metabolic rates (Lee and Kenyon 2009). In fact, Hansen *et al.* (2006) found that the lifespan of *C. elegans* can be extended by treatment which shifts cells from states favouring growth to states favouring maintenance and stress resistance; this includes down-regulation of insulin/IGF-1. They observed that protein synthesis is a process linked to growth and aging, where reducing levels of ribosomal proteins during adulthood of *C. elegans* extends their lifespan. Thus, growth at a higher temperature would mean a greater rate of metabolism, which increases the rates of protein synthesis, and in turn causes the lowering of the life span. Moreover, Garigan *et al.* (2002) conducted an experiment by using mutants with reduced insulin/IGF-1 signaling. This resulted in doubling the life span of *C. elegans*, which indicates that the endocrine system has an influence on the rate at which tissues age. Such studies may explain why the growth rate (the steepness of the population increase over time) is much greater at 25˚C compared to the lower temperatures, and why the 25˚C curve plateaus quickly.

Despite the statistical significance of the results, there are some sources of error; elimination or minimization of these errors that would greatly enhance the accuracy of the results. Some sources of error include the overestimation of the number of adult *C. elegans* when counting them. In the petri dish, most of the *C. elegans* were observed to be concentrated in the centre, with far fewer adults in the outer regions. Since our counting method used the grid system to estimate the number of adults in the petri dish, the numbers may be overestimated since the adults are not uniformly distributed on the petri dish. Although we used a certain method of counting (refer to Methods), as an attempt to minimize the overestimation, this error could be

greatly minimized if more time were allotted for the experiment; in this way, the data collection would be more accurate, with fewer variation in replicates and potentially smaller 95% confidence intervals, since we would count more than 5 boxes (such as 10) or simply the entire petri dish. Another source of error includes the unpreventable damage to the adults during the transfer procedure, using the platinum pickup wire. Depending on the amount of damage, some of the adults that were transferred may have died, or were unable to produce and lay eggs; such a result would cause the variation in the data to be high, and cause the 95% confidence intervals to be large. Furthermore, in wild-type *C. elegans*, the frequency of egg-laying in adults is greatly reduced with time (Trent 1982; Mendel *et al.* 1995; Koelle and Horvitz 1996; Schafer 2005). In the first few days, when the adults were transferred to different plates, in order to distribute their egg densities uniformly among a few petri dishes, it was observed that there were fewer eggs in the later petri dishes; this caused the 95% confidence intervals to be very large as the population of adults boomed in the first few petri dishes, while the population of adults were much lower in the later plates due to fewer eggs. In addition, although there was excess food available for the worms to feed on, we are unable to ensure the equal food distribution to each worm. In other words, we cannot confirm if the food was readily available to each individual worm. If the petri dishes were plated with food equally, and not just the centre, then such an error would be minimized, as we would be more confident in knowing that the food is more equally distributed to each individual worm.

Other sources of error include non-uniform observation periods and genetic variation. Although we strived to keep the observation period consistent, there was some variation in the timing due to circumstances and time constraints of the laboratory. As well, genetic variation may be a significant source of error; for instance, some of the worms may have had genetic mutations (such as heat resistance) during the experiment that may have had a positive or

negative effect on adaptation and distribution. Both of these errors may have caused some inconsistency in the data, leading to large 95% confidence intervals. Moreover, according to Golden and Riddle (1984), there is a certain stage in the life cycle of *C. elegans* known as the dauer stage (containing dauer larvae), which is a developmentally arrested stage. When the conditions are harsh or non-optimal, the larvae of *C. elegans* enter the dauer stage in order to survive harsh conditions, but do not grow as a result (Apfeld and Kenyon 1999). If there was a time in our experiment where the worms entered the dauer stage, they would be unable to grow. Thus, this could affect our data on the total number of results, since some worms may have not matured to the adult stage, but rather shifted into the dauer stage.

Conclusion

There are significant differences among the 25˚C, 17˚C, and 11˚C treatments in the total number of adult *C. elegans*. This indicates that there is also a significant difference in the growth rate between the different temperature treatments. As a result, we can reject the null hypothesis (H_0) and support the alternate hypothesis (H_a); as the temperature decreases, the growth rate of wild-type *C. elegans* decreases.

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