

Effect of temperature on the negative geotaxis response in *Drosophila melanogaster*

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Abstract

In our experiment, we used the species *Drosophila melanogaster* to further elucidate the basis of a known behavioral trait, geotaxis. *Drosophila* are negatively geotactic; they move against the pull of earth's gravity. Using lab-raised flies, we investigated the sensitivity of this attribute to a range of temperatures. Four treatments of 13°C, 18°C, 28°C, 33°C, and two control treatments of 22°C and 23°C, were administered over the course of two lab periods. Ten replicates per treatment were performed, each comprising five males and five females. We recorded the time individuals took to move a distance of four centimeters in an inverted test tube, when exposed to a specific temperature. We found that at 18°C, *Drosophila melanogaster* exhibit the most pronounced negative geotactic response. This temperature was the only significant value found in our data; the differences among the other treatments were not statistically significant. The optimal range of temperature for *D. melanogaster* has been reported as between 23°C to 25°C; this does not agree with our finding that 18°C produces the strongest negative geotactic response, and leads us to consider reasons for this discrepancy.

Introduction

Since T.H. Morgan first characterized *Drosophila* as a model organism in the early 1900s, the fruit fly has become the focal point of diverse experimental analysis and study. Our experiment investigates the effect of temperature on a well-documented fruit fly trait: negative geotaxis (movement against earth's gravitational pull). We used the species *Drosophila melanogaster*, which demonstrates a high sensitivity to temperature, as noticeable through responses in locomotor behavior (Vang *et al.* 2012). Specifically, *D. melanogaster* possess thermosensitivity (the ability to detect temperature change), allowing individuals to seek out desirable habitat (Riveron *et al.* 2009); Newman *et al.* (2004) note that the strength of thermosensitivity a species elicits is dependent on the species' surrounding environment. Couple this with the fact temperature influences the olfactory information an organism receives due to changes in odor concentrations within a specific environment (Riveron *et al.* 2009), the

importance of living in an environment with appropriate temperature becomes clear: temperature impacts the abilities of many animals in locating food, mates, as well as avoiding predators (Riveron *et al.* 2009).

Since knowing that extreme heat can interfere with an organism's physiology, using *Drosophila* as a model may provide a basis for understanding the pathways of various thermally influenced diseases, such as heat-stroke and sudden infant death syndrome (Newman *et al.* 2004). Investigating the effect of temperature on *Drosophila*'s activity level can also be used as a model to predict the response patterns of animals (Kellermann *et al.* 2012), a relevant issue today considering in the growing concern regarding climate change and global warming.

In our experiment, we used wild type *D. melanogaster*, which were cultivated under lab conditions at temperatures of 22-23°C. Our measurements were based on the time it took the flies to move up a test tube, containing standard cornmeal at the top, when exposed to a specific temperature treatment. Temperatures used in this experiment were 13 °C, 18°C, 28°C and 33°C, with laboratory room temperature selected as controls for both days (23°C and 22°C, respectively).

Below are the null hypothesis and alternative hypothesis of our experiment:

Ho: An increase in temperature increases or has no effect on the negative geotactic response of *Drosophila melanogaster*.

Ha: An increase in temperature decreases the negative geotactic response of *Drosophila melanogaster*.

Methods

Prior to the experiment, wild type *D. melanogaster* were cultivated on a standard cornmeal medium, at room temperature of 22-23°C.

Treatments consisted of six different temperatures over the course of two lab periods: 23°C, 28°C and 33°C on the first; and 13°C, 18°C, and 22°C on the second. Due to the differences in room temperature on each day, we had two different controls: 23°C on the first day and 22°C on the second day.

Before starting on each day, we immobilized adult *Drosophila* using carbon dioxide. The length of time for which *Drosophila* were immobilized varied (likely due to changing amount of carbon dioxide used and handling). The adults were separated from larvae, eggs, and pupae, and placed on a petri dish. Under the dissecting microscope, we identified the sex of individual flies by looking at the horizontal stripes on the organism's body: males have thick non-horizontal stripes on their abdomen compared to the thin horizontal stripes of females. We then deposited 25 females and 25 males into two vials, labeled female and male, which contained fresh cornmeal medium. Pregnant females were encountered in small numbers on the second day of the experiment, by the observation they were in the process of producing eggs. In order to limit damage, we used brushes to transfer individuals from the petri dishes to the vials. We waited ten minutes after completing the transfer of all the participating *Drosophila*, to allow them to become fully awake and active. Individuals that did not become active were removed after waiting an additional five minutes.

For each temperature treatment, five females and five males were used. We first measured the males' negative geotaxis and then the females'. Five sample vials with cornmeal medium, labeled 1 through 5, were used to conduct our experiment, each with a line marked at

four centimeters from its opening (Figure 1). We transferred individuals by inverting the sex-labeled vial containing flies to be tested, and connecting it to a numbered vial. Tilting the two tubes until a fly passed into the numbered vial, we minimized external stressors on the flies. After disconnecting the vials, they were again inverted to position all *Drosophila* back on the cornmeal medium, and then resealed. A beaker was used to cover the opening of the numbered vial.



Figure 1. *D. melanogaster* climbing upwards to the marked 4-centimetre line.

For the control treatment, we measured the response time directly at room temperature. For the treatment temperatures, we used an incubator with variable temperature to perform the experiment. As a secondary check, we inverted each replicate three times prior to measurement to ensure flies were active (Figure 2). The incubator temperature changed every time we opened the door. In order to ensure minimal variance, we limited the frequency of opening the incubator.

We used a stopwatch phone application to measure the time it took for a fly to move from the bottom of the tube to the 4-centimeter line we marked. The cut off time for the negative

geotaxis measurement was five minutes. After each replicate was complete, we disposed of each fly as instructed.



Figure 2. We inverted the tubes containing *Drosophila*, while inside the incubator, to ensure the fruit flies were active.

We calculated the mean time of the negative geotactic response; the standard deviation and 95% confidence intervals were also calculated. We then plotted two graphs showing the average times it took for *Drosophila* to climb up four centimeters; Day 1, at temperatures of 23°C, 28°C, and 33°C; and Day 2, at temperatures of 13°C, 18°C, 22°C.

Results

Average Time:

$$\bar{x} = \Sigma(x_i/n)$$

$$\text{Average time at } 33^\circ\text{C} = (8+38+2+5+8+11+40+5+8+21)/10 = 14.6\text{s}$$

Standard Deviation:

The standard deviation at 33°C:

$$s = \left[\frac{\sum (x_i - \bar{x})^2}{n-1} \right]^{1/2}$$

$$s = \left\{ \frac{[(8-14.6)^2 + (38-14.6)^2 + (2-14.6)^2 + (5-14.6)^2 + (8-14.6)^2 + (11-14.6)^2 + (40-14.6)^2 + (5-14.6)^2 + (8-14.6)^2 + (21-14.6)^2]}{10-1} \right\}^{1/2}$$

$$s = 13.8$$

95% Confidence Interval:

The 95% confidence interval at 33°C:

$$C.I = \bar{x} \pm 1.96 * (s / n^{1/2})$$

$$C.I = 14.6 \pm 1.96 * (13.8 / (10)^{1/2})$$

$$C.I = 14.6 \pm 8.6$$

Therefore, [6.0, 23.2]

Table 1. Sample calculation for a 95% Confidence Interval of the means for data at 33°C.

Temperature	Replicates(n)	mean (s)	Standard deviation (s)	Confidence Interval (s)
33°C	10	14.6	14.8	8.6

Each treatment had ten replicates, with the exception of day 1. Average times were calculated from seven control replicates from day 1 and ten control replicates from day 2 (Table 1). Replicates one through five of a treatment were male; replicates six through ten were female. In addition, for our controls, three males and four females were used from day 1, and five males and five females from day 2.

For day 1, the confidence intervals of the three treatments (23°C, 28°C, and 33°C) were 24.3, 31.8, and 8.6 (Figure 3). For day 2, the confidence intervals of the three treatments (13°C, 18°C, 22°C) were 31.0, 9.4, and 42.2 (Figure 4).

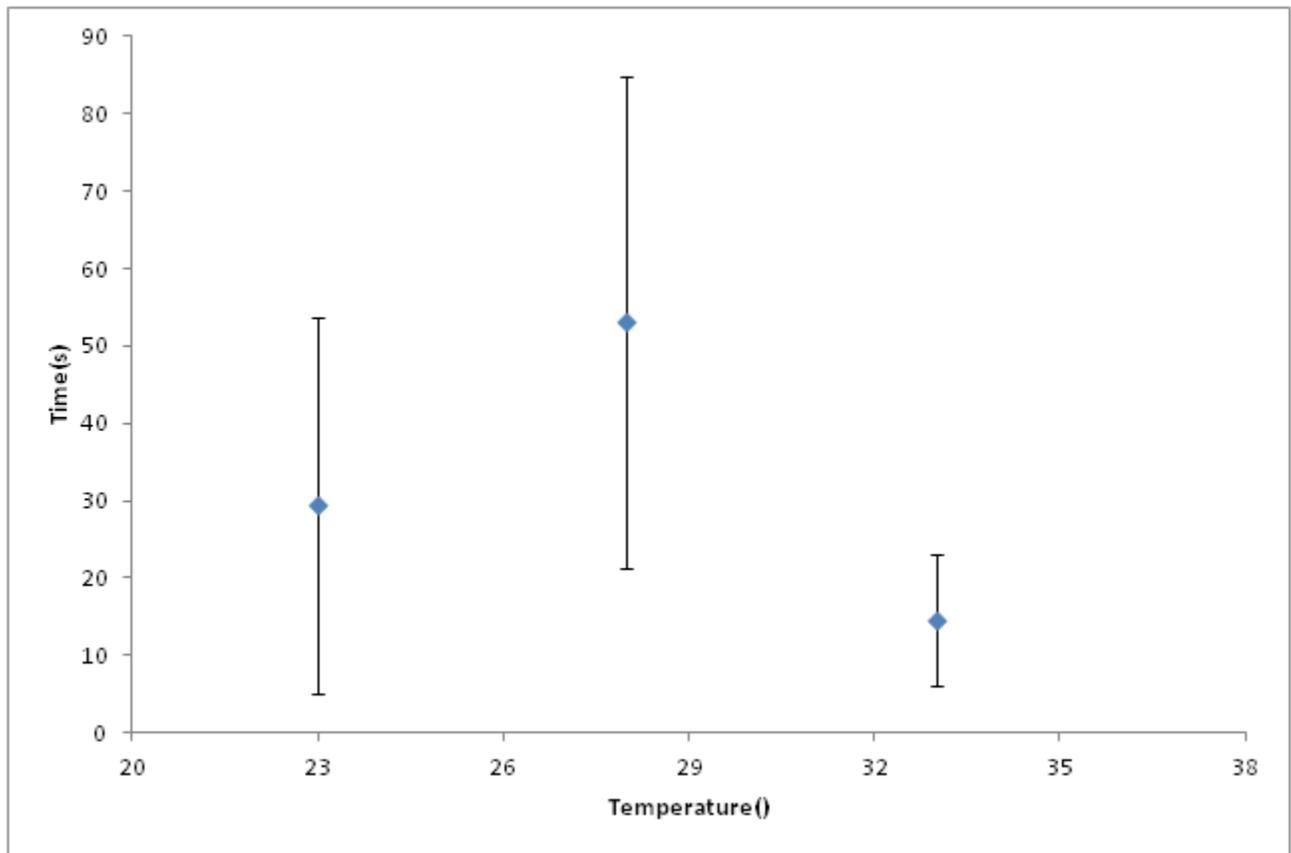


Figure 3. Day 1 average time interval for *D. melanogaster* wild type to move up the test tube a distance of four centimeters. The temperatures used are 23°C, 28°C, and 33°C. Error bars represent the 95% confidence intervals. The intervals for 13°C, 23°C, and 28°C were [5.1, 53.7], [21.3, 84.9], and [6.0, 23.2], respectively.

An increase in mean time from 23°C to 28°C was seen; however, a noticeable decrease in mean time from 28°C to 33°C was evident. Variation from the means was most pronounced in at 23°C and 28°C, though overlap between the times were present in all three.

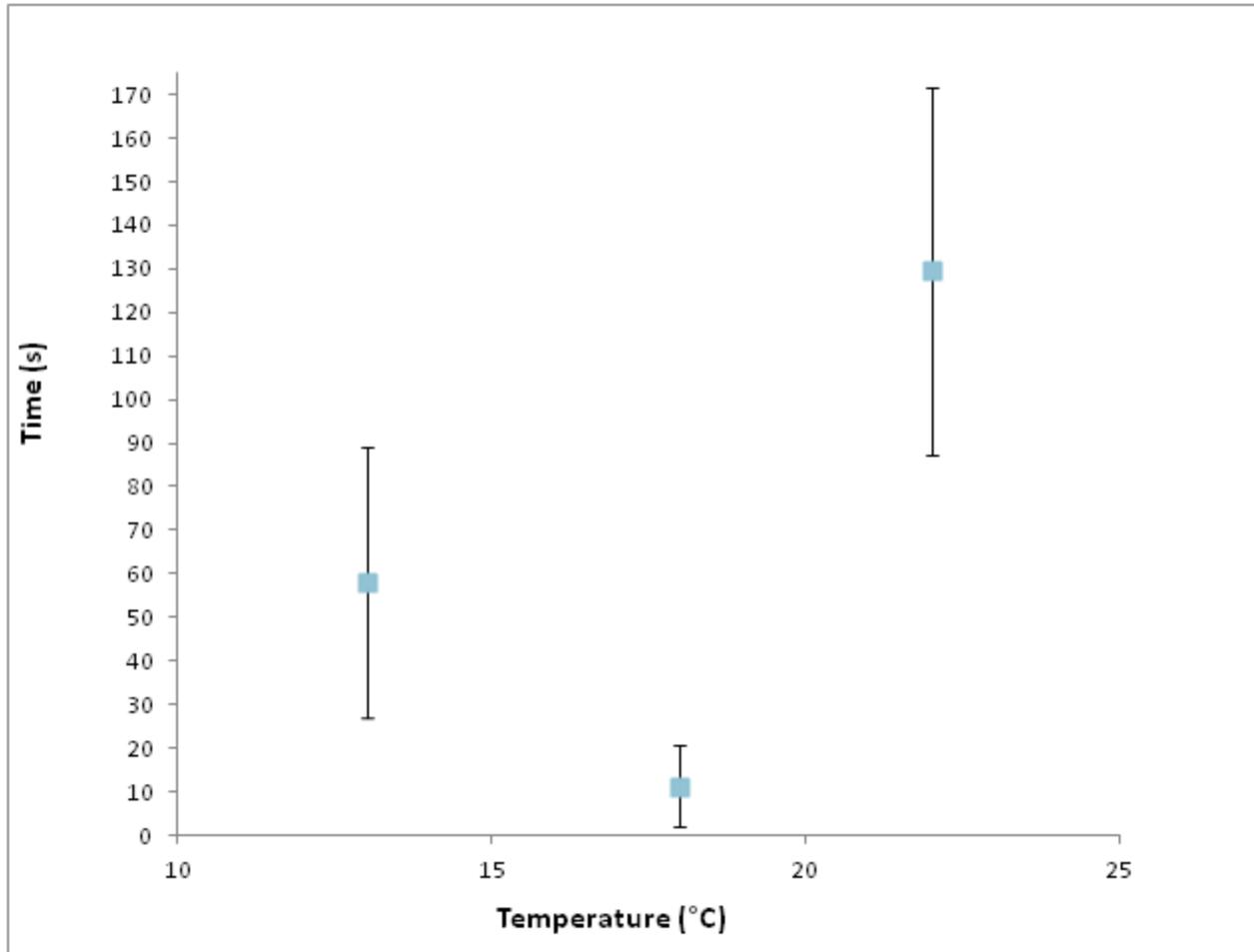


Figure 4. Day 2 average time interval for *D. melanogaster* wild type to move up the test tube a distance of four centimeters. The temperatures used are 13°C, 18°C, and 22°C. Error bars represent the 95% confidence intervals.

For day 2, the confidence intervals of the three treatments (13°C, 18°C, 22°C) were 31.0, 9.4, and 42.2 (Figure 4). Mean time decreased between 13°C and 18°C, though a large increase between 18°C and 22°C was observed. Variation from the means was less than that seen in day 1, though still large for 13°C and 22°C.

From day 1, overlap was observed in all three treatments, indicating that none of our mean values were statistically significantly different (Fig. 3). The results from day 2 were different: overlap between 13°C and 22°C is present; however, there was no overlap between

18°C and the other treatments (Fig. 4), suggesting statistically significant differences in the means.

Discussion

The optimal temperature for *Drosophila melanogaster* is at room temperature. Described by Dillon *et al.* (2009), fruit fly activity declines substantially below 15°C or above 33°C. With these boundaries in mind, two temperatures above and two below the control (room temperature) were chosen as treatments: 13°C and 18°C; 28°C and 33°C.

The data demonstrate overlapping in the confidence intervals for both Day 1 and Day 2. In Figure 3, it is observed that the 95 percent confidence intervals did overlap for all treatments. In Fig. 4, only 18°C did not overlap with other treatments. This suggests the difference at 18°C is significant; while this value represents the optimal temperature for *Drosophila* activity -overall - the results are not significantly different, therefore our null hypothesis cannot be rejected.

Hamada *et al.* (2008) discuss the mediation of temperature preference by internal thermal sensors in the brain. Small-warmth activated anterior-cell (AC) neurons sense changes in temperature, triggering a thermally-sensitive pathway to decide if temperature is suitable. While Hamada *et al.* (2008) looked at various stages of development in *D. melanogaster* - not just adult flies - their findings help to explain why the negative geotactic response is greatest at 18°C: a large increase in AC neuron activity would be expected at the flies' optimal range of temperature. This would heighten the *Drosophila*'s thermosensitivity and facilitate greater motor activity (Hamada *et al.* 2008), resulting in shorter movement periods up the test tube at 18°C.

The decreased response at 13°C is consistent with contemporary literature. Kelty and Lee (2001) found that at temperatures close to 10°C, the limbs of *Drosophila* begin to freeze. Our 13°C treatment varied by two degrees: it is likely our flies experienced freezing under the coldest conditions in the incubators - producing longer response times. Gilchrist and Huey (1999) showed that at higher temperatures, *Drosophila* lose their ability to cling onto inclined surfaces. This event was observed

during high temperature treatments, as the flies began to slide and fall down the test tube walls. We expect that such a stress would produce longer response intervals, yet our 33°C treatment disagrees with this notion, a finding likely influenced primarily by procedural inconsistency.

During the experiment, various errors were present which may have affected the results. Deviation from the chosen treatment temperatures was present at room temperature and within the incubators. The experiment was performed over two days with room temperature as our control; yet, the control temperature for both days was different. This could skew the analysis as the treatments on each day have different reference values (23° and 22°C).

The incubators were pre-set to the desired temperature prior to each treatment; however, these temperatures were found to be inconsistent throughout the experiment. Fluctuation was prevalent at lower temperatures - from continued exposure to room conditions - as a result of opening and closing the incubator door for each replicate; uncertainty of the temperature was as high as +/- 2°C for some treatments.

Furthermore, procedural human error was abundant. Throughout the experiment, confusion among group members existed on the method to measure the negative geotactic response of the *Drosophila*; timing did not always start at the same designated point with each replicate, with measurements being taken from when *Drosophila* first exhibited movement and when they began to climb the tube. With each member using variations of a common method until the second day of the experiment, our first day results may be inaccurate, as the mean times from day 1 are not reliable indications of fruit fly response time. Finally, many flies were injured in the process of being transferred: many were unable to move after being placed in the test tube, and a few were fatally wounded. In addition, three replicates went beyond our cut-off time for Day 1 and were not used in our calculations.

An additional factor that may have impacted response time is light intensity. *Drosophila melanogaster* locomotion is highly dependent on light and research has demonstrated that *Drosophila* activity shows a positive correlation with light intensity (Collins *et al.* 2003). For our experiment, the light fixture within the incubator was inactive; this lack of light could have exaggerated response times

for our flies, and further restricted the negative geotaxis of flies experiencing harsh temperatures (namely the 13°C treatment).

Lastly, research has shown that somatic mutations are inherent in aging *Drosophila* (Garcia *et al.* 2010). Since there was a week between the first experimental day and the second, the flies were allowed to age, leading to the possibility of somatic mutation development in some individuals. Although the chance of mutation within two weeks may be small, there is a possibility that the mutations may have disrupted the negative geotactic movement of *D. melanogaster*, and could have been a factor (Garcia *et al.* 2010).

We suggest that for further experiments, a consistent control for all treatments, and performing the replicates in a confined space, to limit temperature fluctuation, will greatly benefit the outcome of the experiment.

Conclusion

The mean response times of *D. melanogaster*'s negative geotactic response were shorter at 18°C compared to the five other treatment temperatures. We maintain this represents an optimal temperature of activity for our organism, and warrants further investigation. Overall, the results from five of six treatments were not significant, and we failed to reject our null hypothesis: an increase in temperature increases or has no effect on the negative geotactic response of *Drosophila melanogaster*.

Acknowledgements

We would like to thank Dr. Carol Pollock for supporting and encouraging us to pursue this project, Diana Rennison for proofreading our report, and Katelyn Tovey for providing guidance and insight throughout the experiment. Finally we would like to thank University of British Columbia for giving us access to the experimental space.

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