

The effect of temperature on time spent in the dark by *Drosophila melanogaster*

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Abstract

The purpose of this study was to determine whether a long-term (48 hour) exposure to suboptimal temperatures would affect the time spent in the dark by *Drosophila melanogaster*. Many studies found that exposure to warmer temperatures led to a higher light intensity preference by *D. melanogaster*. Our prediction was that an exposure to cooler temperature would lead to less time spent in the dark and warmer temperatures would lead to more time spent in the dark. The fruit-flies were incubated at temperatures of 20°C, 24°C and 30°C for 48 hours before testing. The incubated flies were temporarily immobilized by anaesthetizing them with CO₂ and then individually introduced to a T-maze apparatus, with one side having a light intensity of 2700 lux and the other having 0 lux. We found the amount of time spent in the dark during the three-minute test increased slightly, but not significantly (p -value = 0.8629) for the 20°C and 30°C treatments as compared with the 24°C treatment. Therefore, we conclude that the time *D. melanogaster* spent in the dark is not affected by 48-hour temperature changes.

Introduction

Navigation of terrain and adaptability to environmental changes are of utmost importance in a highly motile organism's lifespan such as *Drosophila melanogaster* (common fruit fly). Small organisms such as *D. melanogaster* allow us to study the dynamics of how environmental changes affect habituation duration. *D. melanogaster* are known to be poikilothermic, where their internal temperature can vary depending on the environment (Lee and Montell 2013). This is in contrast to homeotherms, who exhibit internal thermal homeostasis. Dillon *et al.* (2009) found that *D. melanogaster* do not have a single thermal preference, but instead modify their thermal preference according to environmental changes. Markow (1974) found that *D. melanogaster* exhibit slight photonegative behaviour when introduced to a light gradient maze; however, individual variability exists. Rieger *et al.* (2007) also found that *D. melanogaster* favour low light intensity for resting, feeding, grooming and short term movement. This is further supported by Kawanishi and Watanabe (1979), who found that *D. melanogaster* show a

strong preference to dim light and avoid high light intensities in a natural setting. Although the change in behaviour in response to light (phototactic) and temperature (thermotactic) of *D. melanogaster* have both been researched extensively, there is a lack of research on how these two factors interact with each other.

To stress the importance of behavioural change of *D. melanogaster* with changes in environmental temperature, we addressed a set of hypotheses, with our H_A being the time spent in the dark of *D. melanogaster* is affected by the long term (48 hours) exposure to suboptimal temperatures and our H₀ being the time spent in the dark of *D. melanogaster* is not affected by the long term (48 hours) exposure to suboptimal temperatures.

Previous studies found that *D. melanogaster* show the highest activity level under dim light conditions in nature, but show a preference for light when introduced to high temperatures above 30°C (Rieger *et al.* 2007; Markow 1979). However, these studies observed immediate behaviour with regards to temperature change and did not reflect long-term behavioural adaptability with temperature change in *D. melanogaster*. In order to address this issue, we incubated the flies for 48 hours at different temperatures to allow them to acclimate and then examined the phototactic changes in flies' behaviour as a result of these treatments. Despite the different experimental settings from the prior studies, we predicted that the time spent in the dark side of the experimental apparatus by *D. melanogaster* will increase as the incubation temperature increased based on results from Markow (1979). This behavioural change in *D. melanogaster* is likely to be a result of the physiological interactions between its thermoreceptors in combination with photoreceptor signalling and motor function. Although the molecular processes behind physiological and behavioural responses of *D. melanogaster* when sensing innocuously low temperatures have not yet been characterized, sensing warmth is dependent on a

cation channel termed *Drosophila* Transient Receptor Potential-A1 (dTrpA1) (Dillon *et al.* 2009). When the flies are exposed to temperatures over 25°C, the dTrpA1 protein functions as a mediator that activates neurons which allows the flies to establish temperature preference (Barbagallo and Garrity 2015). Out of the eight photoreceptors in a fly’s eye, the R7 and R8 photoreceptors are involved in sensing light and sending the signals to the medulla (Katz and Minke 2009). Interestingly, protein channels called TRP and TRPL that play roles in cool avoidance are also known for their role in phototransduction (Dillon *et al.* 2009). These proteins could be a key link between thermosensory response and change in phototactic behaviour, but the way the two functions of TRP and TRPL proteins are managed is distinct from one another. The specific mechanism behind how exposure to different temperatures causes changes in phototaxis remains unknown.

To summarize the dynamics of temperature variation on phototactic sensation of *D. melanogaster*, we propose the following model (Figure 1).

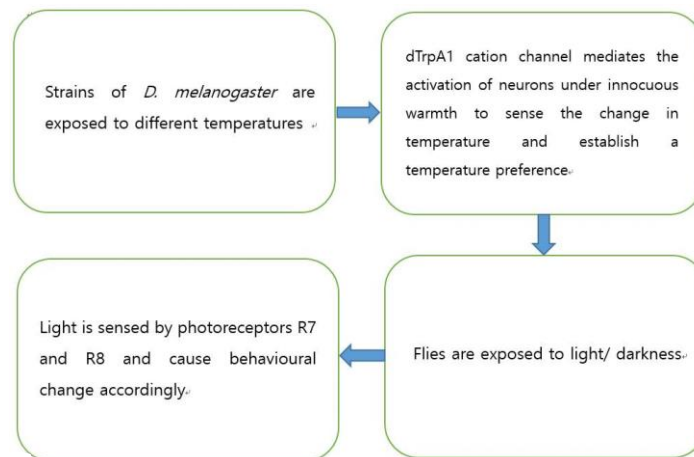


Figure 1. A flowchart of the research model of the cellular mechanisms followed after the experimental input.

Methods

In our experiment, we defined recovery time as the time it took to regain any sign of mobility after an immobilization treatment. We noted that after introducing CO₂ gas into the vial

for 10-20 seconds, the recovery time of *D. melanogaster* was roughly 3-5 minutes. Mee *et al.* (2014) found that immobilization of *D. melanogaster* through heat exposure was ineffective, as they had almost an instantaneous recovery after being placed in a 40°C incubator for 7 minutes. From these results, we decided that CO₂ immobilization was the most reasonable method for our experiment as it allowed us enough time to transport individual flies effectively and collect enough data in a limited time.

Prior to beginning the experiment, we considered what would be the most suitable condition for the flies immediately after recovering. We found that the flies had little to no movement after recovery, likely due to the lingering effects of the CO₂ treatment. To minimize this variable, we decided to utilize the flies' characteristic of having negative geotaxis (Toma 2002) by creating an upward path for them to travel. By doing this, we confirmed that the fly had recovered enough motility to move on its own, increasing the efficiency of our experimental design.

Lastly, we researched what would be the appropriate temperature and light intensity for our experiment. Markow (1979) and Rako and Hoffman (2006) stated that *D. melanogaster* will exhibit a state of cold coma when exposed to temperatures below 20°C, resulting in a recovery time of roughly one hour. These studies also showed that when the flies were exposed to temperatures above 38°C their lifetimes decreased dramatically, dying after only a few hours. Therefore, we decided to subject the flies to treatments of 20°C, 30°C and room temperature of 24°C.

We raised our *Drosophila* in vials containing a cornmeal medium that was solidified using agar. They were raised at room temperature, being only exposed to mild changes in temperature during their lifetime. They were also exposed to lab light during transfers and stored

in the dark until needed for experimentation. To mimic the flies' dark culturing environment, we enclosed the vials inside a box before being placed in the incubators. Three vials consisting of roughly 12 flies each were placed in a 20°C incubator, a 30°C incubator, and a dark room at a temperature of 24°C. , All samples were incubated for 48 hours prior to experimentation.

After incubation, we treated each vial with CO₂ and placed 16 samples from each of the three treatments into individual three-mL test tubes. Each test tube was then sealed off with cotton to prevent the flies from escaping and placed on a rack to let each fly recover. After the fly's mobility was confirmed in the test tube, we introduced it into our light apparatus.

For our experimental setup, we used a retort stand with a clamp held at 30 cm, supporting a 9 x 14 cm T-maze tube (Figure 2). We placed a lamp on one side of the tube and set it to maintain a constant light intensity of 2700 lux at the position of the tube. We then sealed the exit with cotton. For the other side of the T-maze tube, we sealed off the entire side with tin foil, resulting in that side having a light intensity of 0 lux. We also measured the temperature on each side of the T-maze to ensure that light was the only variable within the apparatus. We set up four T-maze apparatus and used them simultaneously during the experiment.

To introduce the fly, we took off the cotton seal and connected the test tube with the middle extension of the T-maze tube. The negative geotaxis characteristic of *D. melanogaster* resulted in it crawling up the test tube and into the T-maze. Upon entry, we sealed the T-maze entrance with cotton and started two timers; we recorded total time and time spent on the dark side. After three minutes, we removed the fly from the apparatus and placed the fly in a separate container so it would not be used again.

We repeated this procedure 16 times for the flies incubated at 20°C, 30°C, and 24°C, resulting in a total of 48 recorded samples. Any flies that showed no movement (due to death or individual differences), were not recorded in our data.

We calculated the means, standard deviations and 95% confidence intervals. We used a Kruskal-Wallis ANOVA to calculate a p -value.

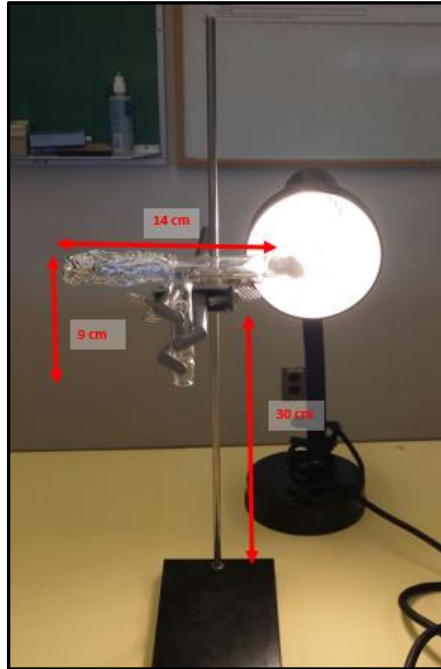


Figure 2. Experimental setup used for all treatments. A retort stand was used to hold a T-maze tube having one side with a light intensity of 2700 lux and the other with 0 lux.

Results

As shown in Figure 3, *D. melanogaster* spent more time in the dark in the 30°C treatment (mean = 114.75 +/- 70.28 s), compared to the 20°C treatment (mean = 94.56 +/- 77.24 s) and the 24°C treatment (mean = 91.31 +/- 83.36 s). As both the 30°C and 20°C treatments resulted in longer mean time spent in the dark region, there is no observable trend among the three treatments for increasing or decreasing temperature; rather, changing the temperature from the standard growth temperature resulted in longer mean times. Also, all three treatments had large confidence intervals, with the 24°C treatment showing the largest range (83.36 s), and

smaller ranges shown in the 20°C and 30°C treatments (70.28 and 77.24 s, respectively).

Statistical analysis resulted in a p -value of 0.8629. This is much greater than 0.05, and thus, the differences among the means of the three treatments were not statistically significant. We discarded eight individuals (due to death or no movement); all other *D. melanogaster* used to collect data had relatively high levels of activity and were able to climb up to the top of the apparatus.

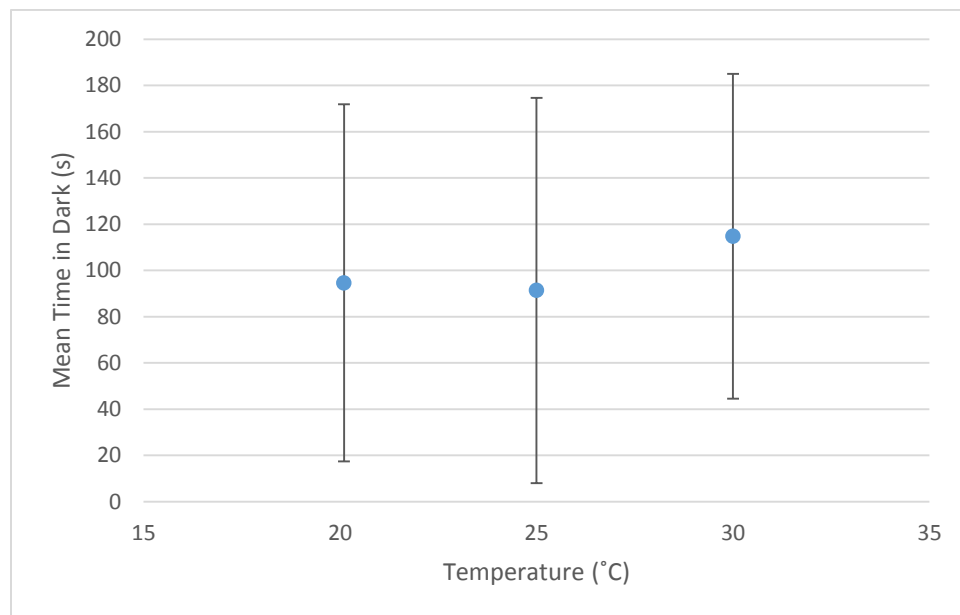


Figure 3. Mean time spent in the dark region for each incubated temperature (20°C, 24°C, and 30°C). Each treatment was incubated at the temperature indicated for 48 hours prior to the experiment. Mean time in dark is shown in seconds. Error bars shown represent +/-95% confidence intervals for mean values. For all treatments, $n = 16$. $p = 0.8629$.

Discussion

The statistical analysis resulted in a p -value of 0.8629. Since our p -value is greater than 0.05, we failed to reject H_0 , and unable to provide support for H_A . We found no significant difference for time *D. melanogaster* spent in the dark in the different temperature treatments.

This result contrasts with the findings from previous studies, in which *D. melanogaster* preferred lower light intensities when incubated at lower temperatures and higher light intensities

when incubated at higher temperatures (Markow 1979; Gong 2012). This trend is explained by a preference for lower light intensities for the larval stage of a fly's lifespan, and a preference for higher light intensities in the adult stage of a fly's lifespan (Gong 2012). However, no firm evidence exists to support the physiological mechanisms responsible for this phenomenon (Gong 2012).

One factor that may have caused the inconsistency between our results and those proposed in literature is related to the plasticity in the development of visual systems found in this species. This plasticity refers to the shift in preference for higher light intensities in the adult stage of a fly's lifespan (Gong 2012), which is dependent on environmental conditions and the genetic makeup of these flies (Zhou *et al.* 2010). This, in turn, may affect the development of photoreceptor cells or the neural processing of light stimuli (Hall *et al.* 1982, cited by Sawin *et al.* 1994; Zhou *et al.* 2010). Moreover, if flies are blinded or kept in the dark during a critical stage in the development of their visual system, plasticity will be nearly nonexistent and may produce no change in preference towards higher light intensities (Zhou *et al.* 2010). Thus, it is possible that these flies were subject to such conditions, and had incomplete development in their visual systems. In such a case, the activity of the NP-394 neurons and TRP and TRPL proteins would facilitate avoidance behaviour from high light, as seen with flies in their larval stages (Dillon *et al.* 2009; Gong *et al.* 2010).

Nevertheless, it is interesting to note that under natural conditions, adult flies are normally seen under leaves or decaying fruit, away from intense light (Rieger *et al.* 2007). This is consistent when considering the natural circadian rhythms found in this species. *D. melanogaster* are known to be diurnal organisms, being the most active during the night and where light intensities are sufficiently low during the day (Rieger *et al.* 2007). Given that our

current study was done in the early afternoon, *D. melanogaster* may have tended toward lower light intensities, regardless of temperature changes due to its natural tendencies based on the time of day (Lu *et al.* 2008). Blanchardon *et al.* (2001) support this by finding that the small ventral lateral neurons located in either hemisphere of the brain would control motor activity to move towards lower light intensities.

Alternatively, we recognize several other factors that could have had potential impact on our results. To begin with, one possible factor is the change in sample size. The flies that did not manage to climb up the entrance test tube, died, or clearly observed to have an injury were omitted from data collection (see Methods). As a result, we omitted eight specimens, decreasing the population size for our statistical analysis.

Additionally, only initial temperature values were measured. From this, another source of error could originate from the increasing temperatures from the lamp heat on our high-light side of the T-maze tube as our experiment was carried out. The increasing heat levels may have also affected the other side of the T-maze, as tin foil was utilized to simulate darkness. Tin foil can act as a heat conductor, which trapped heat within that side of the tube, increasing the temperature and further contributing to error. Dillon *et al.* (2009) found a correlation between temperature and walking speed of *D. melanogaster*.

A third factor contributing to uncertainty is the freedom of movement of the specimen when climbing up the entrance tube into the T-maze. Since the fly can climb up any side of the entrance tube, it will enter the T-maze closer to one side unless it climbed up directly in the middle of the entrance tube. The flies may have been biased to remain on the side of the tube they were on upon entering the T-maze.

Lastly, our experiment utilized 4 T-maze apparatus. Although light intensity and temperature were initially controlled, the close proximity of the four apparatus may have caused some interference by influencing the light intensity or temperature of adjacent apparatus.

Conclusion

Our experiment tested if long term exposure to suboptimal temperatures has an effect on the time spent in the dark of *D. melanogaster*. Although the results of our study showed a trend of increased dark time spent in the dark at higher temperatures, our analysis concluded that the time spent in the dark by *D. melanogaster* did not change significantly when exposed to temperatures of 20°C and 30°C for 48 hours as compared with 24°C. Further studies are necessary to find the correlation between thermal response and phototaxis of *D. melanogaster*.

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