

The effect of temperature on the number and gender of fruit flies (*Drosophila melanogaster*) reaching adulthood

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Abstract

In *Drosophila melanogaster*, gender and duration of heat exposure can alter the level of the induced heat shock protein Hsp70, which in turn may influence individual heat tolerance. It has not been fully explored whether gender and long-term heat exposure affect the time from larval stages to adulthood in *D. melanogaster*. To examine this further, wild-type *D. melanogaster* larvae were incubated at 17 °C, 25 °C or 30 °C until the adult stage was observed. The larvae were divided into 12 food vials, each containing 5 larvae. From these 12 vials, four food vials were incubated for each temperature treatment. We recorded the gender and maturation time daily for 18 consecutive days except on weekends and found that 18 days were insufficient to observe adult stage *D. melanogaster* in the 17 °C treatment. Our results showed that the 25 °C treatment had a higher male to female ratio (12:3) than the 30 °C (6:4) treatment. Furthermore, *D. melanogaster* at 25 °C showed a longer developmental time to reach adulthood than 30 °C treatment. From our results we did not find statistical support for an effect of temperature on the number of males and females reaching the adult stage. We did however, find that temperature has a significant effect on developmental time to reach adulthood, and may relate to the role of Hsp70, but this effect was not observed between genders.

Introduction

Drosophila melanogaster (common fruit fly) are small poikilotherms, organisms whose body temperature can vary (Sokolova 2008). Fruit flies, in particular, have a high surface-area-to-volume ratio, which allows for a rapid heat exchange with the environment, and for this reason, their physiological processes are easily influenced by subtle changes in ambient temperature (Sayeed and Benzer 1996, Dahlgaard *et al.* 1998, Zars 2001, Sokolova 2008).

One of such temperature-dependent processes is metabolism and the trend of temperature-dependent metabolic rates is conserved across many strains of wild-type *D. melanogaster* (Berrigan and Partridge 1997). Although the same trend is also conserved across many organisms in general (Gillooly *et al.* 2001), the small size and poikilothermic qualities of *D. melanogaster* allow for the

metabolic rate of the fruit fly to be more readily altered by the changes in the ambient temperature than is the case for many other larger organisms. For example, an ambient temperature of 14°C is said to greatly reduce metabolic rate and, consequently, halt development in *D. melanogaster* (Petavy *et al.* 2001). Imasheva *et al.* (1998) and Trotta *et al.* (2006) observed faster developmental rates at higher temperatures within a temperature range permissible for *D. melanogaster*.

While higher temperatures could promote a faster development time from one life stage to the next, temperatures deviating far from the optimal temperature can lead to increased mortality from heat stress (Petavy *et al.* 2001). To counteract the damage from heat stress, a widely conserved chaperone, heat shock protein (Hsp70), is typically elicited and can assist in refolding denatured, aggregated and misfolded of proteins or function in proteolytic pathways (Lindquist 1986, Craig *et al.* 1994, Dahlgaard *et al.* 1998, Hoffman *et al.* 2003). The findings of Krebs *et al.* (1998) highlighted the importance of Hsp70 in thermotolerance as they showed that genetically modified flies overexpressing Hsp70 have improved thermotolerance. The overexpression of Hsp70, however, has been observed to vary with gender and with the duration of heat exposure in *D. melanogaster* (Dahlgaard *et al.* 1998, Krebs *et al.* 1998).

To determine if there is an interaction between long-term exposure to various temperatures (17°C, 25°C, and 30°C) and the number and gender of wild type *D. melanogaster* that reach adulthood, we tested the following hypotheses:

H₀₁: Temperature has no effect on the developmental time of *D. melanogaster* from larva to adulthood.

H_{a1}: Temperature has an effect on the developmental time of *D. melanogaster* from larva to adulthood.

H₀₂: There is no effect of temperature on the number of male and female *D. melanogaster* that reach adulthood.

H_{a2}: There is an effect of temperature on the number of male and female *D. melanogaster* that reach adulthood.

The findings from testing the above hypotheses would contribute to the knowledge that is presently lacking on the effects of long-term heat exposure on *D. melanogaster* during the larval to adult stage.

From summing knowledge from all the literatures and studies aforementioned in this section, we propose the following model (Figure 1).

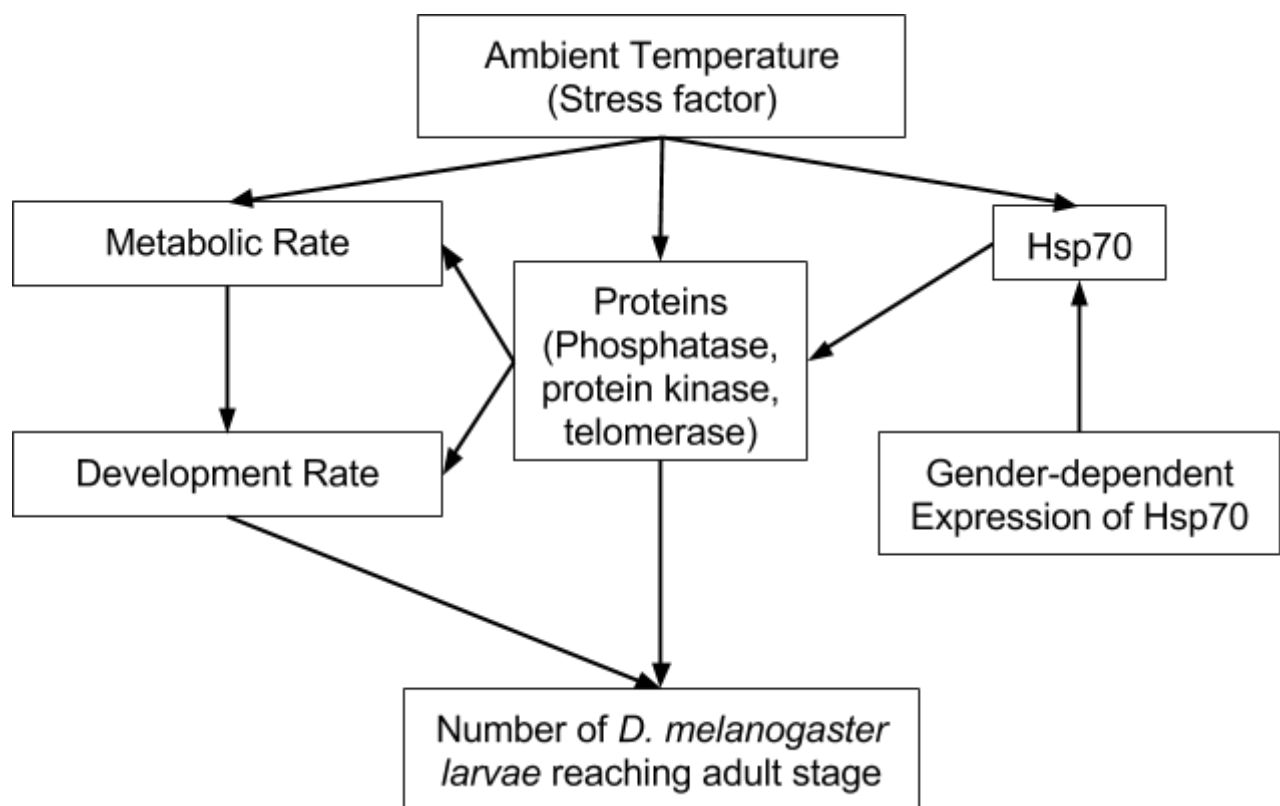


Figure 1. The cascading effects of temperature on *Drosophila melanogaster* resulting in gender-related variation of Hsp70 expression. The expression of Hsp70 can be affected by temperature, which in turn can affect pathways involved in metabolism and development. Specific proteins involved in growth of *D. melanogaster* are considered (Morimoto 2002; Morrow and Tanguay, 2003; Tower 2009).

Methods

Our group initially isolated 60 *D. melanogaster* larvae from the growth medium provided by the instructors. All growth medium in this experiment was made of cornmeal with solidifying agar.

We isolated the larvae by emerging culture medium in a petri dish filled with an 18% sucrose solution (Figure 2). We identified the larvae by their white-colored body which moved peristaltically in the solution. We also selected for larvae that were approximately equal to or less than 1 mm in length using small metal loops (Figure 2). Next, we transferred the isolated larvae into 12 identical vials containing the growth medium. In the larval stage, we could not identify the gender of the organism hence there was only random selection of sexes for each vial. We sealed each test tube with cotton after loading five larvae to allow for sufficient air ventilation yet prevent mature flies from later escaping. We then divided the 12 vials into three treatment groups where each group was comprised of four replicates. We then placed each group into three different incubators with the temperature of 17°C, 25°C and 30°C where the 25°C served as the control group. To ensure that the growth conditions were as even as possible among incubators we placed each test tube for every treatment group separately into a non-translucent cardboard box to minimize the light intensity variation among the group (Figure 3). However, we did not seal the boxes completely due to the concern of air ventilation, so minor variation in light intensity might have still persisted.

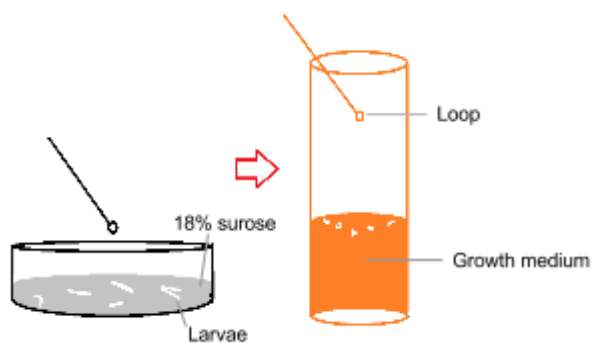


Figure 2. Isolation of larvae to growth medium



Figure 3. Experimental setup for incubation.

In choosing a control temperature, we chose to incubate the *D. melanogaster* larvae at 25°C since has been previously determined as the optimal temperature (Siddiqui and Berlow 1972; Sayeed and Benzer 1996). Research has shown that metabolic rates almost cease at temperatures below 14°C (Petavy *et al.* 2001) so an incubation temperature lower than 14°C could not be selected. Similarly, development in *D. melanogaster* has not been observed above 32°C (Chakir *et al.* 2002)

There was only a limited selection of incubator temperatures (4°C, 11°C, 15°C, 17°C, 20°C, and 30°C) available for us in the lab room. For the above reasons, our available choices were 15°C, 17°C, 20°C, and 30°C incubators. For the warmer incubation treatment, we had to choose the 30°C incubator since it was the only one that was warmer than 25°C. Similarly, for the colder incubation we chose 17°C to further separate the treatment condition from the 25°C control group temperature. This temperature was ideal since it did not reach the temperature of complete metabolic cessation.

Our group took the first observation one week after the first day of incubation. We selected this first observation time based on the average *D. melanogaster* developmental time of 10 days at temperature of 25 °C (Sang 2001). After the first observation, we observed the flies once per day on weekdays. Before each observation, we first transferred the mature flies to a new vial so we could minimize the impact on the development of other immature flies. For each identified adult fly, we anesthetized it using carbon dioxide in order to record its gender, observed it under a dissecting microscope and took a digital image using a DinoXcope™ camera attached to the ocular of the microscope. Once we had observed the fly, we removed it from the microscope stage and discarded it into morgue bottles to prevent recounting. We identified the sex of the flies based on the widths of the dark bands near the abdomen as males tend to have thicker bands than females (Lande 1980). To reduce bias, we recorded time in hours as we made some observations in the mornings while

others in afternoons due to the lack of availability for lab access. Because of the time limitation, we made the last observation on the 18th day.

After the data collection, we calculated the average development time for both male and female flies, and performed a one-way ANOVA. We calculated the p-value to confirm the significance in developmental time among the different temperatures. Lastly, since there were two independent variables being studied, gender and temperature, we performed two-way ANOVA to see if there is a significant difference between each temperature treatment on the number of male and female adult *D. melanogaster*.

Results

At 25 °C and 30 °C, 15 and 10 larvae reached adulthood, respectively. As shown in Figure 4, larvae grown at 25 °C took longer to reach adulthood (11 days on average ± 0.3 days) when compared to larvae at 30 °C which only took only 9 days ± 1 day. We did not observe any adults at 17 °C throughout the observation period of 18 days and this is noted as 0 developmental time in the graph (Figure 4).

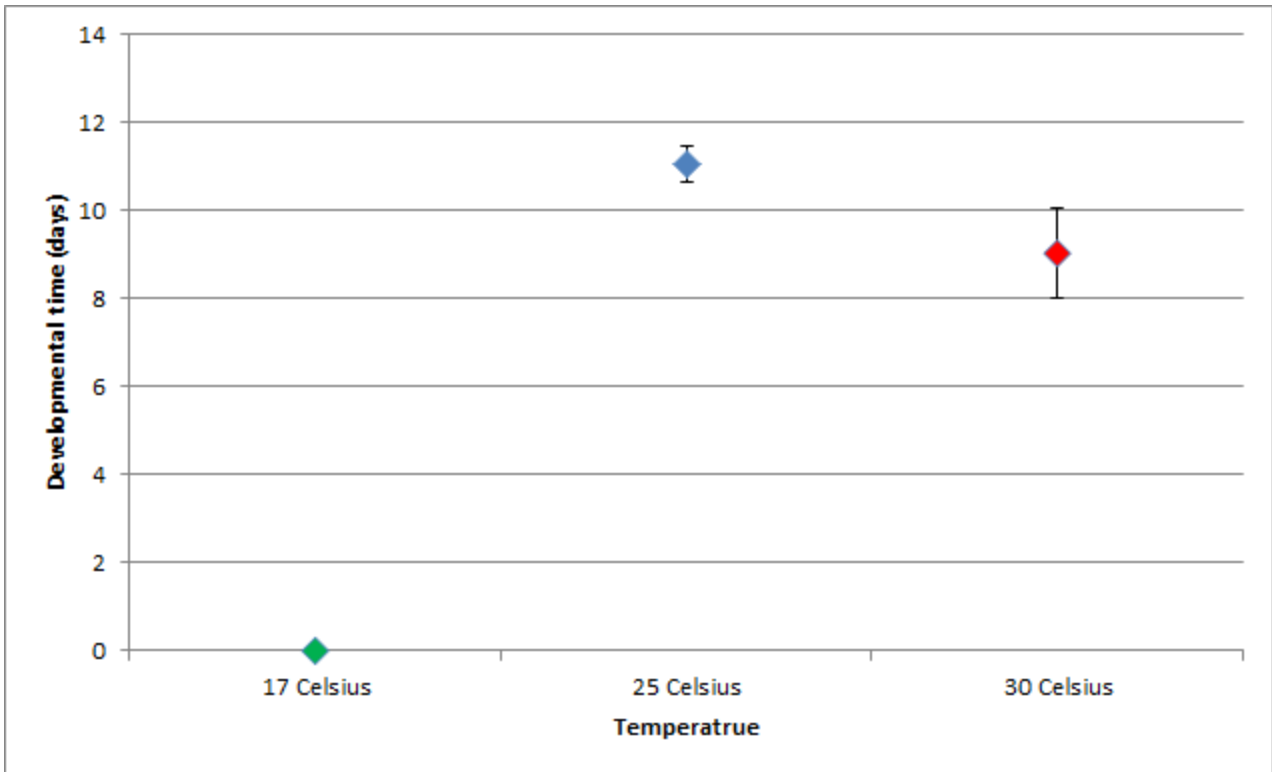


Figure 4. Mean developmental time from larval to adult stage at 17, 25 and 30 °C temperature treatments. Error bars represent 95% confidence interval ranges (n =4 replicates per treatment).

Between all 3 treatments, one-way ANOVA shows that there is a significant difference ($p = 3.9 \times 10^{-12}$) in developmental time to reach adulthood. Furthermore, the 95% confidence intervals do not overlap suggesting that there is a significant difference between temperature treatments.

The mean number of male and female *D. melanogaster* larvae reaching adulthood is 2.25 and 0.88 flies, respectively (Figure 5). Although Figure 5 shows that the confidence intervals overlap, the p-value obtained from two-way ANOVA was smaller than 0.05 ($p=0.024$). As a result, the number of males and females reaching adulthood is significantly different. It should be noted that data from 17 °C treatment was omitted for two-way ANOVA because missing values are not permitted in the calculation.

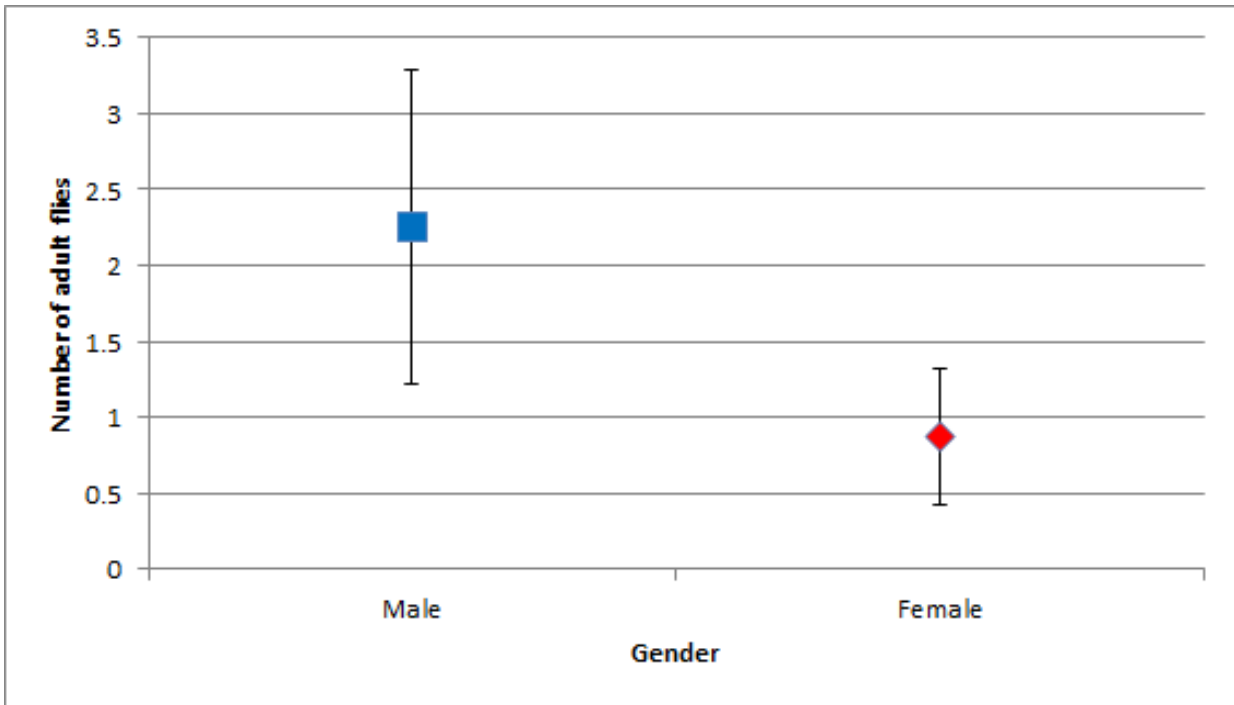


Figure 5. Relationship between gender and number of adult flies. Bars represent 95% confidence interval ranges of 1.2 to 3.3 flies and 0.4 to 1.3 flies respectively with n= 4 replicates per treatment.

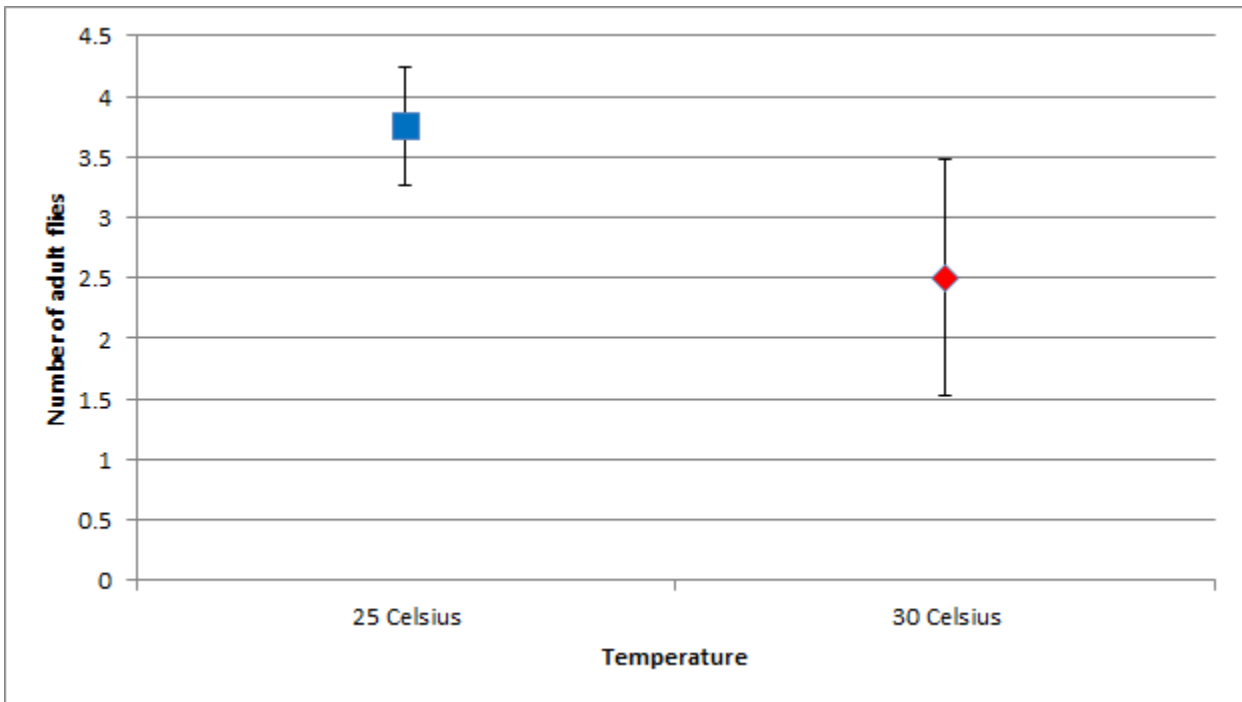


Figure 6. Relationship between temperature and number of adult flies. Bars represent 95% confidence interval ranges of 3.3 to 4.2 flies and 1.5 to 3.5 flies respectively with n= 4 replicates per treatment.

The mean number of *D. melanogaster* adults for each replicate at 25 °C was 3.8 flies and at 30 °C was 2.5 flies (Figure 6). The p-value obtained from the two-way ANOVA was larger than

0.05 ($0.26 > 0.05$), suggesting that the number of flies reaching adulthood at these two temperature is not significantly different. The 95% confidence intervals also overlap supporting this finding.

At both 25 °C and 30 °C, more males were found eclosed (mature) compared to females (Figure 7). Only 25 °C treatment had significant difference between the genders, though. At 25 °C, an average of 12 male and 3 female flies eclosed while 6 male and 4 female flies eclosed at 30 °C. The two-way ANOVA suggested that the developmental time of males and females to reach adulthood between 25 °C and 30 °C is not significant ($p\text{-value} = 0.13 > 0.05$).

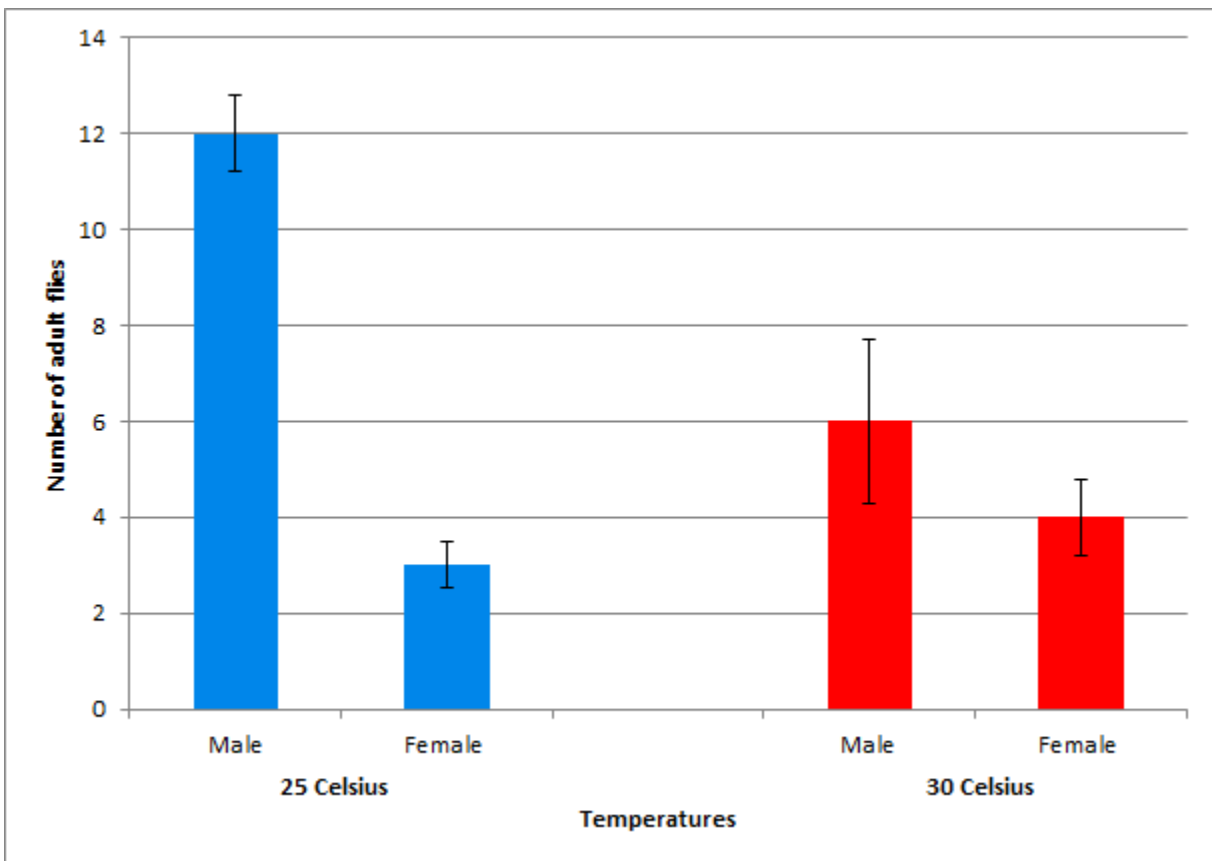


Figure 7. The total number of adult female and male flies at 25 and 30 °C. The vertical error bars represent 95% confidence intervals with $n=4$ replicates per treatment. The 95% confidence interval shows 11 to 13 of males and 2.5 to 3.5 females in 25 °C whereas 4.3 to 7.7 of males and 3.2 to 4.8 of females were observed in 30 °C.

We also observed cracking of medium due to dryness in 25°C and 30 °C whereas 17 °C remained moist without cracks. On our last observation day, we observed 11 pupae at 17 °C and 2 pupae at 25 °C that were close to eclosion. There was no sign of pupae at 30 °C.

Discussion

We analyzed the effect of temperature on the number of male and female *D. melanogaster* larvae reaching adulthood and the time it took them to reach adulthood. Using a one-way ANOVA, we found that temperature had a significant effect on the developmental time. Therefore, the alternative hypothesis (H_{a1}) is supported. Using two-way ANOVA, we failed to reject our null hypothesis (H_{02}) supporting that temperature does not affect the number of male and female *D. melanogaster* larvae to reach adulthood. In fact, Figures 5 and 6 show that gender may have an effect on number of larvae reaching adulthood while temperature does not.

The absence of adult flies at 17 °C may be to the result of a slowed metabolic rate commonly observed at lower temperatures (Petavy *et al.* 2001). Petavy *et al.* (2001) found that *D. melanogaster* required approximately 19 days to mature into adult at 17 °C while we only observed pupae until the 18th day. Therefore the absence of adults at this temperature may be due to the differences in experimental length. Petavy *et al.* (2001) also used sugar-agar medium with baker's yeast, and 12 hours of constant temperature followed by 12 hours of alternating temperature (10 °C-27 °C) to simulate daily fluctuations in their natural habitats. They also added water to the medium to prevent dryness. In contrast, we used oatmeal mix medium, which appeared quite dry and may not have been as favourable for growth, and applied constant temperature over 24 hours without addition of water. In any case, we could not include data from the 17 °C treatment for statistical analyses.

In general, we observed a higher number of flies at 25 °C compared to 30 °C. However, the first 6 adult flies to emerge were observed at 30 °C. We had expected 25 °C to yield the flies first since it is the optimal temperature known for *D. melanogaster* (Hamada *et al.* 2008). Interestingly, another study explains that although 25 °C is the optimal temperature, 29 °C is the temperature with a faster maximum developmental rate, followed by 28° C and 30 °C (Petavy *et al.* 2001). While high temperature (but still below 32 °C) could induce heat stress on *D. melanogaster* larvae, it could also increase metabolic rate (Petavy *et al.* 2001). Conversely, the authors observed a decrease in the developmental rate below 29 °C. Higher metabolic rate increases energy expenditure of an organism and could result in an increased rate of food intake and shortened developmental time (Mueller *et al.* 2005). Therefore, observing the first 6 flies from the 30 °C treatment seems to be consistent with literature.

Overall, more flies eclosed at 25 °C compared to 30 °C. This may be due to higher heat stress. In *D. melanogaster* larvae, heat induces the expression of Heat shock protein (Hsp), which is involved in thermotolerance (Dahlgaard *et al.* 1998). Specifically, these enzymes assist in folding and unfolding of proteins and degradation of denatured proteins (Sorensen *et al.* 2003). Hsp proteins can form complexes with other chaperone proteins, including components of signaling pathways such as phosphatases, protein kinases, and telomerases (Morimoto 2002, Morrow and Tanguay 2003, Tower 2009). The growth and survivability of larvae may then be disturbed under heat stress if Hsps are impaired (Sorensen *et al.* 2003). Additionally, mortality rate may increase above 28 °C (Petavy *et al.* 2001). This is partially explained by the impacts of overexpression of Hsp resulting in the shutdown of normal cell functions, extensive use of energy in expressing the proteins, interrupted fertility, and retarded growth and cell division (Sorensen *et al.* 2003, Feder *et al.* 1992). Petavy *et al.* (2001) concluded that overexpression of Hsp can influence fitness under

non-optimal environmental conditions. These reasons may explain why *D. melanogaster* larvae at 30 °C showed lower proportion of eclosion relative to 25 °C.

We observed statistically more males than females at both 30 °C and 25 °C treatments. This might be due to specific adaptive responses towards heat resistance observed in males but not as significantly in females (Moskalev *et al.* 2009). Such responses allow better protection of the cells from heat, resulting in males to have more resistance to heat stress (Moskalev *et al.* 2009). Higher surface area to volume ratio of male flies due to their smaller size may also enhance their survivability in warmer environments (Partridge *et al.* 1994). It is also important to note that the male to female ratio was more dramatic at 25 °C than at 30 °C. Tantawy and Mallah (1961) observed a 1:1 ratio at 25 °C and deviating from this temperature pushed towards lower male to female ratio. A possible reason why we might not have seen the same result may be due to the differences in experimental design. Tantawy and Mallah (1961) allowed a generation of *D. melanogaster* to produce offspring at 25 °C and worked with this new, acclimated generation, in which their gender was determined during the experiment. However, we were given larvae whose gender was unknown.

D. melanogaster larvae can vary biologically leading to individuals that grow faster and better under different conditions. This variation may explain why all larvae did not eclose at the same time and is supported by the different ranges of 95% confidence intervals in Figure 4. Also, since the incubators were shared by other students this could have potentially exposed the replicates to varying temperature conditions, possibly provoking different Hsp70 levels.

Furthermore, different life stages of larvae may have been used. Rubin (1988) suggested that larvae have three different stages and the age of each stage vary by a day. We picked random larvae therefore we could have easily used third stage larvae in our experiments which may

contributed to the shorter developmental time we observed at the 30°C. Lastly, the recorded time is not an accurate representation of true developmental time and could vary by one or two days since we did not have access to the lab during the weekends and were not present at all hours of the day. Therefore, reducing these uncertainties and variations would have reduced errors in our experiment.

Conclusion

In this experiment, we observed the effect of temperature (17 °C, 25 °C and 30 °C) on the number of male and female *D. melanogaster* larvae. We could not obtain any data from 17 °C and using our results from 25 °C and 30 °C we found that these two temperatures do not seem to affect the number of male and female larvae reaching adulthood although it does seem to affect developmental time. Because the effect of long-term heat exposure on females and males has not been studied extensively compared to short-term exposures, our experiment might encourage other scientists to study further this topic in more detail.

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