The effect of temperature on development time and body size in male and female wild-type Oregon-R and mutant *ort-1 Drosophila melanogaster*

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Abstract

The purpose of this study was to examine the effects of differing temperatures on average development time and body size in male and female, wild-type Oregon-R, and mutant, *ort-1*, *Drosophila melanogaster*. *D. melanogaster* were raised from larval to adult stage in three temperature treatments, 17˚C, 25˚C and 30˚C, each containing five replicates. Body size was measured for the adult flies, and date of emergence and gender were recorded. The presence of the mutation at the different temperatures showed no significant difference in both average body size and development time (with a two-way ANOVA, p=0.60; p=0.24; between 17˚C and 25˚C). However, when examining individuals based on fly type, there was a significant difference in average development time for *D. melanogaster* between 17˚C and 25˚C (p<0.0001). Through a comparison of the means, the same trend was present between 17˚C and 30˚C, but absent between 25˚C and 30˚C. Average body size was not affected by temperature in the analysis on fly type (p=0.89). The effects of temperature on average body size (p=0.90) and development time (p=0.74) was the same between genders, however, gender did exhibit an effect on average body size (p=0.0004). Additionally, average development time within males and within females was significantly longer at 17˚C compared to 25˚C (males and females: p<0.0001) and 30˚C (males and females: p<0.0001). Temperature had an effect on average body size and development time of *D. melanogaster* when grouping individuals by gender (p=0.016; p<0.0001, respectively). Additionally, the effects of temperature on average development time and body size was the same between fly types (p=0.24; p=0.60, respectively) and between genders (p=0.79; p=0.90, respectively). As such, we failed to support that gender affected average development time, the mutation affected average body size or average development time, or there being a differential effect of temperature between wild-type and mutant, and males and females on average body size and average development time.

Introduction

The negative relationship between temperature and average development time is well documented for insects (Davidson 1944; Al-Saffar *et al.* 1996). The average development time and body size of *Drosophila melanogaster*, the common fruit fly and member of the family Drosophilidae, have been observed to decrease as temperature increases (Davidson 1944; Al-Saffar *et al.* 1995; Hartwell *et al.* 2011; Gilbert and De Jong 2001; Petavy *et al.* 2001). Limited research has been conducted on the relationship between these factors with regards to the *ort-1* mutant, since it is a relatively new mutation. This presents an opportunity to investigate the differences and commonalities between the Oregon-R wild-type and *ort-1* mutant with regards to temperature's effect

on average development time and body size, alongside its effect on average development time and body size in males and females.

D. melanogaster are holometabolous insects whose larval and pupal stages precede the adult stage. Larvae hatch from eggs laid in fermenting fruit after approximately 24 hours (Hartwell *et al*. 2011). There are three stages of larval development, lasting approximately 4 days at 25˚C, during which individuals feed on the fruit in which the eggs were laid (Reaume and Sokolowski 2006). This leads to the pupal stage during which metamorphosis occurs over 3-4 days, followed by eclosion (the emergence of adults), with adults typically living for over 10 weeks (Hartwell *et al.* 2011).

The mutant *Drosophila* contains the *ort-1* mutation - a result of a deletion in exon 2 and 3, and all of intron 2 in the *ort* gene (Iovchev *et al.* 2012). This affects the histamine-gated chloride channels, and results in an abnormal temperature preference in mutant flies (Hong *et al.* 2006). Because the mutant is relatively new, limited research on its impact on phenotype is found. As such, it is unclear if a similar temperature relationship in wild type on average body size and development time will hold for the *ort-1* mutant. Compared to the wild-type, the mutation is thought to affect temperature tolerance, since during the initial evaluation of this fly type; they experienced delayed recovery after 10 minutes of incubation at 40˚C.

Average development time of *D. melanogaster* decreases exponentially as temperature increases, but rapidly increases as the lethal temperature is approached (Davidson 1944). This transition point has been observed at 31˚C, with 34˚C resulting in incomplete development (Davidson 1944). As such, 30˚C was chosen as the maximum temperature for this experiment. Similarly, the average body size of *D. melanogaster* decreases as temperature increases, with the maximum occurring at 16-17˚C, while the inverse relationship occurs below 16˚C (Partridge *et al.* 1994; Karan *et al.* 1988). Therefore, 17˚C was chosen as the lower temperature limit for this experiment.

Furthermore, sexual dimorphism is displayed in adult *D. melanogaster* as males have a smaller average body size than females (Handa *et al.* 2014). This is attributed to the female reproductive organs taking up more abdominal space than in males (David 1979, as cited in David *et al.* 2011). The difference in average body size between the sexes is minimal at low temperatures, but increases as temperatures increase (David *et al.* 2011).

Our study investigated the effects of temperature on average body size in the wild-type and mutant *ort-1 D. melanogaster* (Figure 1). The hypotheses were:

H_{o1}: Temperature has no effect on *D. melanogaster* average body size.

H_{A1}: Temperature has an effect on *D. melanogaster* average body size.

H_{o2}: The presence of a mutation has no effect on *D. melanogaster* average body size.

H_{A2}: The presence of a mutation has an effect on *D. melanogaster* average body size.

H_{o3}: The effect of temperature on average body size of *D. melanogaster* is the same in the wild-type and mutant.

H_{A3}: The effect of temperature on average body size of *D. melanogaster* is not the same in the wild-type and mutant.

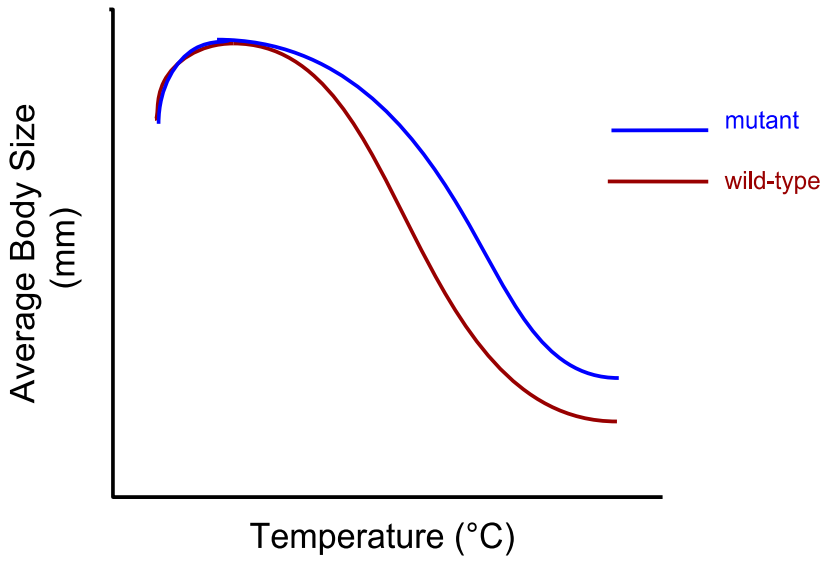

Figure 1: The Predicted Effects of Varying Temperatures on Average Body Size measured in millimetres for *D. melanogaster*.

Furthermore, the effects of temperature on average development time was investigated in both wild-type and *ort-1* mutant *D. melanogaster* (Figure 2).

H_{o1}: Temperature has no effect on *D. melanogaster* average development time.

H_{A1}: Temperature has an effect on *D. melanogaster* average development time.

H_{o2}: The presence of a mutation has no effect on *D. melanogaster* average development time.

H_{A2}: The presence of a mutation has an effect on *D. melanogaster* average development time.

H_{o3}: The effect of temperature on average development time of *D. melanogaster* is the same in the wild type and mutant.

H_{A3}: The effect of temperature on average development time of *D. melanogaster* is not the same in the wild-type and mutant.

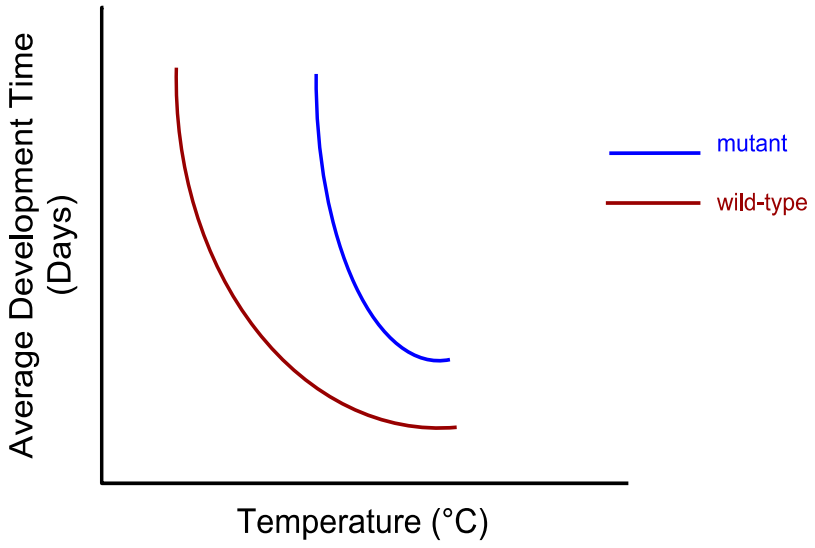

Figure 2: The Predicted Effects of Varying Temperatures on Average Development Time measured in days for *D. melanogaster*.

The effect of temperature on male and female average body size (Figure 3) was also investigated with the following hypotheses:

H_{o1}: Temperature has no effect on *D. melanogaster* average body size.

H_{A1}: Temperature has an effect on *D. melanogaster* average body size.

H_{o2}: Gender has no effect on *D. melanogaster* average body size.

H_{A2}: Gender has an effect on *D. melanogaster* average body size.

H_{o3}: The effect of temperature on average body size of *D. melanogaster* is the same for males and females.

H_{A3}: The effect of temperature on average body size of *D. melanogaster* is not the same for males and females.

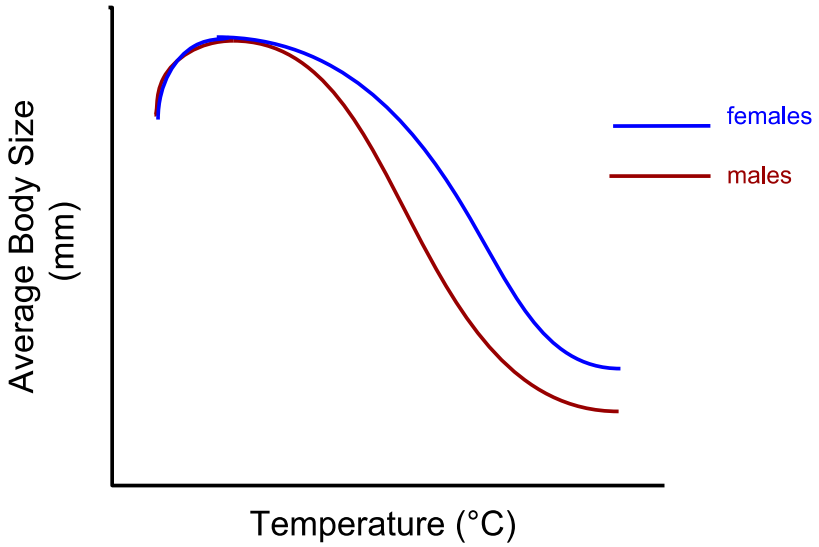

Figure 3: The Predicted Effects of Varying Temperatures on Male and Female Average Body Size measured in millimetres for *D. melanogaster*.

Lastly, we examined the effects of temperature on male and female average development time (Figure 4):

H_{o1}: Temperature has no effect on *D. melanogaster* average development time.

H_{A1}: Temperature has an effect on *D. melanogaster* average development time.

H_{o2}: Gender has no effect on *D. melanogaster* average development time.

H_{A2}: Gender has an effect on *D. melanogaster* average development time.

H_{o3}: The effect of temperature on average development time of *D. melanogaster* is the same for males and females.

H_{A3}: The effect of temperature on average development time of *D. melanogaster* is not the same for males and females.

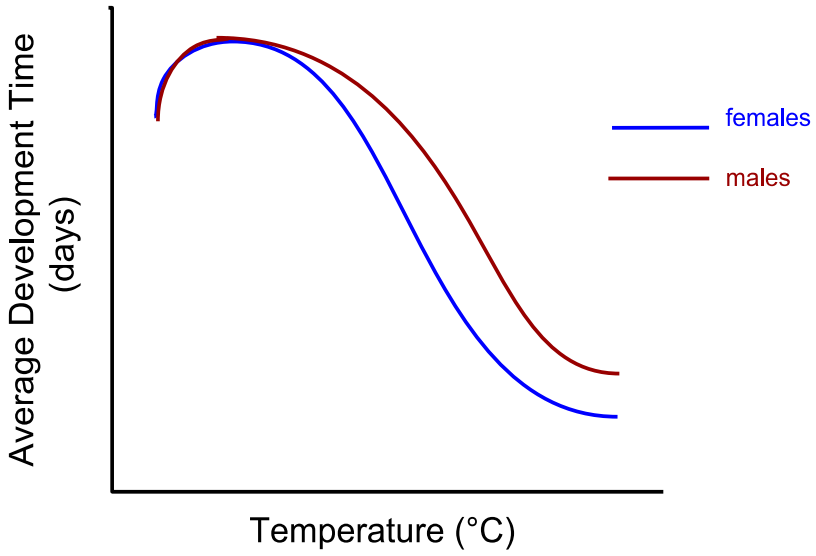

Figure 4: The Predicted Effects of Varying Temperatures on Average Development Time of Male and Female *D. melanogaster* measured in days

Materials and Methods

To determine the effects of temperature on average development time and body size of mutant and wild-type *Drosophila melanogaster*, we used three treatments of 17˚C, 25˚C, 30˚C with five replicates for each. The analysis for gender utilized these same treatments by combining the data for individuals of the same sex regardless of their fly type (wild-type or mutant). *D. melanogaster* individuals were deemed mature upon emergence as adults, and we defined average body size to be from the tip of the head to the end of the abdomen (see Figure 5).

Figure 5: Photo of *Drosophila melanogaster* taken with the Dinoscope under 8x magnification. The line represents the length, which ImageJ used to calculate actual body length.

We collected larvae from the BIOL 342 stock grown in a cornmeal medium and placed five larvae into each of the five replicate vials for each treatment, which contained cornmeal medium. This was done for both wild type and mutants. We then placed the replicates into boxes for their corresponding incubation at 17˚C, 25˚C, and 30˚C (Figure 6). The 17˚C incubator contained a light source, so we placed a box over these replicate vials to block the light to ensure identical conditions within the incubators. To control for humidity we placed a clean petri dish filled with water up to the lid line in each incubator.

Figure 6: Experimental set up. Mutants and wild-type replicate vials for 17˚C, 25˚C, and 30˚C (left to right).

We ran the experiment from October 21, 2014 to November 10, 2014, performing daily observations (excluding weekends) starting three days after commencing the experiment. We anesthetized adult flies that had emerged using CO_2, and removed them from their respective vials in order to observe them under the dissecting microscope. We noted the date of emergence along with gender, and took pictures of each individual from the dorsal and lateral views using a Dinoscope. We identified males based on the presence of sex combs and rounder abdomens, which had a higher concentration of black pigment at the tip; females were identified by their more elongated abdomens with more numerous black bands on the dorsal surface. After observations were made, we disposed of the flies into soapy water, and put the vials back in their corresponding incubators. We refilled the petri dishes in each incubator every day with equal amounts of water to keep the humidity constant for all treatments.

We measured body length using the Dinoscope photos in conjunction with ImageJ.

At the higher temperatures, specifically 30°C, the cornmeal medium seemed to shrink causing some *D. melanogaster* to become stuck between the cornmeal and the side of the vial. If the flies were fully emerged, we picked them out of the medium. In the case of partial emergence, flies were labeled as emerged if the majority of it was developed and it was able to fly.

Statistical Analysis:

We calculated the number of days required for each individual to emerge, and averaged the values for each replicate. We calculated the mean development time for each treatment by using the averages from their corresponding replicates. There was insufficient data for the mutant 30˚C treatment due to the lack of emergent adults, so we excluded this temperature from the statistical analysis, but we used mean values to make comparisons to 17˚C and 25˚C. We ran two two-way

Analysis of Variance (ANOVAs) to determine the effects of temperature on average body size and development time in wild type and mutant in the 17˚C and 25˚C treatments. Additionally, we ran two two-way ANOVAs to determine the effects of temperature on these variables in males and females in the 17˚C, 25˚C, and 30˚C treatments. Four one-way ANOVAs were run to examine differences in average development time and average body size across the temperature treatments within each gender. For the tests that yielded significant results, we conducted a post-hoc analysis using the Tukey-Kramer Method to determine which combination of treatments was significantly different. We generated graphs using JMP9, which shows the means and 95% confidence intervals for each variable of interest.

Results

Due to the emergence of flies in only two vials (n=2) for the mutant 30˚C treatment, no statistical analysis could be made with regards to this treatment, but general trends using mean values were made alongside the statistical data for 17˚C and 25˚C.

Neither the presence of a mutation (p=0.53), or the variation in temperature between 17°C and 25°C (p=0.89) had a significant effect on average body size of *D. melanogaster* (Figure 7). Additionally, the effect of temperature on the average body size was the same for both mutant (2.68 mm) and wild-type (2.74 mm; p=0.60). Comparing the means from the 30˚C treatment to 17˚C and 25˚C indicated that the above trend continued, with there being little difference in average body size between fly types or between treatments.

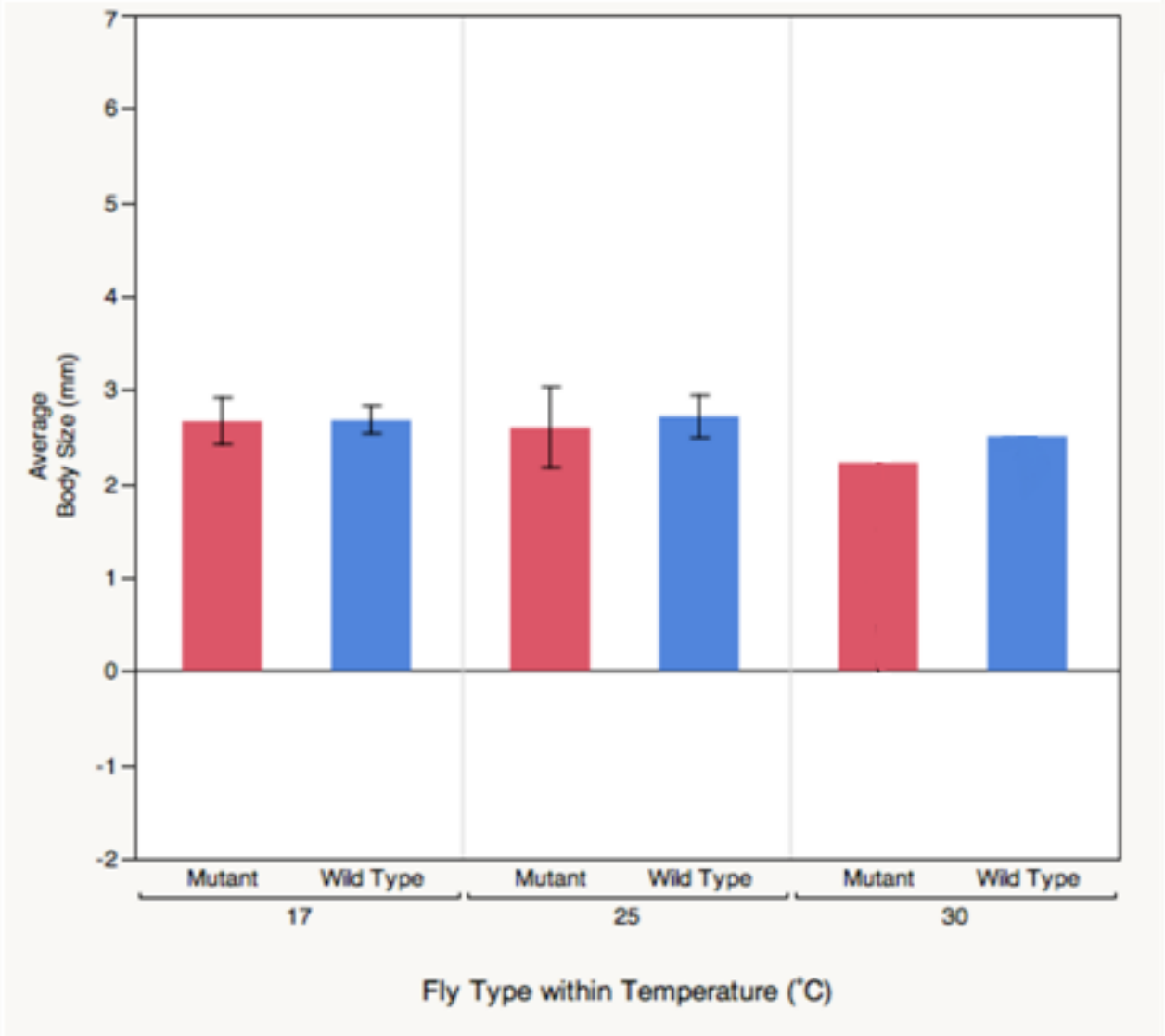

Figure 7: The Effect of Fly Type on Average Body Size of *D. melanogaster* at Various Temperatures. The average body size for Oregon-R wild-type and *ort-1* mutant *D. melanogaster* at 17, 25, and 30°C in millimetres as measured in mean ± 95% confidence interval for 17˚C and 25˚C and mean values for 30˚C. n= 5 at 17˚C and 25˚C; n=2 and n=5 at 30˚C for mutant and wild-type, respectively. Note: 30˚C treatments lack confidence intervals as there was insufficient data to perform statistical analysis.

There was a significantly longer average development time for the individuals in the 17˚C treatment (17.38 days) compared to those at 25˚C (8.03 days; $p<0.0001$) (Figure 8). The presence of the mutation had no effect on the average development time of *D. melanogaster* ($p=0.42$). By comparing the means, there appeared to be a trend for a longer average development time for both the wild-type and mutant at 17˚C (wild-type=18.27days; mutant=16.50days) compared to 30˚C (wild-

type=6.71days; mutant=6.75days). A slightly longer mean development time was noted for both fly types at 25˚C (wild-type=7.87days; mutant=8.20days) compared to 30˚C, however this difference was minor. There was found to be no differing effect of temperature on the average development time between mutants (12.35 days) and wild type (13.07 days; p=0.24) at the above temperatures.

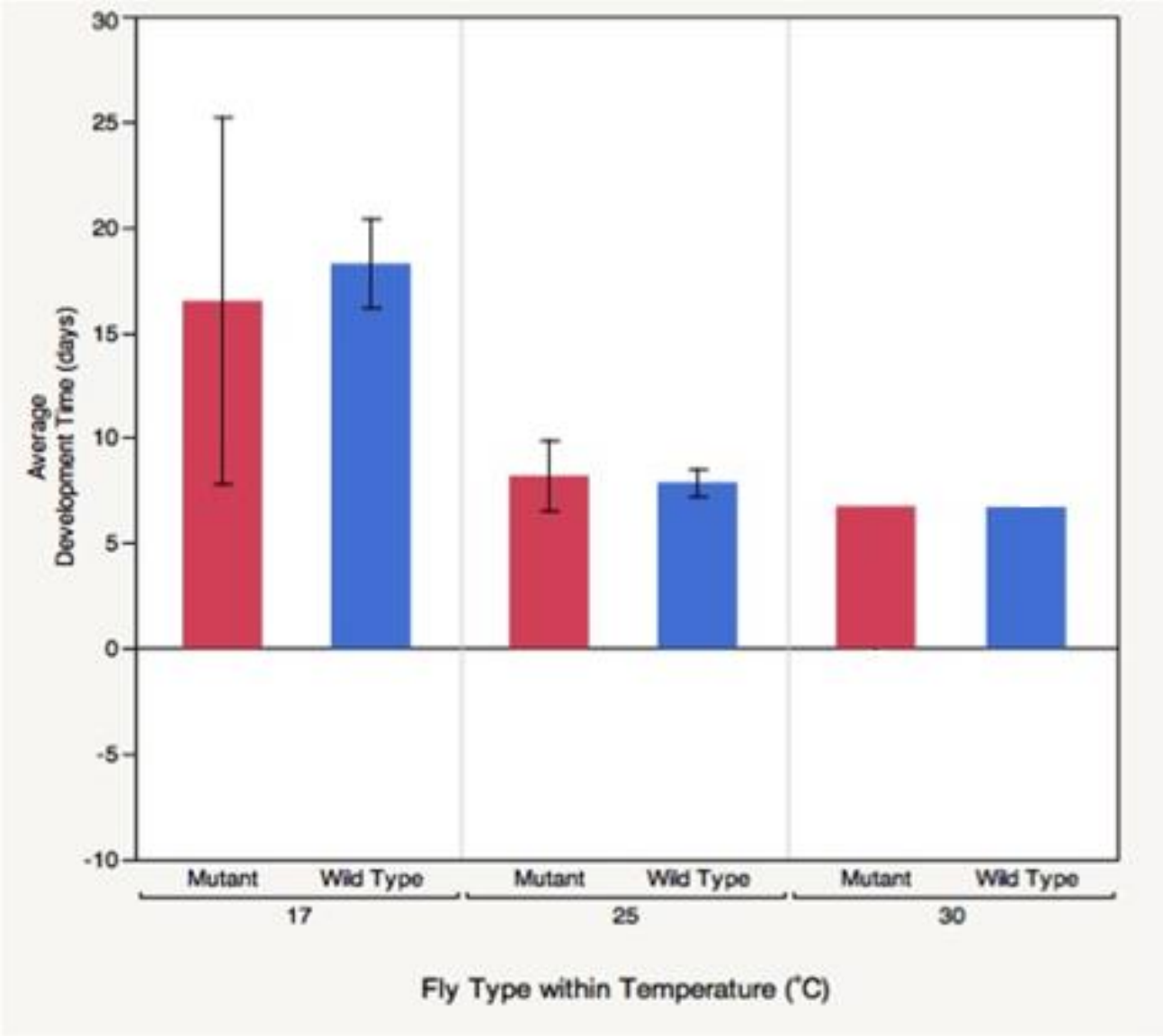

Figure 8: The Effect of Fly Type on Average Development Time of *D. melanogaster* at Various Temperatures. The average development time for Oregon-R wild-type and *ort-1* mutant *D. melanogaster* at 17, 25, and 30°C in days as measured in mean ± 95% confidence interval. n=5 17˚C and 25˚C; n=2 and n=5 at 30˚C for mutant and wild-type, respectively. Note: 30˚C treatments lack confidence intervals as there were insufficient replicates to perform statistical analysis.

The analysis based on gender indicated that temperature had an effect on *D. melanogaster* average body size (p=0.016). Average female body size (2.92 mm) was significantly larger than males (2.57 mm; p=0.0004) (Figure 9), however, the effect of temperature on average body size was the same for males and females (p=0.90). There was no significant difference found within each gender between temperature treatments with regards to average body size (males: p=0.09; females: p=0.15).

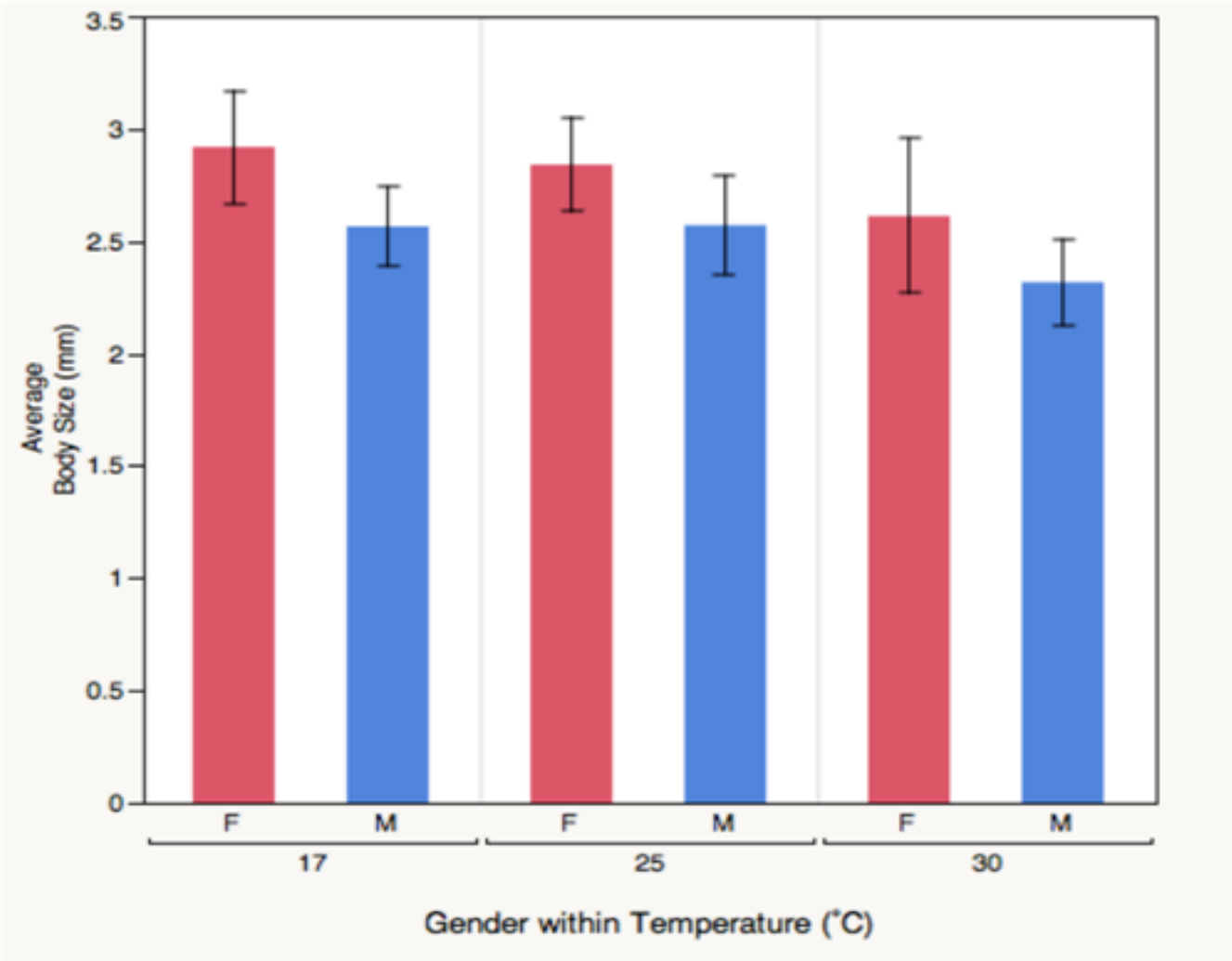

Figure 9: The Effect of Gender on Average Body Size of *D. melanogaster* at Various Temperatures. The average body size of female and male *D. melanogaster* at 17, 25, and 30°C in millimetres as measured in mean ± 95% confidence interval. n=6 at 17˚C; n=8 at 25˚; n=5 and n=7 at 30˚C for females and males, respectively.

Temperature was also found to have an effect on *D. melanogaster* average development time ($p<0.0001$) when analyzed by gender. There was no difference observed between the males (17.61 days) and females (17.86 days) with regards to average development time ($p=0.88$), and the effect of temperature on this variable was the same for males and females ($p=0.79$) (Figure 10). Analysis of the differences among males between treatments indicated a significantly longer average development time at 17˚C (17.61 days) compared to both 25˚C (7.68 days; $p<0.0001$), and 30˚C (7.00 days; $p<0.0001$). Similarly, females had a longer average development time in the 17˚C treatment (17.86 days) as compared to 25˚C (7.81 days; $p<0.0001$), and 30˚C (6.37 days; $p<0.0001$). There was no significant difference between the 25˚C and 30˚C treatments for either males ($p=0.78$) or females ($p=0.16$).

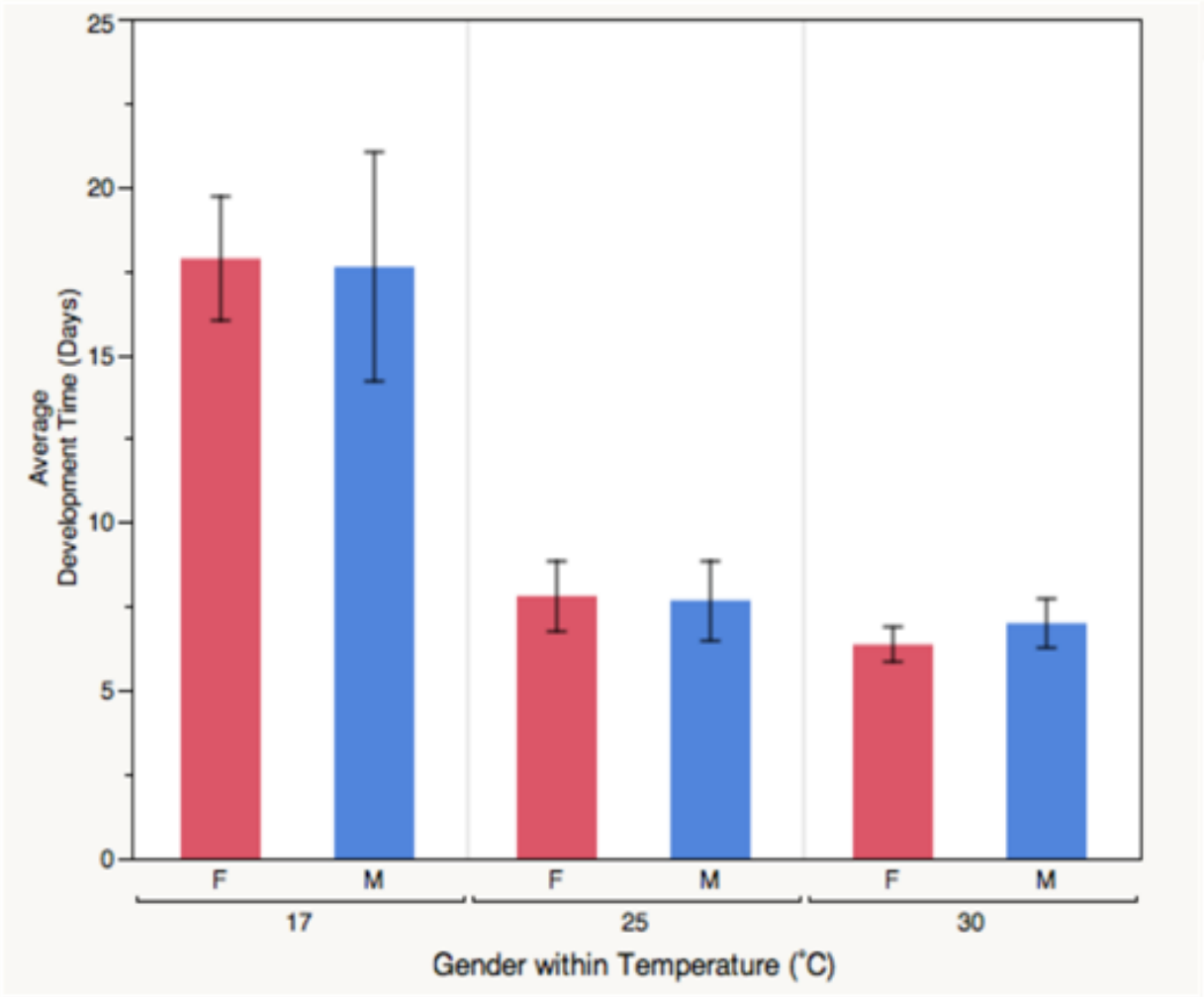

Figure 10: The Effect of Gender on Average Development Time of *D. melanogaster* at Various Temperatures. The average development time of female and male *D. melanogaster* at 17, 25, and 30°C in days as measured in mean ± 95% confidence interval. n=6 at 17˚C; n=8 at 25˚C; n=5 and n=7 at 30˚C for females and males, respectively.

Discussion

We rejected the null hypothesis that temperature has no effect on average development time and we supported the alternative hypothesis that temperature has an effect on average development time ($p<0.0001$). We also rejected the null hypothesis that gender has no effect on average body size and supported the alternate hypothesis that gender has an effect on average body size ($p=0.0004$) of *D. melanogaster*. Additionally, we supported the alternatives, which stated temperature had an effect on *D. melanogaster* average body size ($p=0.016$) and development time ($p<0.0001$) when individuals were grouped based on gender. However, grouping individuals by type wild type or mutant indicated that temperature affects average development time ($p<0.0001$) of *D. melanogaster*, but not average body size ($p=0.89$). We failed to reject the null hypotheses that the presence of a mutation has an effect on *D. melanogaster* average body size ($p=0.42$), that gender has an effect on average development time ($p=0.88$), or for the effect of temperature on average body size and development time differing between wild type and mutant ($p=0.60$; $p=0.24$, respectively) and between genders ($p=0.90$; $p=0.79$, respectively).

The average body size for both mutant and wild type was relatively consistent throughout all the treatments (Figure 7). This differed from previous research, in which the wild type's average body size decreased with increasing temperature, specifically between 25˚C and 30˚C (Partridge *et al.* 1994; Good 1993). This inverse relationship is due to body cells developing to a smaller size at higher temperatures (Partridge *et al.* 1994). Research has also shown that larvae raised at lower temperatures convert food more efficiently, thus leading to a larger adult size compared to those raised at higher temperatures (Robinson and Partridge 2001). Furthermore, mature larvae tend to have a larger body mass, and a 5-7% higher metabolic rate in comparison to larvae raised at higher temperatures (Berrigan and Partridge 1997).

A decrease in average development time was observed with increased temperatures for both mutant and wild type. At 17˚C, *D. melanogaster* exhibited a longer average development time compared to 25˚C (Figure 8), which is in agreement with Gibert and De Jong's (2001) observation of an exponential decrease in average development time with increasing temperature. Due to the lack of data for the 30˚C treatment, accurate comparisons could not be made between the other treatment temperatures, but a trend towards a lower average development time was seen (Figure 8). The predicted differential effect of temperature on average development time between the wild-type and mutant was not observed in this study. Temperature sensitivity is a common result of mutations in *D. melanogaster* (Foster 1972). The *ort-1* mutants are known to be temperature-sensitive, and exhibit a more delayed recovery compared to wild type after a 10 minute exposure to 40˚C (Hong *et al.* 2006). However, depending on the mutation involved and the vital processes affected, lethality due to temperature sensitivity can occur at various stages of development (Foster 1972; Tarasoff and Suzuki 1970). Therefore, even though *ort-1* mutants exhibit temperature sensitivity, the processes affected may not have altered its average development time as compared to the wild type, or the temperature treatments may not have been extreme enough to induce these severe effects.

It was observed that females were significantly larger than males at all temperatures. This was consistent with previous research, which indicated that males typically have smaller bodies than females (Partridge *et al.* 1994). However, David *et al. (*2011) found this difference to become greater as temperature increases, which was not observed in this experiment. As well, individuals of the same gender appeared to develop to relatively the same average body size regardless of the temperature treatment (Figure 9), which contradicts findings where males raised at lower temperatures had significantly larger bodies than those raised at higher temperatures (Pavkovic-Lucic and Kekic 2013). The lack of difference in average body size between individuals of the same gender at different treatments may be due to some flies not being fully developed when measurements were taken, as

some were observed to lack abdominal bands or have underdeveloped wings. In addition, the significant effect of temperature on average body size of *D. melanogaster* obtained when individuals were grouped by gender contradicts the results observed when they were grouped by type, wild type or mutant. This may be due to the fact that we pooled the mutant and wild-type data together to separate them by gender, resulting in characteristics of both fly types being represented within each gender.

The lack of a significant difference in the average development time between males and females at each temperature treatment (Figure 10) contradicts previous research. It was indicated that the pupa stage of males was longer than those of females, resulting in an overall longer average development time in males (Davidson 1944). Different stages of development have an optimum temperature in which development will be faster, while other stages may remain unaffected (Bonnier 1926). It is thought that males and females may have different temperature optima during different stages of development, such as the pupal stage which is known to be longer for males due to the formation of secondary sexual characteristics (Bonnier 1926).

Research suggests that the optimal survival temperature of *D. melanogaster* is 24-25˚C; therefore survival rate would decrease beyond this point (Hong *et al.* 2006; Al-Saffar *et al.* 1995). This trend was observed in our experiment. The harmful effects associated with high temperatures, such as the accumulation of metabolic waste products and the increased rate of water loss, become greater during the more advanced stages of development, potentially causing development to stall during the later stages (Davidson 1944). This may account for the development of several pupae at 30˚C with very few emerging as adults, particularly for the mutant. Furthermore, this may be a reason why few flies emerged at 30˚C. These results, which are not supported in the literature, could be explained by our pooling the replicates in both wild-type and mutant *D. melanogaster*. This would increase the number of flies representing males and females at each temperature treatment, thus

resulting in a better representation of a population of *D. melanogaster*. For this reason further experimentation for a prolonged period of time may yield more consistent results, which may support that temperature has an effect on *D. melanogaster*.

The accuracy in measuring average body size was a potential source of error in this experiment. Measurements taken on the first day were not done using ImageJ, but were instead done by using a ruler under the dissecting microscope. A comparison between the two methods indicated that using a ruler was a few tenths of millimetres less accurate than the measurements obtained by ImageJ. As a result, 59% and 22% of the data for the wild-type at 30˚C and 25˚C, respectively, were inaccurately measured.

Furthermore, the individual larvae collected were noted to vary in size, which may be due to individuals being at different stages of larval development. This could have potentially influenced average development time as those collected in the first larval stage may have developed into a pupa after 4-5 days, while that process may have only taken 2 days for those collected in the third larval stage (Hartwell 2011). Additionally, inherent genetic differences among individuals might influence their average development time and body size, and may account for some of the variation observed in the data.

Lastly, the lack of data for some of the treatments, such as the 30˚C mutants, may have influenced the accuracy of the results, since the means may have been less representative of the true populations. This was reflected by comparing the proportion of the initial population that emerged as adults in each treatment. This proportion increased with temperature for wild type, however, was highest for mutants at 25˚C, followed by 17˚C then 30˚C. The largest variation between fly types for this proportion occurred for the 30˚C treatments.

Conclusion

Neither the *ort-1* mutation nor gender were not found to affect average development time or average body size of *D. melanogaster*, nor was there a difference between fly types regarding these variables. As such, we failed to reject the associated null hypotheses. We do support gender affecting average body size, temperature affecting average development time of *D. melanogaster*, which was reinforced by the significant decrease in average development time within males and females as temperature increased. These results suggest that the *ort-1* mutation does not cause differences in developmental processes and body size as compared to wild-type *D. melanogaster*, regardless of temperature. Additionally, this research has helped to support previous observations indicating sexual dimorphism in adults with regards to body size.

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