The effect of temperature on the maturing time of temperature-sensitive fruit fly (*Drosophila melanogaster*) mutants

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Abstract

Drosophila melanogaster is a small fly species commonly known as a 'fruit fly'. Temperature-sensitive mutants of *D. melanogaster*, with the mutation *shibire* become paralyzed at temperatures exceeding 29°C. This mutation affects the nervous system of the flies by reducing synaptic vesicles. Both wild type and mutant strains were treated at: 17°C, 25°C, and 29°C to determine if the mutation causes a significant difference in maturation time. It is important to know whether the maturation time of *shibire* mutants are different from the wild type when using *shibire* to manipulate other genes. We incubated vials containing *D. melanogaster* larvae in cornmeal medium at the three treatment temperatures. We examined the time (in days) that it took for the larvae to molt into pupae and for the pupae to become adults. The results indicated that the maturing times between mutant and wild type strains were not significantly different. Also, we observed that the adult mutants did not become paralyzed at the anticipated temperature. This revealed that the sample pool did not have all intended Cha-Gal 4/UAS-*shi* mutants to begin with. Consequently, we do not support our alternate hypothesis that the maturing time for the wild type is shorter than the mutants as temperature increases.

Introduction

The common fruit fly, *Drosophila melanogaster*, is a small ectothermic species used in many genetic studies. We worked with wild type *D. melanogaster* of the Oregon-R strain and temperature-sensitive mutants having the mutation *shibire*. Adults and larvae experience reversible paralysis when subjected to 29°C (Poodry *et al.* 1973). The mutation works by affecting the nervous system, reducing synaptic vesicles at neuromuscular junctions at high temperatures (Poodry and Edgar 1979).

Geneticists can use this temperature-sensitive mutation to manipulate the expression of certain genes (Kelley and Suzuki 1974). By keeping the temperature above and under the restricted temperature, the effect of the gene can be turned

on and off. This allows the researchers to control the expression of the gene easily by adjusting the temperature to which *D. melanogaster* are subjected.

There are different mutant alleles of *shibire*. In this study, the expression of *shibire* was controlled by the GAL4/UAS system. The GAL4/UAS system is a commonly used tool to overexpress a certain gene product (Duffy 2002). GAL4 is a gene that encodes transcription activator protein Gal4, and UAS is a promoter. This UAS was inserted upstream of the *shibire* gene in our mutant strain. Gal4 protein binds to the DNA sequence at UAS and activates the transcription of *shibire* (Duffy 2002).

In this experiment, our objective was to determine whether the maturing time was different between the temperature-sensitive mutants and that of the wild type. This investigation is important because it gives insight into the temperature-sensitive mutation, which is vital when using it to control the expression of other genes. Chen *et al.* (1992) identified that the expression of *shibire* is broadly involved in many parts of *D. melanogaster* development. The *shibire* mutant has also been found to experience developmental defects on legs, wings, and the neuromuscular system when treated with heat pulses (Poodry *et al.* 1973). The expansion of the nervous system caused a lethal defect in *shibire* mutant embryos, when they were exposed to a temperature above the restrictive temperature (Poodry 1990). This indicated that *shibire* mutants cannot survive heat-pulses when developing from eggs to larvae.

The development defect has been identified in several experiments when mutants were treated with a short period of temperature higher than 29°C (Chen *et al.* 1992; Poodry *et al.* 1973; Poodry 1990). However, we expected to see that increasing the temperature also slows down the development in *shibire* mutants,

even at a temperature lower than 29°C. Therefore, we decided to measure the maturing time of *Drosophila melanogaster* to reach the pupae stage and adult stage.

H_0: The maturing time (in days) of wild type *D. melanogaster* is equal to or longer than the mutant as temperature increases.

H_a: The maturing time (in days) of wild type *D. melanogaster* is shorter than the mutant as temperature increases.

Methods

Wild type and mutant *D. melanogaster* larvae were maintained in separate vials. The larvae in these vials were reared in a cornmeal medium. We extracted the larvae from the vials provided by introducing the medium to an 18% sucrose solution brought to room temperature. The sucrose solution separated the larvae from the cornmeal medium. The wild-type and mutant larvae were distributed separately into four replicate vials of five larvae each per treatment (Figure 1). The treatments consisted of subjecting the larvae to temperatures of 17°C, 25°C, and 29°C. Our control treatment was selected to be 25°C, because it is the optimum growth temperature for *D. melanogaster* (Montchamp-moreau 1983). At the start of the experiment we ensured that the volume of medium was same for each of the vials in the treatments to prevent this from potentially influencing *D. melanogaster* growth.

We then placed the treatment replicates into wooden vial racks (Figure 2) and covered the top and sides with cutout cardboard (Figure 3), to prevent the high and varying light intensities of each incubator from influencing *D.*

melanogaster growth. After doing this, we placed the racks at roughly the same time into three different incubators (Figure 4) maintained at our treatment temperatures.

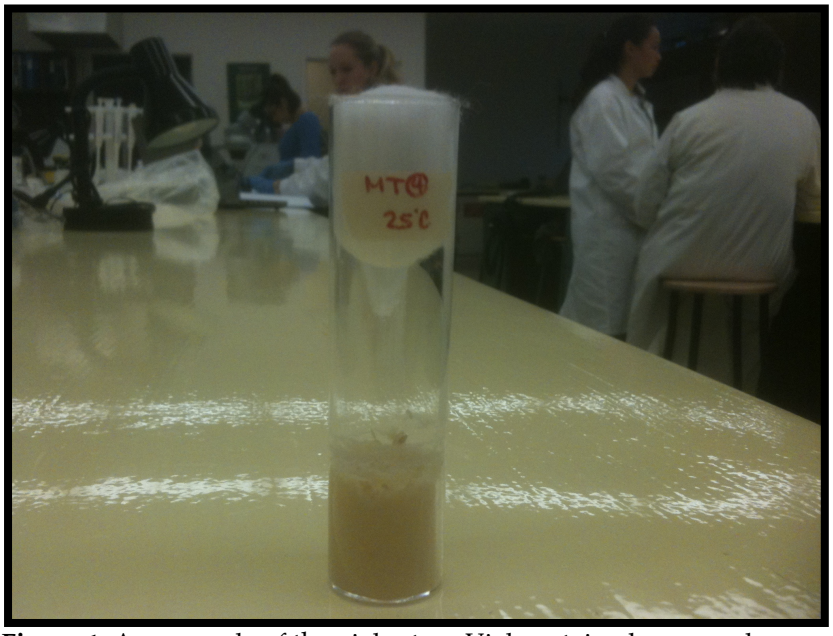

Figure 1. An example of the vial setup. Vial contained cornmeal medium and was covered with a cotton plug.

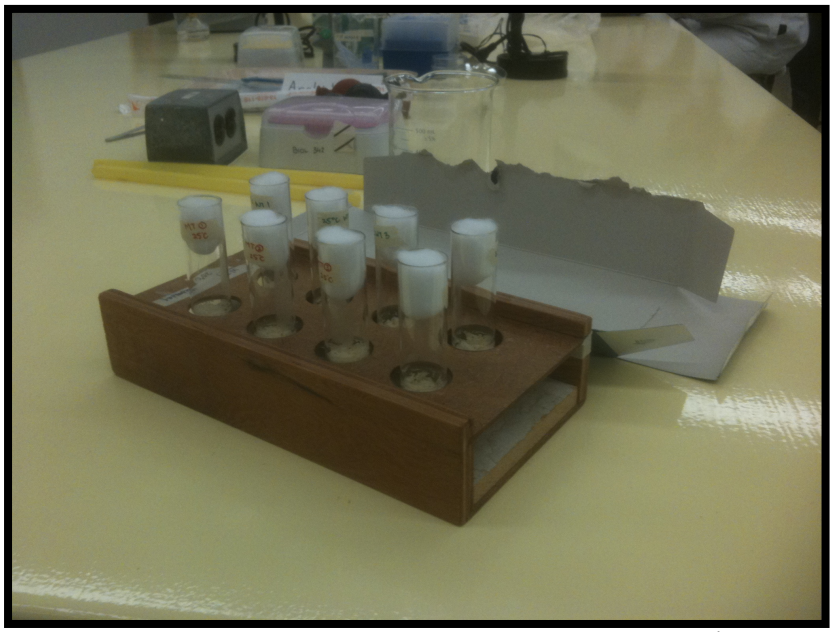

Figure 2. Wooden vial rack for one treatment temperature. The left column contained mutant replicates (4) and the right column contained wild type replicates (4).

Figure 3. Wooden vial racks for one treatment temperature with cardboard cover.

Figure 4. Two of the incubators used in the experiment. Left: 25°C; Right: 29°C.

We observed the flies in each replicate vial of each treatment at random times over 12 school days. The number of larvae, pupae and adults were counted every time. Larvae were white and tiny. They preferred to stay inside the medium where it was difficult for us to see. Pupae were more obvious because

they were distinctly brownish-yellow in colour. Some pupae were present above the medium in the vial. Adults were classified as mature flies, and they were able to fly quickly inside the vial. It was expected that all wild type and mutant larvae that we put in 25°C would mature and become adults. We did not expect mutant larvae in the 29°C incubator to grow at all. At 17°C we expect lower activity and longer maturing time.

After the data were recorded, we calculated the average number of pupae and adult *D. melanogaster* and the 95% confidence intervals for each of the four replicates independently at all the observation times for treatments 17°C, 25°C and 29°C. We subtracted the time at each observation from the time at the start of experiment to give the number of hours spent until the recording was made. We converted the number of hours to number of days and plotted the number of pupae and adults against time for both mutant and wild-type *D. melanogaster*. We compared the maturation time for mutant and wild type *D. melanogaster* and determined if there was a significant difference in the number of pupae and adults between mutant and wild-type at each of the observation time.

Results

Sample Calculation Table

10-Nov 9:30	25°C							
	Wild Type 1	Wild Type 2	Wild Type 3	Wild Type 4	n	Average	Standard Deviation	Confidence Interval
Adult	1	2	1	1	4	1.3	0.5	0.5

Average

$$\bar{x} = \frac{\Sigma x}{n}$$

The average of wild type adult at 25°C = (1+2+1+1) / 4 = 1.3

Standard Deviation

The standard deviation of wild type adult at 25°C:

$$s = \sqrt{\frac{\Sigma (x - \bar{x})^2}{(n-1)}}$$
$$= \sqrt{\frac{((1-1.3)^2 + (2-1.3)^2 + (1-1.3)^2 + (1-1.3)^2)}{(4-1)}}$$
$$= 0.50$$

95% confidence interval

The 95% confidence interval of wild type adult at 25 °C:

$$C.I = \bar{x} \pm 1.96 \frac{s}{\sqrt{n}}$$
$$= 1.3 \pm 1.96 \frac{0.50}{\sqrt{4}}$$
$$= 1.3 \pm 0.49$$
$$= 1.3 \pm 0.5$$

Time

Time from the start of the experiment = (9:30AM, November 10) - (4:00PM, November 2) = 185 hours = 7.73 days

The confidence intervals were calculated based on four replicates for each experimental group. The variation as shown by the confidence interval was within 1.0 in wild type Oregon-R at all observation times. On the other hand, the amount of variation in *shibire* mutants was larger compared to the wild type and the confidence interval varied between 0.0 and 1.5.

There were four replicates and twenty *D. melanogaster* in total for one treatment. An average of the number of *D. melanogaster* were calculated on four replicates and made into six figures. A maximum number of each type of pupae or adults is five. In the 17°C treatment, there was a small increasing trend for the maturing time from larvae to pupae (Figure 5). Only one wild type pupa started

to form on Day 8 and four wild type pupae formed on Day 12. About three mutant pupae formed on Day 12. There were no adults at all from Days 1 to 12 (Figure 6).

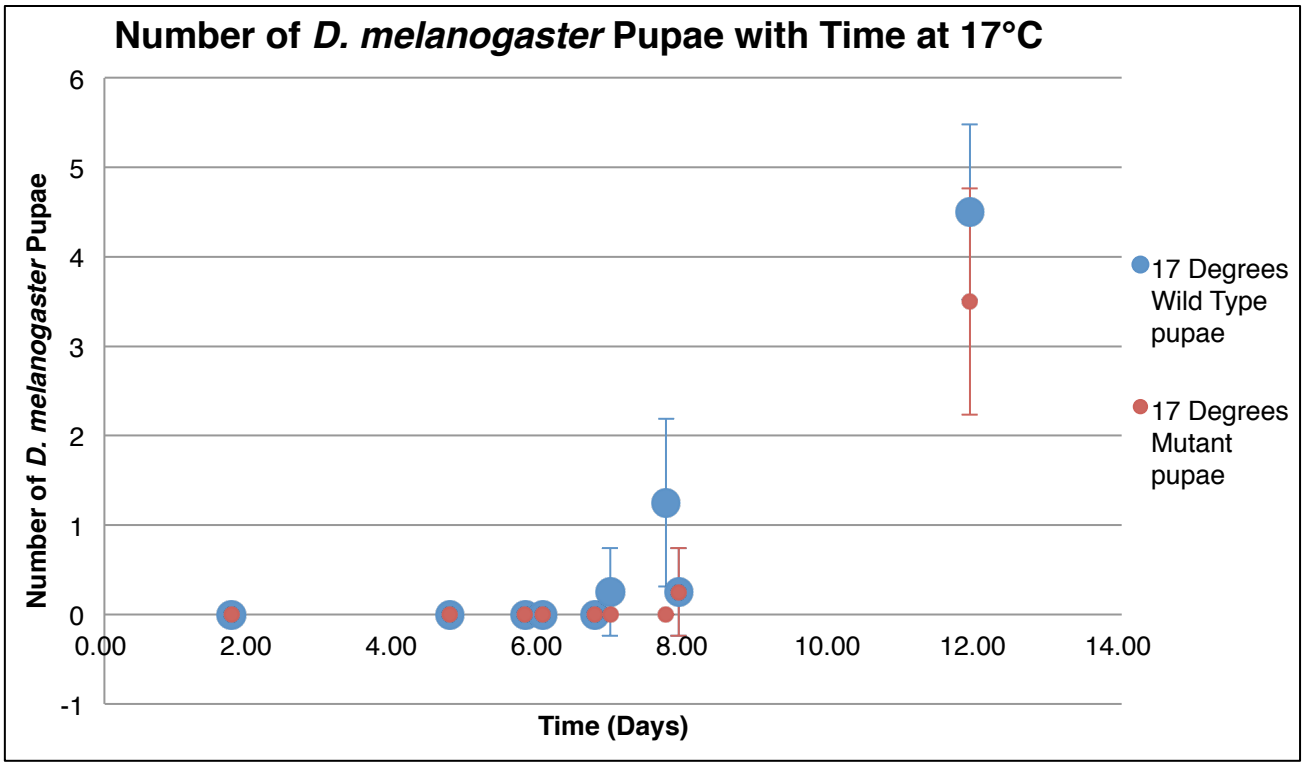

Figure 5. Mutants have a slower maturation time than wild type *D. melanogaster* when subjected to 17°C.

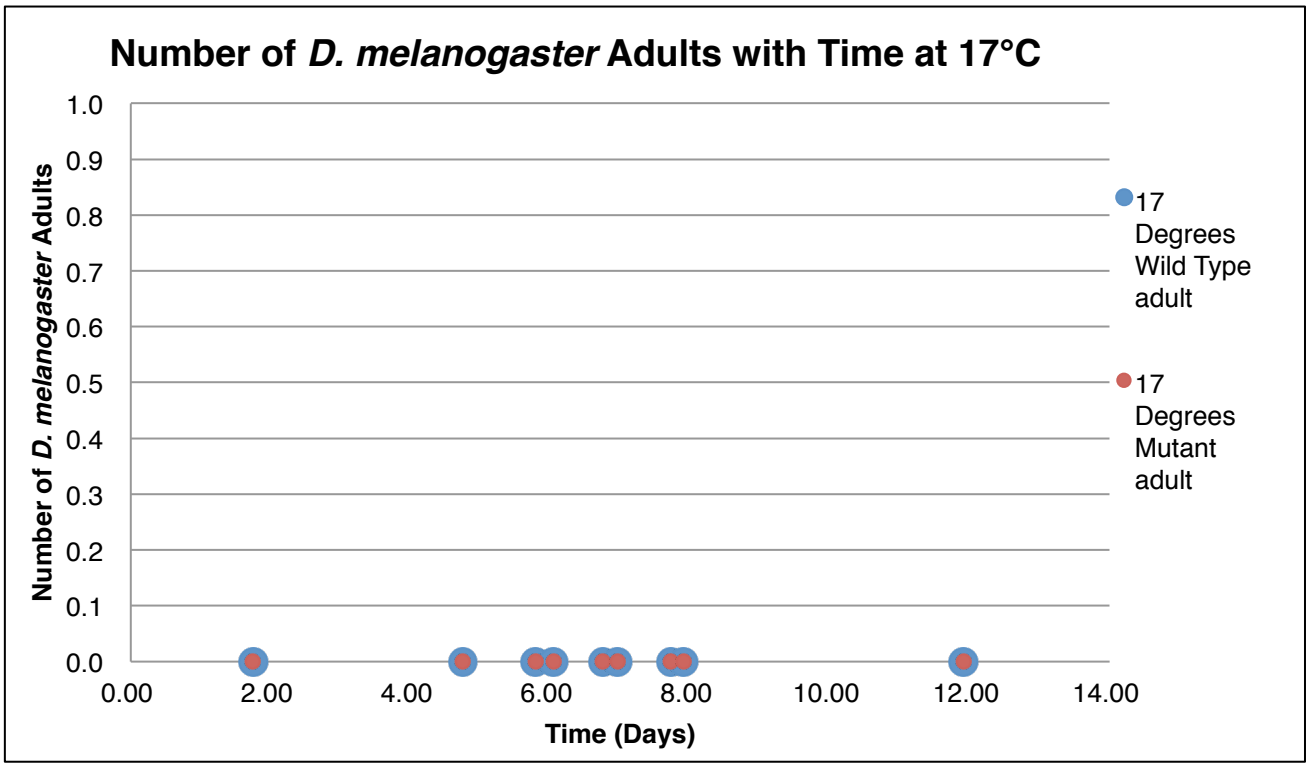

Figure 6. Adults were not observed during the allotted observation period. At 17°C *D. melanogaster* take much longer in order to fully mature compared to optimal temperature.

Figure 7 shows five wild type larvae and three mutant larvae became pupae on Day 5. This shows a faster growth time for larvae at 25°C than at 17°C. Figure 8 shows the number of adult flies; there were two wild type flies and one mutant fly on Day 8. More flies appeared on subsequent days. There was a steeper positive trend, indicating a fast increase in the number of pupae from Day 2 to Day 4 for both mutant and wild type *D. melanogaster* at 29°C (Figure 9). More adult flies were present on the last day at 29°C (Figure 10).

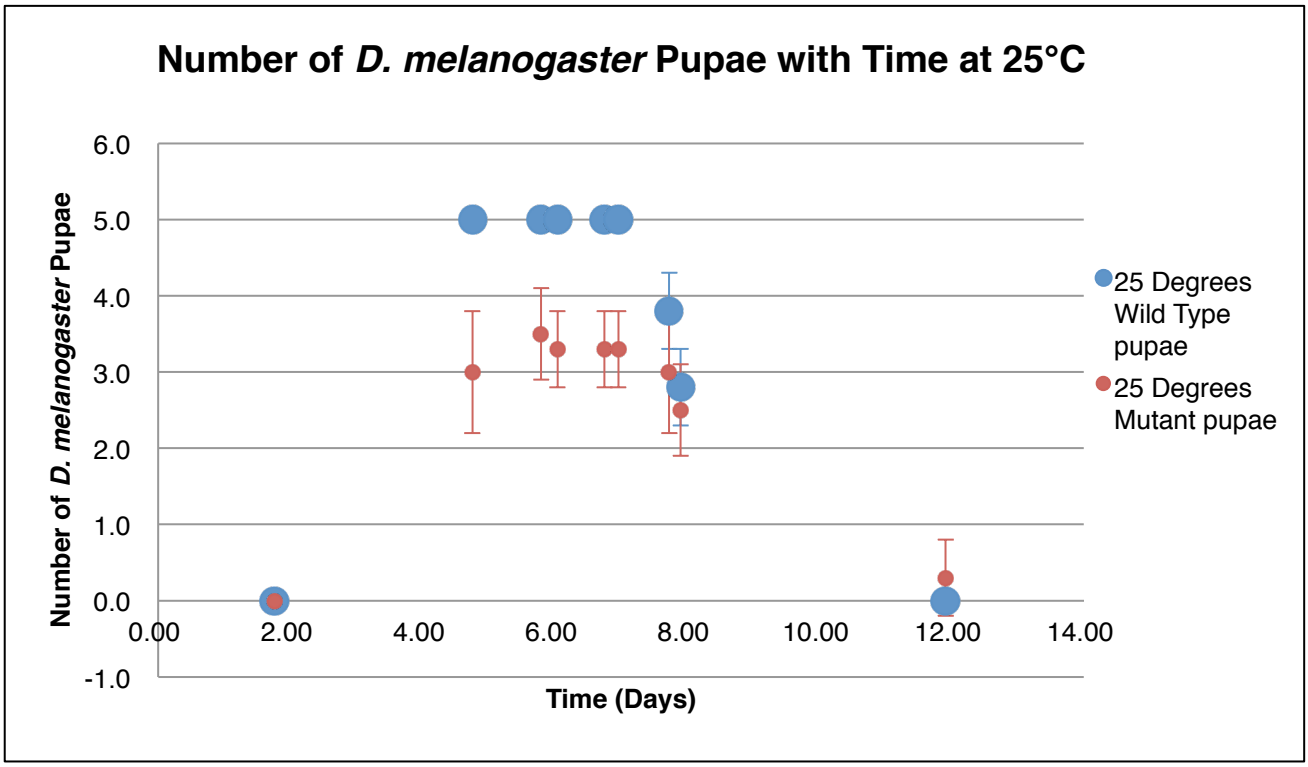

Figure 7. The maturing time of *D. melanogaster* is initially much faster in wild types, but after seven days, the mutant strain caught up and had a very similar time at 25°C.

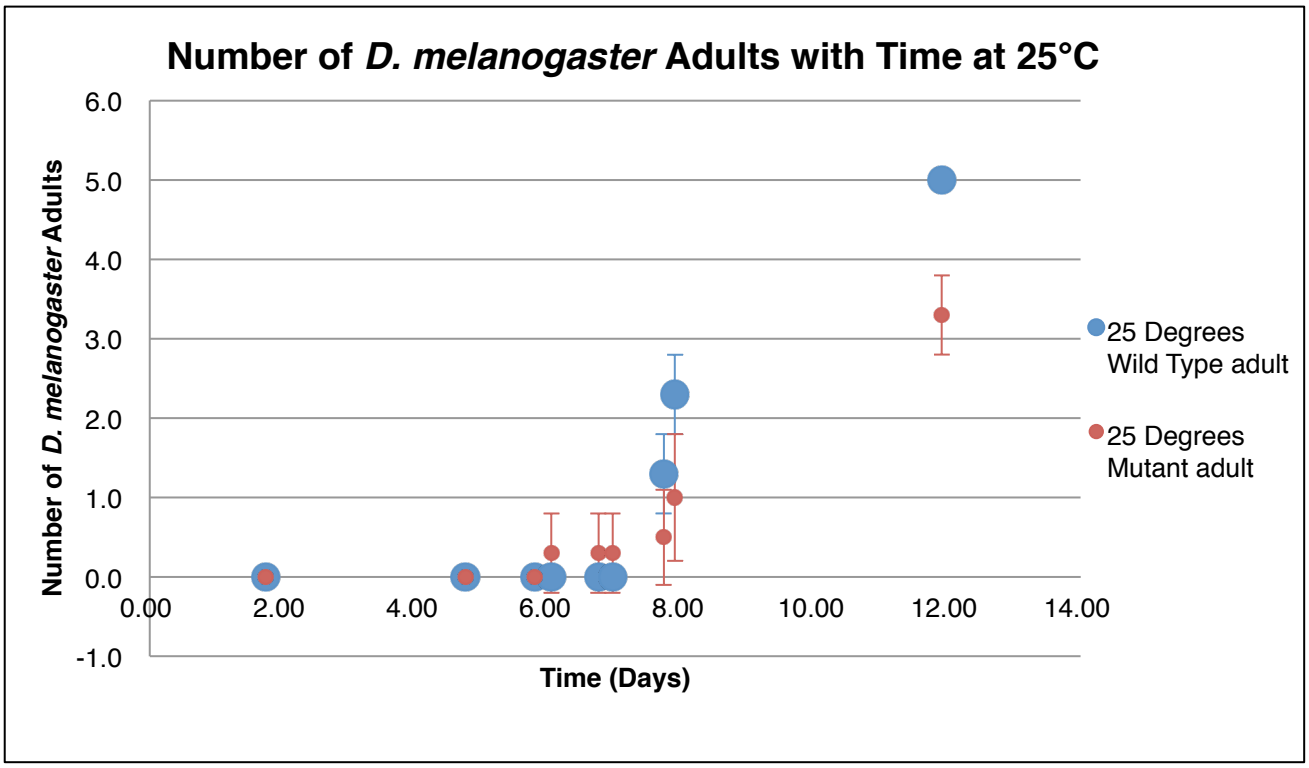

Figure 8. Wild type and mutant strains develop into adults in similar times at 25°C.

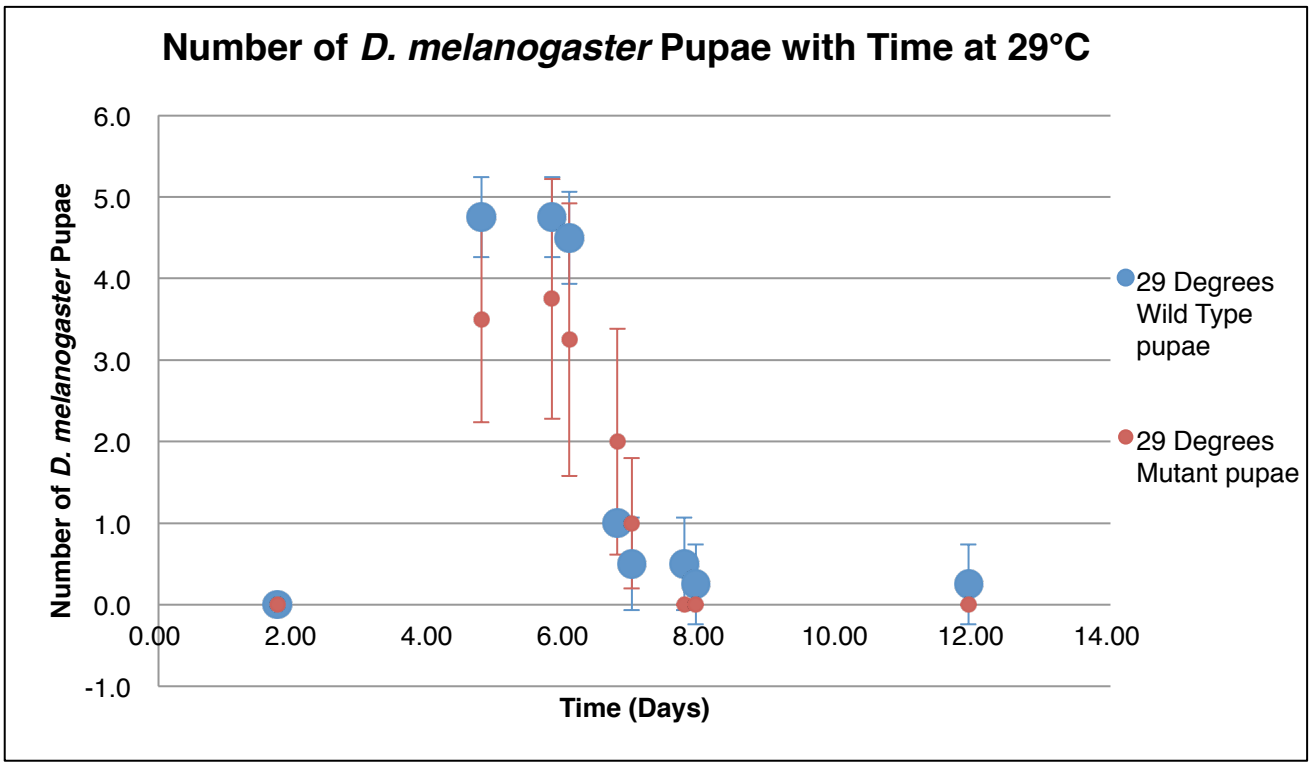

Figure 9. At 29°C mutant and wild type *D. melanogaster* mature into pupae in similar times.

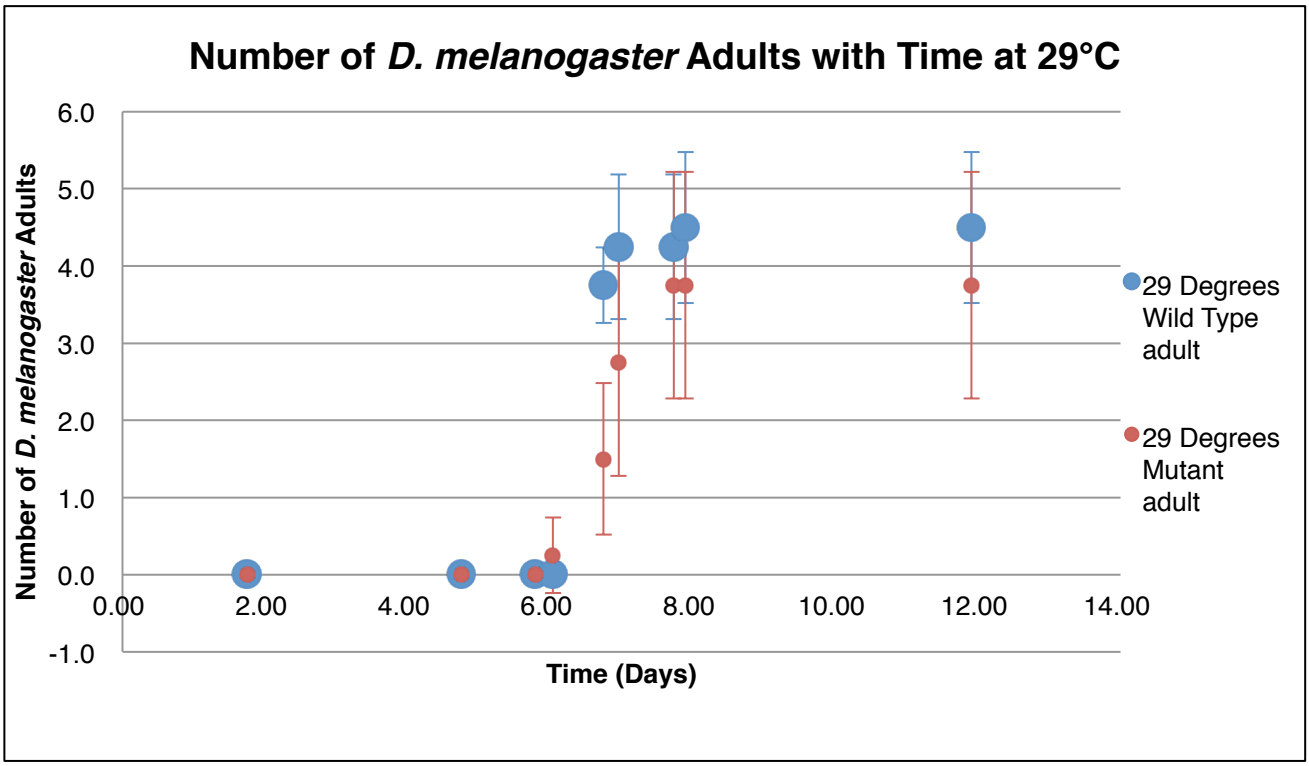

Figure 10. Wild type and mutant strains become adults in similar times when subjected to temperatures of 29°C.

Discussion

We observed some difference in the maturing times for wild type *D. melanogaster* and its mutants. However, based on our statistical analysis, almost all 95% confidence intervals overlap, therefore we fail to reject our null hypothesis. The maturing times that did differ with statistical significance were only observed at 25°C. Moreover, the control treatment at 25°C was unreliable due to incubator malfunctioning. It was brought to our attention that the temperature in this incubator fluctuated between 25°C and 32°C at certain times during a four day period. This temperature fluctuation has likely skewed the results substantially. As a result, we also do not have sufficient evidence to support our alternate hypothesis.

The temperature sensitive mutant *shibire* had 3.3±0.5 individuals that

turned into adults at 25°C and 3.8±1.5 individuals that turned into adults at 29°C. The number of mutant *shibire* matured to adults, however, was expected to be zero in the 29°C mutants and we expected a low survival rate in the 25°C mutants, because the temperature induces lethal development defects as the larvae mature into adults (Poodry 1990). Also, the adults were expected to become paralysed when exposed to temperature higher than the restrictive temperature at 29°C for five minutes (Poodry *et al.* 1973). The surviving mutant adults at 29°C were, however, actively moving without experiencing any sign of paralysis. This observation suggested that the temperature-sensitive phenotype was not expressed in these mutant *shibire* adults. According to our experimental set up, we expected to see *shibire* being expressed in all mutants of *D. melanogaster* adults because we assumed we were provided with first generation *shibire* larvae.

In our experiment, we mated a strain of female Cha-Gal4-shi to a strain of male UAS-shi, with both parental strains not expressing the *shibire* gene. It gives rise to Cha-Gal4/UAS-shi first generation, in which the GAL4 was transcribed and the gene *shibire* was expressed because of the binding of Gal4 to UAS (Duffy 2002). This is however not the case because, unexpectedly, the first generation of mutant flies reproduced to give a second generation, with only 9/16 of which are expected to contain both GAL4 and UAS-shi. The region containing GAL4 and UAS-shi needs both to be present for the *shibire* to be expressed (Kitamoto 2001). The larvae we used in this experiment were very likely to be second, third generation or beyond, which explained why the mutant adults did not show the temperature sensitive phenotypes because they only carried either the GAL4 or UAS-shi gene.

According to Kosaka and Ikeda (1983), both the mutant and wild type *D. melanogaster* behaved the same at 19°C. No perturbation on neuronal activities occurred for the mutant at this temperature (Kosaka and Ikeda 1983). *D. melanogaster* is ectothermic and grows slower but matures at a larger body size in colder environments (Angilletta Jr. *et al.* 2004). This information supports our results, which was that the growth rate of *D. melanogaster* being slower at 17°C and quicker at increasing temperatures.

Kitamoto (2001) showed that an average of 75% of the mutant *D. melanogaster* turned into adults inside a 22°C incubator. In our experiment, roughly 65% of the mutant larvae turned into adult flies at 25°C. There is a 10% difference because the 25°C incubator that the larvae were kept in had temperature fluctuations as mentioned previously. Some of our mutant larvae died at the high temperatures caused by the malfunction because they were temperature sensitive (Wang *et al.* 2004). A shift of temperature could cause rapid perturbation on neuronal activities (Kitamoto 2001). It is found that 27-29°C is the restrictive temperature range for mutant *D. melanogaster* carrying the gene *shibire*, but those that carried the gene but did not express this mutated gene could survive in this high temperature (Kitamoto 2001). Kitamoto (2001) showed that 15.3% of his mutant larvae became adults in a 30°C incubator. It was the same for our experiment, as 75% of our mutant *D. melanogaster* eventually became adult flies because their *shibire* genes were not expressed. The percentage was much higher because our larvae were second or third generation (Duffy 2002).

There are several sources of error and variation that may have impacted the results we obtained. An important source of error is our observation periods.

The vials placed in the 25°C and 29°C incubators had to be taken out in order to be observed. This necessitated that they be exposed to room temperature for roughly ten to 15 minutes on every observation day. Similarly, as mentioned previously, the 25°C incubator had malfunctioned. Thus, temperature fluctuation is greatly magnified for the 25°C treatment.

Another crucial source of error that we witnessed affected the 17°C treatment. We noticed that someone visiting that incubator between our observation periods had left the cardboard cover for the vials open. This incident caused ramifications in our experiment because larvae and pupae are light sensitive and a high light intensity will retard their development (Bruins *et al.* 1991).

Conclusion

Based on our results, we fail to reject our null hypothesis, which states that the maturing time (in days) of wild type *D. melanogaster* is longer or equal to the mutant as temperature increases. Since we did not have enough mutant *D. melanogaster* carrying Cha-Gal 4/ UAS-shi for our experiment to start with, we do not have support for our alternate hypothesis, which states that the maturing time for the mutant was longer than that of the wild type.

Acknowledgments

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