Survival in Oregon-R and *shibire* mutant of *Drosophila melanogaster* in response to Temperature

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ABSTRACT

Using wild-type Oregon-R and *shibire* mutant *Drosophila melanogaster* as the organism of study, we were interested in investigating how different temperatures would affect growth, as measured in female thorax length, and the survival rate of the two different *D. melanogaster* subtypes. These two populations show phenotypic variation when exposed to high lethal temperatures, especially the *shibire* mutant at 29ºC, where paralysis or death may occur. To study their growth and survival rate, we initially started with larvae of the two populations and placed them in three different temperature treatments of 17ºC, 25ºC, and 29ºC. We analyzed our two main factors of interest shortly after the larvae metamorphosed into adults. Our findings showed that there was no significant difference in growth when *D. melanogaster* were exposed to different temperatures. We found that there was a significant difference between the treatments of the mutant population in terms of their survival rate, but no other trends were found that related to our hypotheses. Our results do not support our hypotheses which is that increased temperature will decrease female thorax length and decrease survival rate. Two major sources of error that may have contributed to our results were the fluctuation of temperature in one of our incubators and the genetics of the mutant.

INTRODUCTION

The relationships among temperature, adult body size, and organism growth are quite fascinating in *Drosophila melanogaster.* Several *D.melanogaster* species show geographic variation in body size where generally larger flies are found at higher altitudes than the equator (Neat *et al.*1995). This type of cline has also been reported in the honey bee, *Apis mellifera* (Alpatov 1929) and in housefly, *Musca domestica* (Bryant 1977).

Ectotherms produce a larger adult at lower experimental temperatures (Atkinson 1994). Traits such as increased thorax length and wing area have been demonstrated in *D. melanogaster* if they are exposed to low temperatures (Partridge *et al*. 1994). Similar traits have also been reported in endothermic animals that follow Bergmann's rule; with larger individuals found at higher latitudes and altitudes (Mayr 1963).

Stressful environments to any organism result in a wide variation of their development and survival rate. David *et al.* (1983) suggested that temperatures of 12ºC and 31ºC are very stressful environments to *D. melanogaster*. Cooler temperatures are advantageous to *D .melanogaster* but approaching the near-lethal lower temperature will cause a reduction in their size and an increase in mortality (David and Clavel 1967). At near-lethal higher temperatures, there is more phenotypic variation of morphological characters such as reduction in thorax length hence reduction in their size and an increase in mortality (Imasheva *et al.* 1998).

A lot of research has been done on mutations in *Drosophila melanogaster* affecting a variety of behavioural characteristics such as response to light (Pak *et al.* 1969; Hotta and Benzer, 1969) and temperature (Griglliatti *et al.* 1973). In our particular experiment, our mutant of interest was the *shibire* mutant which is a temperature sensitive mutant that experiences paralysis at very stressful temperatures (non-permissive); about 29ºC, and is reversed on lowering to negative stress temperatures (permissive); 22ºC (Tweedie *et al*. 2009; Griglliatti *et al.* 1973). Paralysis continues until death after 12-14 hours of exposure to the non-permissive temperature (Griglliatti *et al.* 1973). At any time before this, *D. melanogaster* could recover mobility if temperatures are brought back to 22ºC (Griglliatti *et al.* 1973).

Since we know that 31ºC is lethal for the wild-type Oregon-R flies and 29ºC is lethal for the *shibire* mutants, our first aim of the study was to determine the effect of high non-permissive temperatures on the survival rate of both Oregon-R and *shibire* mutant of *Drosophila melanogaster* when examining the adult stage that developed from well-fed larvae stage. Our first two hypotheses are:

H_{a1}: An increase in temperature would lead to a decreased survival rate of wild-type Oregon-R or *shibire* mutant *Drosophila melanogaster.*

H_{01}: An increase in temperature would lead to increased or no change in survival rate of wild-type Oregon-R or *shibire* mutant *Drosophila melanogaster.*

The second aim of the study was to determine the effects of increased temperature on size of Oregon-R and *shibire* mutant of female *Drosophila melanogaster* at their adult stage that developed from well fed larvae stage. Our third and fourth sets of hypotheses are;

H_{a2}: An increase in temperature would lead to decrease in thorax length of female wild-type Oregon-R or *shibire* mutant *Drosophila melanogaster* populations.

H_{02}: An increase in temperature would lead to increase or no change in thorax length of female wild-type Oregon-R or *shibire* mutant *Drosophila melanogaster* populations.

We chose female flies because they live longer than males at any rearing temperature (Alpatov and Pearl, 1929) and also females are larger than males so they would be easier to measure. The trend of having larger adult *D. melanogaster* from their larvae stage increases with decrease in developmental temperature (Partridge *et al.* 1994) but at extremely low temperatures, growth of *D. melanogaster* is limited (Robinson and Partridge, 2001).

METHODS

Details of Procedure

In order to start the experiment, larvae of *D. melanogaster* were collected from large vials containing flies at all stages of growth, from egg to adult flies. We used CO_2 gas to temporarily paralyze any active adults and transferred them in to the morgue, since the adults were not of interest. Fly larvae tend to be burrowed in the agar, therefore a spatula was used to scoop them out of its burrow together with the least amount of agar. The larvae were then transferred into a petri dish filled with 18% sucrose solution, where the agar could be separated from the larvae.

After separation, the larvae were picked up via a bacterial loop and transferred into its designated vial (Fig. 1). The vials were then covered by cotton to prevent any organisms from escaping. This process was done for two populations of *D. melanogaster*, wild-type Oregon-R, and *shibire* mutant. Each of these two populations was separated into three treatments and the treatments were dependent on the availability of incubators. The three temperature treatments chosen were 17˚C, 25˚C, and 29˚C. Within these three treatments, we had 4 replicates in each with 5 samples of larvae. In total, we collected 60 samples for each population for a total of 120 *D. melanogaster* larvae.

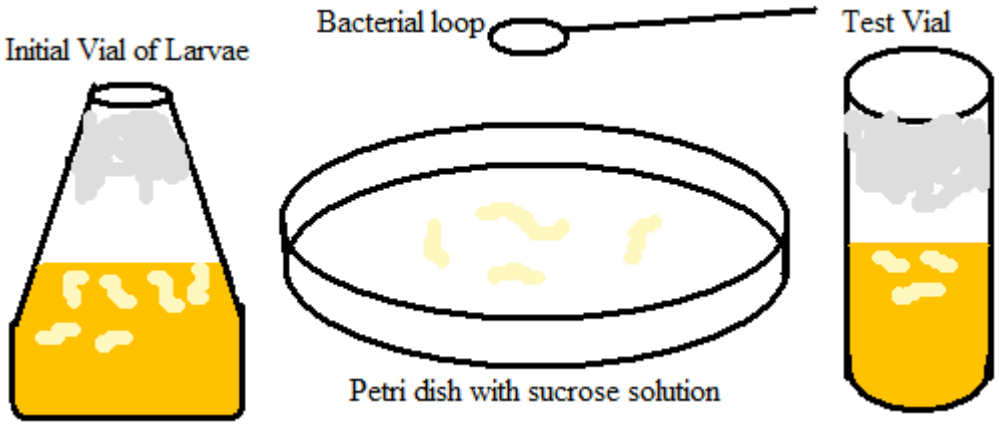

Figure 1. The equipment needed to collect *D. melanogaster* larvae were: the starting vial of larvae, petri dish filled with 18% sucrose solution, a bacterial loop, and test vials with agar where larvae were kept for testing.

Once the organisms in their vials were set up in their respective incubators, observations were taken every 5th and 7th day after being placed at their respective temperatures. The number of larva, pupa, and adults was noted, as well as dead organisms at their particular stage of metamorphosis. If there were any adult flies present, they were taken out of their vials by first anaesthetizing them with CO_2 gas. We then placed them on a white surface so they could easily be seen through the dissecting microscope. The microscope was fitted with an ocular camera and

objective set to 10X magnification. Pictures were taken with the ocular camera that was compatible with software called DinoXcope.

The pictures of female *D. melanogaster* were later analyzed through another program called ImageJ, an image processing and analysis program. The dimensions of the actual view were measured from a control picture using a transparent ruler. These dimensions were then applied to the pictures of the flies on the computer. The actual dimensions were 8mm by 6mm; using a key function of interest in ImageJ, we were able to set the digital pictures to be scaled to the actual dimensions. Parameters were set to these values in the Set Scale menu: Distance in pixels – 354.99, Known distance – 2.22, Pixel aspect ratio – 1, and Unit of length – mm. A line extending from the posterior end of the thorax of *D. melanogaster* to the anterior was drawn using the program and its actual length was determined.

To calculate the survival rate, we took the ratio of flies that developed into adults to the total number of larva we put in each vial, which were 5. We calculated 95% confidence intervals for thorax length and survival rate for both populations to determine statistical differences between temperature treatments. Any overlapping of the intervals would result in no significant difference between the temperature treatments.

Some key factors that were controlled and maintained throughout the experiment were the storage of the vials that contained *D. melanogaster* and the time of exposure to room temperature during analysis. Because the incubators and cooler had varying intensities of light, vials were covered by a Styrofoam box to ensure no light enters the vials in all of our treatments. Light may contribute to an increase in temperature in the vials and disrupt the flies' behaviour. Additionally, the response to light is one of the behavioral characteristics researched on in regards to mutation (Pak *et al.* 1969; Hotta and Benzer, 1969). To ensure the samples did not

undergo temperature shock when being introduced to room temperature, we used minimal time to observe the organisms and to take photographs.

RESULTS

The effect of increased temperature in decreasing fly size was not confirmed: the wild-type population of female *D. melanogaster* had all treatments with overlapping confidence intervals (17˚C mean = 1.97 ± 0.12 mm (95%), n = 4, 25˚C mean = 1.97 ± 0.44 mm (95%), n = 2, 29˚C mean = 2.07 ± 0.36 mm (95%), n = 3), thus showing no significant difference (Fig.2). The same results occurred in the female mutant population, where all treatments had all confidence intervals overlapping (17˚C mean = 2.36 ± 0.20 mm (95%), n = 4, 25˚C mean = 1.91 ± 0.32 mm (95%), n = 2, 29˚C mean = 2.38 ± 0.32 mm (95%), n = 2), and had no significant differences throughout all treatments (Fig. 3). The average thorax length for the wild-type female population was relatively similar throughout all treatments. For the mutant population, female thorax length in the temperature treatment of 25˚C average was lower than other treatments, but not significantly. We were unable to identify any trends in varying temperature on thorax length.

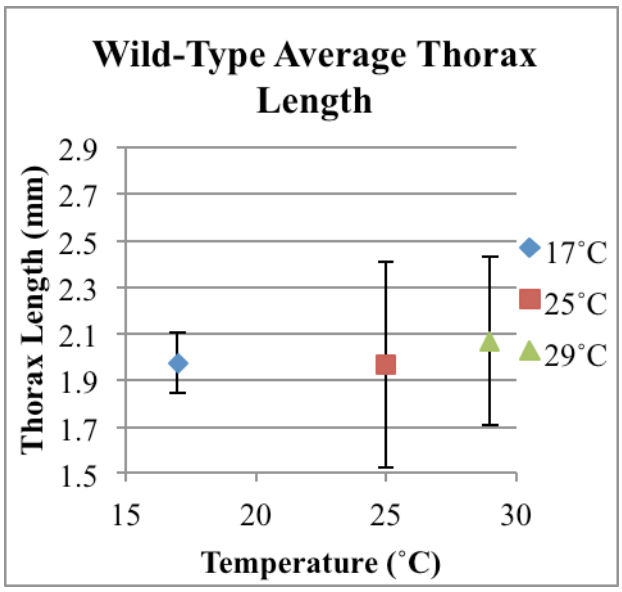

Figure 2. 95% confidence intervals of the average female thorax length of the replicates in the wild-type population of *D. melanogaster* within each treatment. The bars represent CIs of the means of three temperature treatment levels: 17˚C, 25˚C, and 29˚C (from left to right). All confidence intervals overlap.

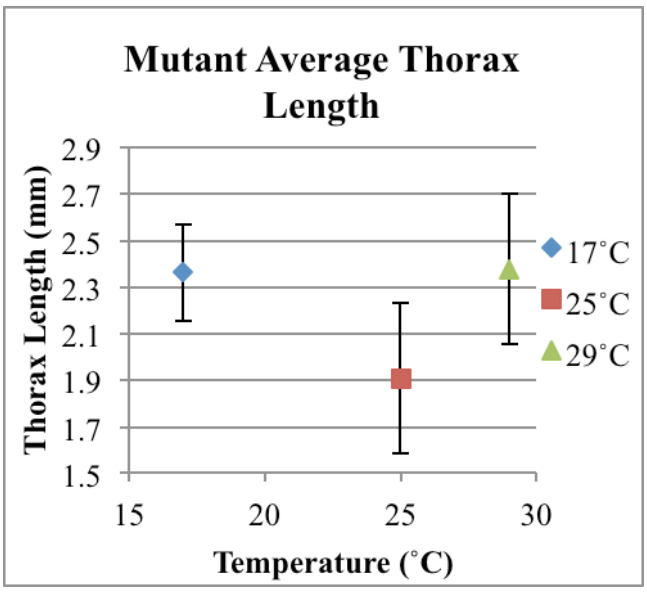

Figure 3. 95% confidence intervals of the average female thorax length of the replicates in the mutant population of *D. melanogaster* within each treatment. The bars represent CIs of the means of three temperature treatment levels: 17˚C, 25˚C, and 29˚C (from left to right). All confidence intervals overlap.

With increase in temperature, there was a significant difference in survival rate when looking at the results of the mutant population, but not the wild-type population. The wild-type population had all treatments with overlapping confidence intervals (17˚C mean = 0.75 ± 0.10 (95%), n = 4, 25˚C mean = 0.55 ± 0.33 (95%), n = 4, 29˚C mean = 0.4 ± 0.28 (95%), n = 4), thus showing no significant difference in the results (Fig. 4). However, in the mutant population, there is a significant difference between the 17˚C and 25˚C treatment, but not with the 29˚C treatment (Fig. 5). With these results (17˚C mean = 0.50 ± 0.25 (95%), n = 4, 25˚C mean = 0.10 ± 0.11 (95%), n = 4, 29˚C mean = 0.25 ± 0.18 (95%), n = 4), there is no specific trend due to the survival rate of the mutant *D. melanogaster* in 29˚C treatment being at a higher temperature and not showing significant differences to other treatments.

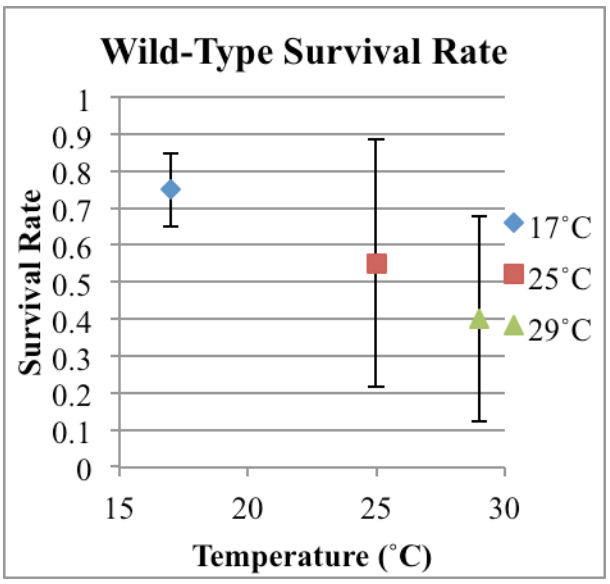

Figure 4. 95% confidence intervals of the average survival rate of the replicates in the wild-type population of *D. melanogaster* within each treatment. The bars represent CIs of the means of three temperature treatment levels: 17˚C, 25˚C, and 29˚C (from left to right). All confidence intervals overlap.

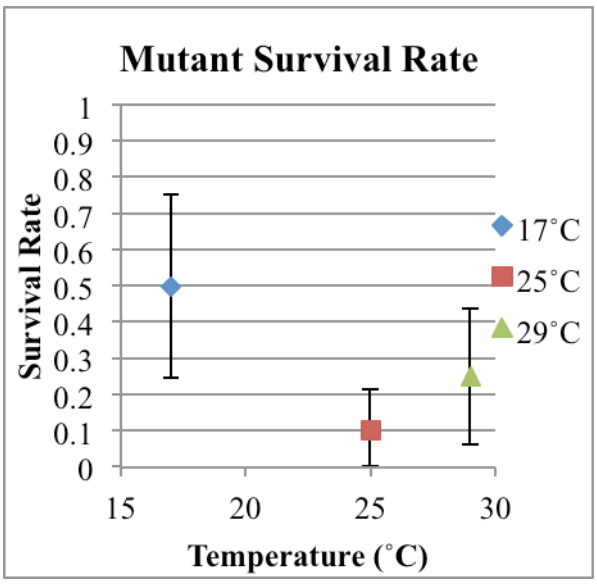

Figure 5. 95% confidence intervals of the average survival rate of the replicates in the mutant population of *D. melanogaster* within each treatment. The bars represent CIs of the means of three temperature treatment levels: 17˚C, 25˚C, and 29˚C (from left to right). Confidence intervals of temperature treatments, 17˚C and 25˚C, do not overlap

DISCUSSION

The pattern of having a low survival rate at higher temperatures was not fully supported by our study. However, the team did observe a general trend in the survival rate of Oregon-R flies decreasing at 25˚C when compared to 17˚C and much lower at 29˚C when compared to the other two. This trend could not support itself in our study because the survival rates of the *D. melanogaster* in the three temperature treatments were not significantly different from each other because the confidence intervals overlap each other. With this finding, we failed to reject our first null hypothesis (H_{01}), which states that an increase in the temperature would lead to an increase or no change in survival rate of wild type Oregon-R *Drosophila melanogaster*. This is not supported by other researchers. Imasheva *et al.* (1998); Nunney and Cheung (1997) suggest

from their studies that the survival rate of *D. melanogaster* species decreases as the temperature increases. This could be due to disturbances in cellular growth (small cells at higher temperature) and delayed maturation (Angilletta *et al*. 2004).

The survival rate of the mutants is interesting since we see a significant difference as temperature increases. Data show that the mutant *shibire* has a good survival rate at 17˚C but then a drastic decrease in the survival rate of the flies at 25˚C and then a considerable good increase at 29˚C; this was also unexpected. According to Griglliatti *et al.* (1973), the mutants would show a decline in survival rate at 29˚C because they would be paralyzed and die if exposed in this temperature for a long period of time; however, this was shown at 25˚C instead of 29˚C. This is because of the depletion of vesicles in the synaptic terminal of the neuron. It is possiblly due to the inability of endocytic vesicles to be separated from parent membranes, neurotransmitters would not be passed on to other neurons, and this would lead to death after prolonged exposure (McMahon 2004). When comparing the survival rate of the *shibire* mutant in the three temperature treatments, we can see that there is a significant difference only between the 17˚C and the 25˚C treatments, but there is no significant difference between 25˚C and 29˚C or 17˚C and 29˚C. We failed to reject our second null hypothesis (H_{02}) that states that an increase in the temperature would lead to increase or no change in survival rate of *Drosophila melanogaster* for the shibire mutant.

The general cline of larger flies at lower temperature was not supported by our study. The wild-type Oregon-R flies showed an almost flat trend with no significant difference in thorax length at different temperatures because the confidence intervals were all overlapping with each other. According to Imasheva *et al.* (1998) study findings, in ideal conditions, the temperature at which they observed the largest flies was around 21˚C, and at both stressful temperatures, the

thorax length was the smallest. With this, we would consider to have flies treated at 17˚C to have really close thorax length to flies treated at 25˚C, while flies treated at 29˚C to have the shortest thorax length. From our findings, we fail to reject the third null hypothesis (H_{03}) that states that an increase in temperature would lead to increase or no change in the thorax length of female *Drosophila melanogaster* for wild-type Oregon-R.

This trend was again portrayed by the *shibire* mutants. Our findings show a sharp decline of mean thorax length at 25ºC when 17ºC and 29ºC are of pretty much equal length. Having flies hatch at 29ºC was a surprise to begin with since there was expected to be no hatching of adult flies from eggs and pupae of *shibire* mutants at 29ºC due to the fact this temperature is fatal to the organism (Grigliatti *et al.* 1973). With these findings we failed to reject the fourth null hypothesis (H_{04}) that states that an increase in temperature would lead to increase or no change in the thorax length of female *Drosophila melanogaster* for shibire mutant. There could be only one major error that could lead to *shibire* mutant *D. melanogaster* providing results like this, as further discussed below. We would expect the adult flies to have a larger thorax length at lower temperatures rather than at higher because the larvae take more time to develop into adults (Imasheva *et al*. 1998) and also they convert food better into adult body size at low temperature (Robinson and Partridge, 2001).

One of the main complications in this experiment was the malfunction of the incubator that was set to 25˚C. Instead of the actual reading, the temperature was actually 32˚C, 7˚C hotter than normal. This mistake was corrected on the 15th day of incubation, and the exact time and day of when this malfunction occurred is unknown; however, we may safely assume the total time of incubation of 32˚C was over 12-14 hours, as *shibire* mutant population was unable to tolerate the non-permissive temperature and resulted in a high mortality rate (Griglliatti *et al.*

1973). If the temperature treatment of 25˚C was changed to 32˚C, we would see a negative correlation with increased temperature and lower survival rate of the shibire mutant population. For the wild-type population, we do not see this trend since the incubator was set back to the temperature of interest, 25˚C. Wild-type Oregon-R *D. melanogaster* were most likely to be affected by the lethal temperature of 32˚C as stated by David *et al.* (1983), but presumably not as much as the *shibire* mutant, as we do not see a drastic drop in survival rate.

In addition, according to Griglliatti *et al.* (1973), all *shibire* mutants would die when exposed to lethal temperatures of 29˚C or higher for more than 12-14 hours. This was not the case as we have two mutant survivors out of twenty in the 25˚C treatment, with exposure to 32˚C for a prolonged period of time, and five mutant survivors out of twenty in the 29˚C treatment. This could be the case because we retrieved the adult flies before they were exposed to the lethal temperature of 32˚C. The other organisms that did not metamorphose in time before the malfunction of the incubator were killed. Upon further investigation, an alternative reason to this dilemma was that the *shibire* mutant *D. melanogaster* were of the wrong genotype and displayed the wild-type phenotype. In order to display the phenotype of the *shibire* mutant, one must have dominant alleles of the Gal4 gene, or the A gene for simplicity, and the UAS or upstream activation sequence gene, or the B gene. Having the dominant alleles of both of these genes is vital to activating gene transcription of the *shibire* trait. If the mutant does not possess at least one dominant allele of the two genes, the phenotype of the *shibire* mutation will not show and the fly will appear as wild-type. When we were given the *shibire* mutants in the initial vial, the generation in which the *D. melanogaster* were in was crossed by mutants of AaBb and AaBb genotype, when initially; they were supposed to be crossed by flies with AAbb and aaBB genotype in order to ensure all offspring express the mutant phenotype. Once mutants with AaBb

and AaBb genotype mate, the chances of having the offspring display the mutated phenotype is 9/16, with a 7/16 chance of appearing wild-type, due to the absence of the two dominant alleles. This correlates with our data as the flies in the 29˚C and 25˚C or 32˚C treatment that managed to metamorphose to the adult stage are the offspring that appeared to be wild type, while the majority of the *D. melanogaster* that did not survive or reach the adult stage were ones that expressed the *shibire* genotype.

Other errors that could have arisen were from our methods that may have affected our results of the survival rate and the female thorax length of the *D. melanogaster*. Since we predetermined survival rate to be a ratio of adult flies to the total number of initial larva put in each replicate, the problem lies with the number of *D. melanogaster* organisms in each replicate. From our observations, some vials had a total of six or more pupae present, which is more than what was expected. During *D. melanogaster* larvae collection, there were some larvae that were significantly smaller than the larger sized larvae that we collected. Because of their size, they may have been unseen when being initially transferred into the sucrose solution and again transferred to the vial via the bacterial loop. This increased the initial number of larvae, thus falsely increasing the chances of more larva surviving and the survival rate. Determining the thorax length using ImageJ was subjective in terms of finding its actual length. All photos had the adult fly positioned differently, such as on its side, posterior, or anterior. The thorax length may have been altered by the way the organism curls in the different positions.

To further improve this experiment, many factors would need to be changed to ensure the turnout of accurate results. We suggest to have temperature treatments set at lethal temperatures and a temperature where thorax length to be the longest according to literature. We may be able to see a trend in size using a wider range of temperatures iAlso, a better method used to paralyze

or kill the *D. melanogaster* adults during analysis would be beneficial as it would be easier to manoeuvre them to be positioned in the same orientation for all pictures. Because we use CO_2 gas for paralysis, there was a limited amount of time before the flies regained mobility and we could not get a picture that accurately represented their thorax length. Time was also an issue in completing this experiment. Because Imasheva *et al.* (1998) studied that *D. melanogaster* larvae take more time to develop and grow, there were still some living pupae when we terminated data collection due to lack of time. To gather all data possible, we suggest initiating the experiment as early as possible if time is a constraint, to again gather more accurate results.

CONCLUSION

In summary, the results of our work are not in agreement with the view that exposure to higher temperature can have a substantial negative effect on survival rate and female thorax length of wild-type Oregon-R and *shibire* mutant *Drosophila melanogaster.*

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