The effects of temperature on the time to maturation of *Drosophila melanogaster*

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ABSTRACT

Insects typically grow faster, but are smaller in size when raised at warmer temperatures, while at cooler temperatures, their growth rate is slower but produces a larger body size. The aim of this study is to examine how long it takes for *Drosophila melanogaster* to mature in a range of temperatures. This study was conducted by raising *D. melanogaster* from the larval stage to adulthood at different temperatures. Four replicates each containing ten larvae were placed within each of the three temperature treatments: 12°, 20° and 29° C. The number of adults and the day they emerged were recorded. Although it was not the main focus of the experiment, the lengths of the emerged adults were also recorded due to the correlation between temperature and the size of the *D. melanogaster.* The average proportions of *D. melanogaster* that matured into adults were 0 for the 12° C treatment, 1 for the 20° C treatment and 0.8+/-0.216 in the 29° C treatment after thirteen days. The 20° C treatment's average body size of 2.607+/-0.00765 mm was found to be significantly different from the 2.547+/-0.0299 mm average body length in the 29° C treatment. With these results, we determined that there is a negative correlation between the temperature and the length of time to maturation, and a positive correlation between the growth time and body size.

INTRODUCTION

There is strong scientific support for a positive correlation between temperature increases and increased rates of development for insects (Angilletta *et. al*. 2004, Davidson 1944). The relationship between body-size and development rate in ectoderms is also well documented – insects raised in cold temperatures tend to be larger and develop slowly, whereas insects raised in high temperatures have a tendency to be smaller but develop more quickly (Angilletta *et al.* 2004). Understanding the temperature-dependent maturation of insects is beneficial knowledge for designing experiments in which large batches of insects are raised at a time.

This study focuses on the temperature-dependence of maturation for *Drosophila melanogaster*. *D. melanogaster* is a homometabolus insect, as characterized by the fact that its larval stage and adult stage are separated by a distinct pupae stage where metamorphosis occurs.

Larva develop through 3 distinct stages, or instars, each larger than the last; at the end of its third instar, the larva crawls from its food medium and pupates (Ransom 1982). At 29°C, puparation usually occurs approximately 4 days after larva has hatched, and eclosion (emergence of adult) generally occurs at around 8 days (Roberts 1998).

Previous studies on temperature-dependant development for *D. melanogaster* suggest a positive relationship between temperature and development (Partridge *et al.* 1994, AL-Saffar *et al.* 1995). AL-Saffar *et al.* found that development time steadily declined for *D. melanogaster* as temperature was raised from 15°C to 30°C (1995). Maximum development rates have historically been observed between 30°C and 28°C (Davidson 1944, Ashburner and Thompson 1978).

We examined the effect of the three different temperatures, 12°C, 20°C, and 29°C, on the time required for *D. melanogaster* to develop from larva to adult. We hypothesized the following:

H_a: Lower temperature increases the amount of time required for *Drosophila melanogaster* larvae to reach adulthood.

H_0: Lower temperature decreases or has no effect on the amount of time that *Drosophila melanogaster* larvae take to reach adulthood.

We based our hypothesis on basic biological considerations. First, metabolic rate decreases at lower temperatures (Zuo *et al.* 2012). In the case of our flies, this suggests that we should observe longer development times at lower temperatures. We also assume the inverse — that shorter development times should be observed at high temperatures, up to a limit, at which point the heat denaturation of proteins should negatively affect growth and survival rate (Berrigan and Partridge 1997). Additionally, with prolonged exposure to a cold temperature, we

expect to see *D. melanogaster* enter a period of diapause - a state of dormancy for insects, as characterized by a suspension of development and growth, and a reduction of mobility induced by extreme temperature, (Schmidt *et al*. 2005).

We chose 12°C to reflect an estimate for the lower limit of adult development rate, and 29°C to represent an estimate of the upper limit on development rate; we based these values on upper and lower level limits found in previous studies. One study found that at 10°C no adults developed, and at above 30°C, development is significantly reduced or halted (Davidson 1994). We chose 20°C as a mid-point between these two values. Because temperature has been demonstrated to have an inverse relationship with body size, we also recorded body sizes of adults collected during daily development-checks.

MATERIALS AND METHODS

In order to measure the effects of temperature on the growth time of *D. melanogaster*, three treatments were used: temperatures of 12°, 20° and 29° C, with each treatment consisting of four *D. melanogaster* replicates. Within this experiment, maturity was defined as the emergence into the adult stage of life.

This study started with the specimens in their larval stage after having been raised up to that point in a cornmeal growth medium at room temperature. We removed the *D. melanogaster* larvae from the vials containing a cornmeal growth medium using a wire loop. To separate the larvae from the medium, the portions of the removed medium were placed in approximately 15 mL of 18% sucrose solution in a petri dish. Within the solution, the larvae floated which made separation easier. We counted out ten larvae and placed them in an unused vial containing growth medium- this made up one replicate. We made twelve replicates in total. Four replicates were placed in a box to ensure no light exposure, and then we placed it in an incubator set at 20°

C; we chose this as our control, as it was closest to the temperature at which the larvae were originally raised. The remaining 8 replicates were similarly allocated to incubators set at 12° and 29° C.

Each day at 12 or 1pm, for a span of two weeks, we checked and recorded the status of the *D. melanogaster*, with an exception of weekends due to lab access times. Larvae, pupae and adult *D. melanogaster* were counted in each vial, and abundances were recorded. If adult *D. melanogaster* were present, we anaesthetized them using CO_2 gas, and removed from the vial in order to prevent breeding. Upon removal, we separated adults by sex; males were distinguished by a large horizontal stripe at the inferior portion of the abdomen, while females were distinguished by thinner, and more frequent horizontal stripes. We placed the males and females in separate vials before being returned to their respective incubators.

Twice a week, we measured the length of the *D. melanogaster* adults. We once again anaesthetized and removed the adults from the vials. We measured the adult's lengths using ImageJ, a digital image analytics software, in conjunction with a Dinoscope attached to the eyepiece of a dissecting microscope. Once ImageJ was calibrated using a ruler, a line was drawn from the top of the *D. melanogaster*'s head to the tip of its abdomen (Figure 1). ImageJ used the length of the line along with the ruler calibration to give a length in millimeters to three decimal places, but this value cannot be considered accurate to three decimal places due to the possibility of human line-drawing error.

Figure 1. A picture taken using the Dinoscope of a *Drosophila melanogaster* at 8 x magnifications, and a line representing the one used to measure the specimen's length.

The proportion of *D. melanogaster* that matured into adulthood for each replicate was calculated along with the 95% confidence interval. In order to analyze the data, we graphed these proportions against the number of days it took for the *D. melanogaster* to reach maturity. In order to determine if a significant difference exists between data values, we performed a t test; if the t value was calculated to be greater than the theoretical value, the difference between the values can be considered significant. Using the mean body length for the treatments in which adults emerged, we used the 95% confidence interval to determine if the difference in *D. melanogaster* body lengths is significant.

RESULTS

No adult *D. melanogaster* were observed in 12° C treatment throughout the observation days. In addition, as it is depicted in figure 2, there were no adult *D. melanogaster* observed in 20°C treatment for the first seven days from the start of our observation, and the average

proportion reached 100% of *D. melanogaster* larvae eclosing into adults after twelve days. According to Figure 2, the 29°C treatment *D. melanogaster* adults started emerging on the sixth day from the beginning of observation, but the average proportion did not reach 100% of *D. melanogaster* larvae growing into adults at the end of the experiment.

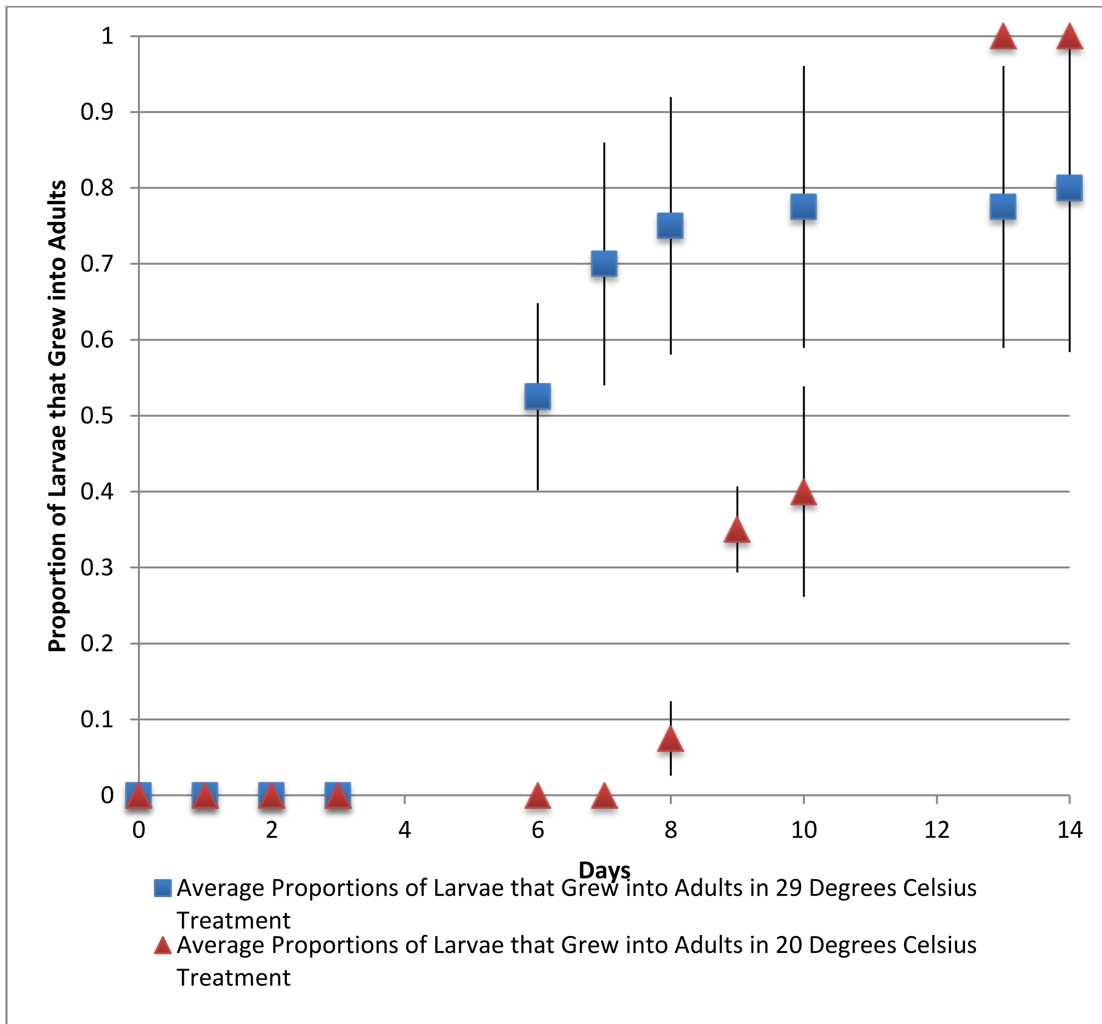

Figure 2. A comparison between the daily average proportions of *D. melanogaster* larvae that grew into adults in 29°C treatment and 20°C treatment. No observations were made during weekends (day 4 to day 5 and day 11 to day 12). Four replicates were in each treatment, and there were ten larvae in each replicate. Bars represent 95% confidence intervals.

There was an increase in the average proportion of *D. melanogaster* larvae growing into adults in both 20°C and 29°C treatments as it is shown in Figure 2. Besides the first four days, which there were no *D. melanogaster* adults observed in both treatments, and the last day, which the 95% confidence interval of 29°C treatment overlaps with the average proportion of *D. melanogaster* larvae growing into adults in 20°C treatment, the average proportions of *D. melanogaster* larvae growing into adults of the two treatments were significantly different for day six to eight and day ten, because there is no overlap in 95% confidence intervals for those days. To further confirm this inference, t values were calculated.

Table 1. Summary of t-values for 29°C and 20°C treatments from day six to day thirteen excluding day nine, day ten, day eleven and day twelve. - *indicates statistically significant =?.

Days	t-values
6	8.34*
7	7.67*
8	7.45*
10	1.56
13	-0.980

The theoretical t-value is 2.447 for the two treatments. According to Table 1, from day six to day eight, the calculated t-values are greater than the theoretical t-value. Therefore, in

those days, the differences between 29°C treatment and 20°C treatment were statistically significant. Finally, since there was no *D. melanogaster* adult observed in 12°C treatment, significant differences in both 95% confidence intervals and t-values are present between 12°C and 20°C treatment from day eight to day ten and from day thirteen to day fourteen, and 12°C and 29°C treatments also show significant differences in 95% confidence intervals and t-values from day six to day eight, day ten, and from day thirteen to day fourteen.

Body sizes of *D. melanogaster* adults were also analyzed. From Table 2, it can be concluded that there was a size difference between 20°C and 29°C treatments, and the average body size for the two treatments were significantly different (95% confidence intervals).

Table 2. A summary table for body size measurement data.

Treatment	Average Body Size (mm)	Standard Deviation of Average Body Size (mm)	95% Confidence Interval of Average Body Size (mm)
29° C	2.547	0.0836	0.0299
20° C	2.607	0.0241	0.00765

Sample Calculations:

Total Adult Flies for Each Vial for Each Day:

Total adults in replicate 1 of 29°C treatment as of Oct 28, 2013= 7 + 0 = 7

Average Proportions of Larvae Grew into Adults in Each Treatment:

Average proportion of larvae that grew into adults in 29°C treatment on Oct 28, 2013 = ((7/10)+(5/10)+(4/10)+(5/10))/4 = 0.525

Standard Deviation for Average Proportions:

Formulae for calculating standard deviation: $\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$

Standard deviation for average proportion of larvae that grew into adults in 29°C treatment on Oct 28, 2013 = $\sqrt{\frac{((\frac{7}{10})-0.525)^2+(\frac{5}{10}-0.525)^2+(\frac{4}{10}-0.525)^2+(\frac{5}{10}-0.525)^2}{4-1}}$ = 0.1258

95% Confidence Interval for Average Proportions:

Formulae for calculating 95% confidence interval: $\bar{x} \pm 1.96\frac{s}{\sqrt{n}}$

95% confidence interval for average proportion of larvae that grew into adults in 29°C treatment= $0.525 \pm 1.96\frac{0.1258}{\sqrt{4}} = 0.525 \pm 0.1233$

t-values for Average Proportions:

Formulae for calculating t-values: $\frac{x1-x2}{s\sqrt{\frac{1}{n1}+\frac{1}{n2}}}$

Formulae for calculating s values: $\frac{\sum(x-x1)^2 + \sum(x-x2)^2}{n1+n2-2}$

The s value for 29°C and 20°C treatments on Oct 28, 2013=
$\frac{0+((\frac{7}{10})-0.525)^2+(\frac{5}{10}-0.525)^2+\left(\frac{4}{10}-0.525\right)^2+\left(\frac{5}{10}-0.525\right)^2)}{4+4-2}$ = 0.089

t-value for 29°C and 20°C treatments on Oct 28, 2013 = $\frac{0.525-0}{0.089\sqrt{\frac{1}{4}+1/4}} = 8.34227101$

Average Body Size for Each Replicate:

Average body size for replicate one of 29°C treatment= (2.172+2.272+2.05+2.5+2.279+2.583+2.782+2.326+2.56+2.742)/10 = 2.4266 mm

Average Body Size for 29°C Treatment:

(2.4266+2.5542+2.6118+2.5943)/4=2.547 mm

Standard Deviation of Average Body Size for 29°C Treatment:

$$\sqrt{\frac{\Sigma(x - \bar{x})^2}{n-1}} =$$

$$\sqrt{\frac{(2.4266-2.547)^2+(2.5542-2.547)^2+(2.6118-2.547)^2+(2.5942-2.547)^2}{4-1}} = 0.0836 \text{ mm}$$

95% Confidence Interval of Average Body Size for 29°C Treatment:

$$\bar{x} \pm 1.96\frac{s}{\sqrt{n}} = 2.547 \pm 1.96\frac{0.0836}{\sqrt{4}} = 2.547 \pm 0.0299 \text{ mm}$$

DISCUSSION

We hypothesized that lower temperature will increase the amount of time required for *Drosophila melanogaster* larvae to reach adulthood. 95% confidence intervals and t-tests were used to test the hypothesis. According our statistical analysis, we reject the null hypothesis, and the alternative hypothesis is supported.

For 12° C treatment, no adults were observed for 14 days. This indicates that 14 days is not a significant amount of time for *Drosophila melanogaster* to progress to adulthood at 12° C. A similar experiment was designed previously to find out if there is a significant effect of temperature on the lifespan of *Drosophila melanogaster*. Mean lifespan of male *Drosophila melanogaster* in 15° C was observed to be 130.3 days, while it was 86.3 for 21° C replicates, 41.6 for 27° C replicates and 20.4 for 30° C replicates (Miquel *et al*. 1976). *Drosophila* originated from tropical locations, so prior to evolutionary adaptation, they did not have the ability to grow, and thus populate temperate regions (Schmidt *et al.* 2005). They explained that the *Drosophila* evolved to express temporary growth dormancy called diapause (2005). When placed under an environmental stress, such as the tropical *Drosophila* would experience when

growing in temperate climates, the specimen's body size, growth, fertility and lifespan all decrease due to diapause expression (Schmidt *et al.* 2005). In our 12° C treatment, the *Drosophila* may have not reached adults due to the effect of diapause.

For the 29° C treatment, adults started to emerge from the sixth day of observation, but only 80% of larva eclosed into adults at the end of the experiment. Even though adults started to emerge from the eighth day of observation in the 20° C treatment, it had forty out of forty larvae turning into adults on the thirteenth day of observation. Possible reason for this outcome is the different fertility rate among the treatments, where the rate increases along with temperature (Economos and Lints 1984).

The developmental period for *Drosophila* varies with temperature. Past research has demonstrated that the shortest developmental period is typically achieved by *Drosophila* at 28° C (Ashburner and Thompson 1978). Male and female adult flies first started to emerge in 29° C replicates, so mating and reproduction may have started earlier in 29°C replicates than 20° C replicates. Due to early addition of reproduced eggs, larval density in a 29° C environment increased, creating competition among the larvae for food and/or space or to chemical alteration of the culture medium as a result of larval metabolism (Economos and Lints 1984). Crowded larvae may release toxic "growth retardation factor" due to restriction of the amount of yeast available to each developing larva (Bakker 1961).

Another possible reason is the mortality rate which is also affected by the rearing temperature, as survival is optimal at 25° C; the survival rate decreased as temperature increased from 15° to 25°C, and increased again with further increase in temperature (AL-Saffar *et al*. 1995). *D. melanogaster* larvae at 29° C might have suffered from the extreme temperature and might have died before they reach adulthood.

There is also a significant difference found in the body size of adult *D. melanogaster* at 20° and 29° C treatments. The temperature and the body size of adult flies were inversely related. Decrease in rearing temperature increases the size of the wings, legs, and eyes through an effect on epidermal cell size (Azevedo *et al.* 2002). Partridge *et al*. also demonstrated that development at low temperatures increased both wing are and thorax length of *Drosophila* (1994).

These studies of *Drosophila* development have provided an explanation for the effect of temperature on duration of development and the body size. The results show that lower temperature increases the amount of time required for *Drosophila melanogaster* larvae to reach adulthood and the body size, as well.

Throughout the experiment we tried to minimize the number of possible errors, yet inevitably there were still some. One possible source of error had to do with the varying light levels the flies were exposed to, as it may have affected the growth rate of the flies. We minimized the amount of light which the flies grew in by placing the vials in a box and closing it prior to incubation, but when it was time to count them we took the vials out of the box, exposing them to light. This occurred every time the flies were counted and each amount of exposure time varied 5-45mins per treatment. Even though this is a source of error, we believe that the varying light exposure had minimalistic effects on the flies' growth rate.

Another source of error was identified in the 29˚C treatment level. In order to measure and count the flies, each time we had to remove the box from the incubator. The replicates were exposed to room temperature for a period of 5-45 minutes depending on how long it took to record the data. Even though we were not able to maintain a 29˚C environment 100% of the time, for the short time it was exposed to room temperature we believe that the effects on the

growth rate are very low. This source of error did not apply to the 12˚C and 20˚C because the incubator for 12˚C was large enough to record the data inside, as for the 20˚C the difference in temperature outside the incubator was minimal.

More errors may have occurred as we counted the flies. When the flies were in the vial prior to removal, there was a possibility that they could have mated resulting in second generation larvae. If the first generation larvae died we may have counted the second generation flies giving us slower growth rates.

In addition, possible human error may have occurred when using the ImageJ software used to measure the flies. It was up to the observer to use his or her judgement to decide exactly where the head and tail of the body were located, the body lengths may vary slightly from the true length.

There is also a possibility of genetic differences among the flies; this could explain why only some of the flies in the 29˚C treatment matured into adults. The biological variation in the flies may have caused some of them to die in either the larvae or pupae stage. This biological variation it may have slightly affected the outcome of our results by showing a lower proportion than the actual value.

Our last possible source of error is that all of the larvae were not at the same stage of their growth cycle. All the larvae varied between first instar stage and second instar lava stage when we started the experiment. As a result, we may have gotten a larger variation for the growth rate data in each replicate. Some replicates might have a larger spread and others may have longer or shorter rates than they should be.

CONCLUSION

Our results suggest a negative correlation between development time for *Drosophila melanogaster* and temperature. Because we observed significant differences between all three treatments, we reject the null hypothesis that lower temperatures are correlated with shorter development times from larva to adult for *D. melanogaster.* More research is necessary to determine the absolute lower temperature limit to development time for *D. melanogaster.*

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REFERENCES

AL-Saffar, Z.Y, Grainger, J.N.R., and Aldrich, J. 1995. Effects of constant and fluctuating temperature on development from egg to adult of *Drosophila melanogaster.* Biology and Environment: Proceedings of the Royal Irish Academy, 95 (**2**): 119-122.

Angilletta, M. J., Steury, T. D., and Sears, M. W. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integr. Comp. Biol. **44** (6): 498-509.

Ashburner, M, and Thompson, J.N. 1978. The genetics and biology of *Drosophila*. Academic Press, London, UK.

Azevedo, R.B.R., and French, V. 2002.Temperature modulates epidermal cell size in *Drosophila melanogaster*. Journal of Insect Physiology, 48 (**2**): 231–237

Bakker, K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Archives Neerlandaises de Zoologie, 14 **(2)**: 200-281.

Barbosa, P., Peters, T.M., and Greenough, N.C. 1972. Overcrowding of mosquito populations: responses of larval *Aedes aegypti* to stress. Environmental Entomology, 1 **(1)**: 89-93.

Berrigan, D., and Partridge, L. 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. Comparative Biology and Physiology, 118 **(4)**: 1301-7.

Davidson, J. 1944. On the relationship between temperature and rate of development of insects at constant temperature. Journal of Animal Ecology, 13 **(1)**: 26-38.

Economos, A.C., and Lints, F.A. 1984. Growth rate and lifespan in *Drosophila*. I. methods and mechanisms of variation of growth rate. Mechanisms of Ageing and Development, 27 **(1)**: 1-13.

Miquel, J., Lundgren, P.R., Bensch, K.G., and Altan, H. 1976. Effect of temperature on the life span, vitality and fine structure of *Drosophila melanogaster.* Mechanisms of Ageing and Development, 5 **(5):** 247-370

Partridge, L., Barrie, B., Fowler, K., and French, V. 1994. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster.* Journal of Evolutionary Biology, 7 **(6)**: 645-663.

Random, R. 1982. A handbook of *Drosophila* development. Elsevier Biomedical Press, Amsterdam, New York.

Roberts, D.B., and Standen, G.N. 1998. *Drosophila*: A Practical Approach. Oxford University Press, Oxford, New York.

Schmidt, P.S., Matzkin, L., Ippolito, M., and Eanes, W.F. 2005. Geographic variation in diapauses incidence, life-history traits and climatic adaptation in *Drosophila melanogaster*. Evolution, 59 **(8)**: 1721-1732.

Zuo, W., Moses, M.E., West, G.B., Hou, C., and Brown, J.H. 2011. A general model for effects of temperature on ectoderm ontogenetic growth and development. Proceedings of the Royal Society B: Biological Science, 279 **(1734)**: 1840-1846.