The effects of limited nutrient availability on the rate of development, body length, and intensity of eye pigmentation in *Drosophila melanogaster*

Brian C. Ahn, Cassie M. Tayler, Warancha V. Tumtaweetikul, Jaclyn A. Wiebe
Biology 342, University of British Columbia, November 29, 2012

ABSTRACT

Many insects encounter periods of nutritional stress during which they must continue to develop and grow despite limited availability of food. Our study organism, *Drosophila melanogaster*, commonly known as the fruit fly, must be flexible and responsive during all developmental stages under a wide range of environmental conditions to survive to adulthood. This study used observations, computer programs, and statistical analysis to study their response to chronic nutritional stress in four treatment levels: 100%, 75%, 50%, and 25% nutrient concentration. We focused on the effects that varying degrees of nutrient availability have on *Drosophila melanogaster* such as: rate of development, body length, and intensity of eye pigmentation. We found that the rate of development and body length did not significantly vary with respect to the nutrient concentration; whereas, the intensity of eye pigmentation decreased with decreasing nutrient concentration. These conclusions were determined from comparing the 95% confidence intervals (C.I.) among the four treatments. For rate of development and body length, the 95% C.I. overlapped which indicated no significant differences. On the other hand, for the intensity of eye pigmentation, the [25%] treatment of the mean eye pigment intensity was 100.182 with an upper range of 108.332. We compared this to the [100%] treatment with a mean intensity of 118.385 and with a lower range of 109.650 and saw that they do not overlap. Moreover, we calculated a t-test value of 2.986, between [25%] and [100%], that was higher than the standard value of 2.009, which showed a significant result. Therefore, these data suggests that organisms may sacrifice less crucial traits, such as eye pigmentation, in order to maintain other metabolic processes such as growth and development.

INTRODUCTION

We conducted our experiment on wild type *Drosophila melanogaster* to gain further insight on the effects that limited nutrient concentration have on their rate of development, body length, and intensity of eye pigmentation. *D. melanogaster* are abundant in number due to their ability to reproduce quickly and lay numerous eggs (Grotewiel *et al.* 2005). In addition, they are relatively inexpensive and have a short life span, making them an ideal organism for this study. All these key factors were crucial for our experiment, as we had limited time and resources.

Our first research question addressed the rate of development of *D. melanogaster* with respect to the nutrient availability.

H_{a1} = Decreasing the nutrient concentration decreases the rate of development of *Drosophila melanogaster*.

H_{o1} = Decreasing the nutrient concentration increases or has no effect on the rate of development of *Drosophila melanogaster*.

By manipulating the nutrient level, we anticipated that the duration of juvenile development could be altered, which would consequently lead to a difference in body size (Tennessen and Thummel 2011). For example, poor nutrient levels could stall larval growth and lead to a smaller body size. Thus, there is a critical period between the larval and pupal development, which could be influenced by nutrient level. This led us to our second objective focusing on the adult size of fruit flies.

H_{a2} = Decreasing the nutrient concentration decreases the size of *Drosophila melanogaster*.

H_{o2} = Decreasing the nutrient concentration increases or has no effect on the size of *Drosophila melanogaster*.

Because of the nutrient stress introduced into their living environment, fruit flies grown under food restriction would result in smaller body sizes (Khazaeli *et al.* 2005). We manipulated the nutrient level predicting that fruit flies at 100% nutrient concentration would have the largest body size. Our final question guided us to investigate the influence that food restrictions might have on fruit flies' eye pigmentation.

H_{a3} = Decreasing the nutrient concentration decreases the intensity of eye pigmentation of *Drosophila melanogaster*.

H_{o3} = Decreasing the nutrient concentration increases or has no effect on the intensity of eye pigmentation of *Drosophila melanogaster*.

Beadle *et al.* (1938) proposed that complete removal of food from the growth medium for mutant *vermilion brown* larvae resulted in flies with less eye pigment production. This

research provides background information for a possible outcome that our flies may experience.

Our results will provide insight into how nutrients and resources are used and distributed by *D. melanogaster* under conditions of restricted nutrient availability.

METHODS

Location & Timing

This study took place in the Bio 342 laboratory, which provided us with the necessary equipment to help conduct our experiment accurately. Our fruit flies were kept in a storage room at a temperature between 20-25°C. We collected our data and observations over a two-week period.

Preparation

We used four treatments (100%, 75%, 50% & 25% growth media nutrient concentration), five replicates per treatment and 10 *D. melanogaster* per replicate for a total of 200 wild-type flies. Before data collection began, we made growth media at four different nutrient concentrations. The recipe for our growth medium came from Khouvine *et al.* (1938). The formula for our control (100%) nutrient concentration was: Purity cornmeal 13g, Rogers Golden brown sugar cubes 16g, Bioshop agar 2g, and distilled water to make 10mL. This recipe was then modified for the other three treatments by taking a percentage of the original amount of cornmeal and brown sugar, leaving the agar constant, while filling the remaining volume (up to 10mL) with distilled water. Next, all media were autoclaved and transferred to the experimental cotton-stopped vials.

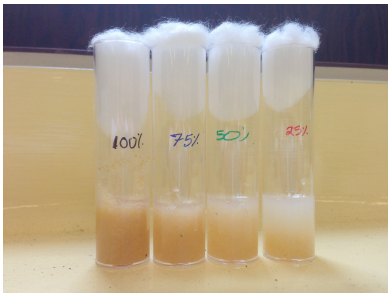

Figure 1: Four cotton-stopped vials at nutrient concentrations: 100%, 75%, 50%, and 25%.

We were supplied with *D. melanogaster* by the University of British Columbia's

Biology Department. On Day 1, we selected and transferred the ten largest larvae visibly available into each of the twenty vials. We tried to ensure that they were all of similar size to minimize age variance. The larvae stock we obtained already had a mixture of larvae and pupae, which meant that they were all laid within a two-day period. Based on this information, we can infer that the largest larvae we selected were approaching the end of their larval stage, and were about to become pupae. We began our pupae and adult counts after the weekend on Day 4 (observation #1), as pupae were beginning to emerge.

Data Collection and Analysis

Date: Nov 6	Observers: Cha T + Cassie T + JW	Temp: 23.0°C	Time: 9:30 A.M.	
PUPAE				
Replicate	100%	75%	50%	25%
1	3	2	0	1
2	4	3	2	0
3	4	2	1	1
4	1	0	3	1
5	0	1	3	2
ADULTS				
Replicate	100%	75%	50%	25%
1	5	9	9	9
2	5	4	7	10
3	6	8	6	9
4	7	9	8	6
5	11	8	7	6

Table 1. A sample of the data table used to collect the number of pupae and adult flies quantitatively.

Our total data collection consisted of ten observations over a fifteen-day period. During this time we observed the number of pupae and adult flies present and visible on the surface of the growth media. We used carbon dioxide gas (CO_2) to anesthetize the adult flies to make them easier to count. For the pupae and adult totals, we used a chart to collect the data (Table 1). The majority of observations were made with at least two group members present in order to minimize inaccuracies while counting.

On the final day of observations, we measured the length of adult flies and the intensity of eye pigmentation of five individuals from each replicate. Using a dissecting microscope and a connected DinoXcope (Figure 2), we took pictures of 100 randomly selected fruit flies. Then using the program ImageJ and the Color Histogram plugin, we measured the body length of *D. melanogaster* (Figure 3a), and the intensity of red eye pigment of each individual (Figure 3b and 3c) (Rasband 1997).

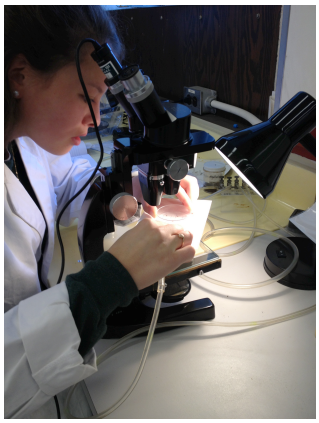

Figure 2: Use of the dissecting microscope and DinoXcope to photograph *D. melanogaster*.

Figure 3a: Measurement of body length using ImageJ. Yellow line represents the length measured in millimeters.

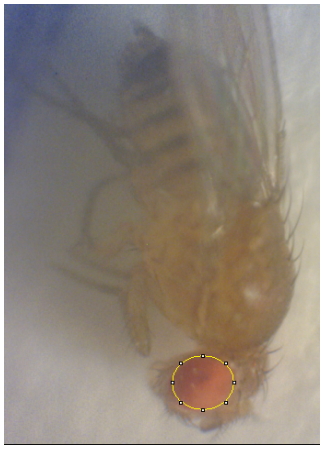

Figure 3b: Measurement of eye pigment intensity using ImageJ. Yellow circle represents the area analyzed.

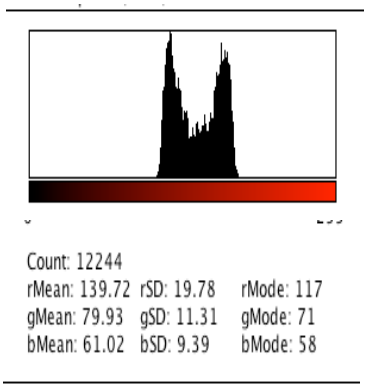

Figure 3c: An example of a ColorHistogram graph of red eye pigmentation of *D. melanogaster*.

Analysis

To statistically analyze our data, we randomly collected five individuals from each replicate, for a total of 25 flies per treatment. We then calculated the mean values for the number of pupae and adults, adult size, and pigment intensity from the randomly chosen 25 flies. By

using the mean values from each treatment, we drew graphs and calculated the 95% C.I. We used the student t-test to determine the significant differences that occurred during the flies' uptake of different nutrient levels.

RESULTS

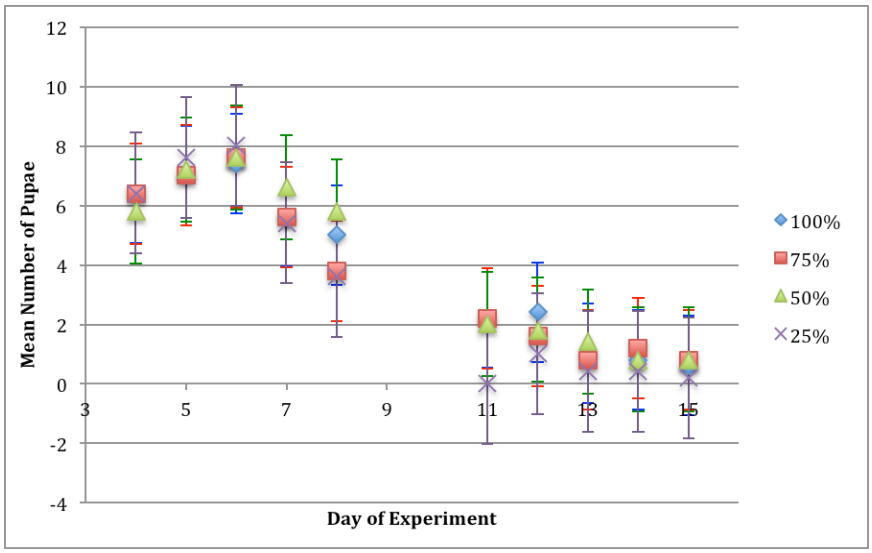

Figure 4. Mean *D. melanogaster* pupae count for each treatment over a two-week period. The number of pupae from the five replicates were averaged to find the mean pupae count for the day for each treatment. Bars represent 95% C.I.

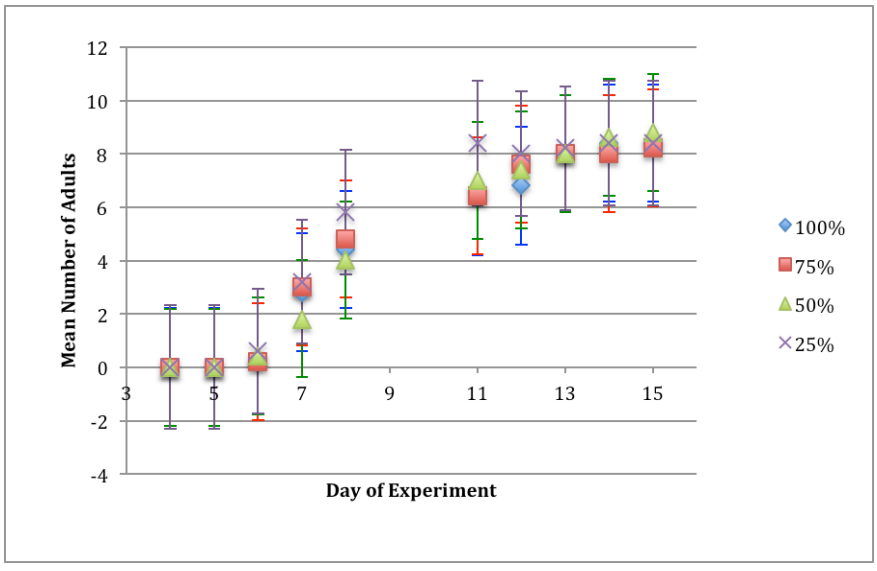

Figure 5. Mean *D. melanogaster* adult count for each treatment over a two-week period. The number of adults from the five replicates was averaged to find the mean adult count for the day for each treatment. Bars represent 95% C.I.

Figures 4 and 5 show the growth patterns of *D. melanogaster* over the course of our experiment. Data are only plotted for the days we observed the flies: weekdays for a two-week period excluding the first day of the experiment. As such there are ten observations over the

course of fifteen days beginning on Day 4 of the experiment. Figures 4 and 5 show no significant difference in growth rate between any of the treatments, as there is overlap in the 95% C.I. for each data point.

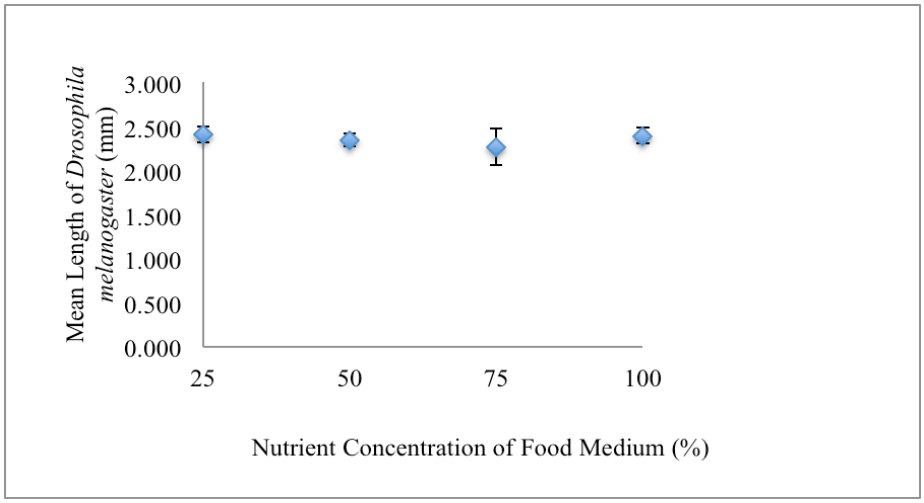

Figure 6. Mean lengths of *D. melanogaster* measured from each treatment over the observation period. Means are calculated from 25 individuals from each treatment. Bars represent 95% C.I.

In Figure 6, we took a sample of five fruit flies from each of the five replicates for the four treatments and calculated the average length. At the concentration of 25%, 50%, 75%, and 100%, we found the means and 95% C.I. to be (2.412±0.092 mm), (2.348±0.074 mm), (2.268±0.209 mm) and (2.393 ±0.092 mm) respectively.

As seen in Figure 6, the 25% nutrient concentration of growth media shows the highest mean length of *D. melanogaster* while the smallest mean length was at the 75% nutrient concentration. However, these means are not significantly different, and the general trend shows no change in *D. melanogaster* length across all four treatments.

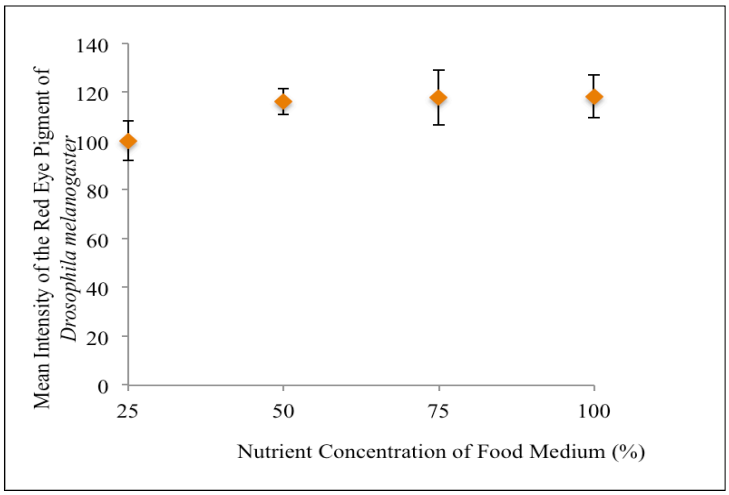

Figure 7. Mean intensity of the red eye pigment of *D. melanogaster* for each treatment over a two-week period. These bars represent 95% C.I.

As we analyzed the data from Figure 7, we determined that there is a trend across all treatments, which indicates that decreased nutrients decrease the intensity of red eye pigmentation. Specifically, we used a t-test to compare the mean of the red pigment at [25%] and [100%] and found a significant difference. Our calculated t-value was 2.986, which is greater than the theoretical value from the table of selected values (i.e. t = 2.009) at P = 0.05 C.I. (MedCalc 2012).

Examples of calculations:

Mean Length at [25%] = $\frac{\text{Sum of the Sizes of 25 flies}}{\text{Total Number of flies}} = \frac{60.300}{25} = 2.412$

Standard Deviation = $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}} = 0.235$

95% C.I. at [25%] = $2.412 \pm 1.96 \frac{0.235}{\sqrt{25}}$

Student t-test at [25%] and [100%] = $t = \frac{\bar{x}_2 - \bar{x}_1}{s\sqrt{\left(\frac{1}{n_2}+\frac{1}{n_1}\right)}} = 2.986$

DISCUSSION

The *D. melanogaster* rate of development did not significantly vary with respect to the

nutrient concentration. As shown in Figures 4 and 5**,** there was an overlap in the 95% C.I. among all treatments. As such, we were unable to reject H_{o1} and unable to lend support to H_{a1}. This was contrary to the findings of Kloss *et al.* (2009) who determined that flies reared on nutrient poor growth media had a slower rate of development than those grown on normal media.

Typically, the final instar for larval growth lasts 48 hours, and we assumed that our selected larvae were from this life cycle stage (Mulinari 2008).Therefore, our larvae had a maximum of two days in which to transform into pupae where they should have stayed for approximately four days, emerging as adults on the fifth day (Mulinari 2008).According to this timeline, the first day of pupal development for our experiment was expected on Day 2 or 3, with the pupa stage ending on Day 5 or 6 and eclosing (transformation of pupae into adult) on Day 7. However, Tu and Tatar (2003) suggested that juvenile *Drosophila* grown in a restricted nutrient environment have an eclosion time delayed by approximately two days. As seen in Figure 4, the number of pupae for each treatment peaked at Day 6, which was consistent with Mulinari's timeline for normal growth, and inconsistent with Tu and Tatar's findings in 2003. This indicated that none of our treatments had any retardation effects on *D. melanogaster'*s rate of development.

We found no significant results or trends with regards to the *D. melanogaster* adult size as demonstrated in Figure 6; thus, we were unable to reject H_{o2} and were unable to lend support to H_{a2}. This was contrary to the results of previous research, in which nutrient limitation in juvenile life stages resulted in smaller adult size. For example, in the study by Tu and Tatar (2003), it was determined that third instar larvae fed a yeast-controlled diet resulted in smaller adults than those fed a yeast-replete diet. Our contradictory findings might be a result of the lack of yeast used in our growth media. Perhaps without the use of yeast, the adults from each treatment were below the average size when compared to individuals grown with yeast, but of similar size when compared to each other.

In the analysis of the intensity of red eye colour of *D. melanogaster* with respect to nutrient concentration, we found significant results between the [25%] and [100%] nutrient media. Between these two treatment levels, our calculated student *t*-test value was 2.986, allowing us to reject H_{o3} at a P-value of 0.05, and lend support to H_{a3}: decreasing the nutrient concentration decreased the intensity of eye pigmentation of *D. melanogaster*. In addition to the significant results between the lowest and highest concentration growth media, there was an observed trend across all concentrations, as seen in Figure 7. This trend showed an increase in pigment intensity with an increase in nutrient concentration. We predicted that this result was due to a redistribution of resources by *Drosophila*. As previously discussed, growth rate and adult body size were not significantly affected by nutrient concentration. Perhaps the flies grown on [25%] media were able to maintain the same growth rate and development of body size as flies from other treatments because they were rerouting their resources from making pteridines, the eye pigment responsible for red eye colour (Ferré *et al.* 1986) to developmental and structural body components. This would result in a slightly duller red eye colour, as observed in the individuals from the [25%] treatment. According to Khouvine *et al.* (1938), in mutant *vermillion* flies, starvation induced "a deviation from the normal metabolism". Similar to Khouvine *et al.* (1938), our subjects may have undergone a redistribution of their metabolic functions.

Sources of error

There were several possible errors that might have affected our data. Firstly, our selected larvae might not have hatched at the same time, and thus, our *D. melanogaster* might not have all been the same age. To minimize this error we selected the largest larvae we could find and rejected those smaller than approximately 2mm. Secondly, during the pupae count, it was hard to differentiate between a real pupa and an empty pupa shell; therefore, some of the pupae might have been miscounted. We attempted to reduce this human error by striving to have at least

two recorders present at each observation. Thirdly, in the ImageJ program, the line that measured the length of the flies was drawn freehand, which could result in some human error in determining where the longest part of the body was. Lastly, we used a lamp to illuminate the microscope; however, as the flies were in different positions on the microscope mount, different amounts of light hit their eyes. This error might affect the results for the intensity of eye pigment measurement.

CONCLUSION

We conclude that while *Drosophila melanogaster* rate of development and adult size did not vary with nutrient concentration, but eye pigmentation did. We found that decreasing the nutrient concentration decreased the intensity of eye pigmentation. By limiting the nutrients available, this organism may have sacrificed developing normal eye pigment intensity in order to dedicate resources to other metabolic processes such as body growth and rate of development.

ACKNOWLEDGMENT

First of all, we would like to graciously thank our Biology 342 instructor, Dr. Carol Pollock, for providing guidance and expertise throughout our project. Secondly, we would like to thank our course technician, Ms. Mindy Chow, for preparing our growth media and ensuring we always had the necessary supplies. Thirdly, thank you to our teaching assistant, Mrs. Diana Rennison, for providing helpful assistance and finding the ImageJ and Color Histogram software.

We would also like to thank the Biology 342 lab at the University of British Columbia for the opportunity to take this course, providing us with the space, subjects and supplies to conduct our experiment, our anonymous peer editors, and lastly, thank you to each colleague who made this study possible and memorable.

LITERATURE CITED

Beadle, G. W., Tatum, E.L., and Clancy, C. W. 1938. Food-level in relation to rate of development and eye pigmentation in *Drosophila melanogaster*. Biological Bulletin, **75**(3): 447-462.

Ferré, J., Silva, F. J., Real, M. D. and Ménsua, J. L. 1986. Pigment patterns in mutants affecting the biosynthesis of pteridines and xanthommatin in *Drosophila melanogaster*.Biochemical Genetics, **24**(7-8): 545-569.

Grotewiel, M.S., Martic, I., Bhandari, P., and Cook-Wiens, E. 2005. Functional senescence in Drosophila melanogaster. Ageing research reviews, **4** (3): 372-397.

Khazaeli, A.A., Van Voorhies, W., and Curtsinger, J.W. 2005. The relationship between life span and adult body size is highly strain-specific in *Drosophila melanogaster*. Experimental Gerontology. **40**(5): 377-385.

Khouvine, Y., Ephrussi, B., and Chevais, S. 1938. Development of eye colors in *Drosophila*: nature of the diffusible substances; effects of yeast, peptones and starvation on their production. Biological Bulletin, **75** (3): 425-446.

Kloss, M., Vijendravarma, R. K., Schwaller, G. and Kawecki, T. J. 2009. Life-history consequences of adaptation to larval nutritional stress in *Drosophila*. Evolution, **63**(9): 2389-2401.

MedCalc. 2012. Values of the t-distribution (two-tailed) [online]. Available from http://www.medcalc.org/manual/t-distribution.php [accessed 28 November 2012].

Mulinari, S. 2008. From cell shape to body shape: Epithelia morphogenesis in *Drosophila melanogaster.* Faculty of Medicine Doctoral Dissertation, Lund University, Sweden.

Rasband, W.S. 1997. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/.

Tennessen, J. and Thummel, C. 2011. Coordinating Growth and Maturation — Insights from *Drosophila*. Current Biology. **21**(18): R750-R757.

Tu, M. P. and Tatar, M. 2003. Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster* [online]. Aging Cell, **2**(6): 327-333.