

The effect of lactose and fructose based-diets on *Drosophila melanogaster* maturation time

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Abstract

Using the model organism, *Drosophila melanogaster*, we studied the effect of lactose, fructose, and dextrose based growth media on maturation time, starting from the larval stage. As *D. melanogaster* is normally cultivated in standard dextrose medium, we used dextrose as our control and chose lactose and fructose as our two treatments. *D. melanogaster* was observed for a total of 12 consecutive days with the exception of days 8 and 9. After 12 days, a statistically equal number of *D. melanogaster* were observed in all treatments, however, between day 5 and day 10 there were significantly more adults observed in the dextrose-based growth medium than in both the lactose and fructose treatments. Our observations were not substantiated by literature, as there was an absence of research on the specific subject matter. However, discussion of biological factors and variance provide possible explanations for our results.

Introduction

The common fruit fly, *Drosophila melanogaster*, is considered to be a suitable model organism for research related to animal nutrition (Bharucha 2009). Its relatively short generation time, well-characterized molecular biology, genome, and physiology, as well as the ease of handling are all major strengths (Piper *et al.* 2005). Studies carried out on *D. melanogaster* have been concerned with caloric intake and dietary restriction and the associated effects on lifespan, metabolic rate and other physiological and biochemical parameters (Bross *et al.* 2005, Lee *et al.* 2008, Min and Tatar 2006, Piper *et al.* 2005).

Wild type *D. melanogaster* cultivated on a basic dextrose cornmeal agar medium have a life cycle of about three weeks and a reproductive cycle of about twelve days (Amirtha *et al.* 2009). In this experiment, our objective was to

determine how a fructose-based growth medium and a lactose-based growth medium affect the maturation time of wild type *D. melanogaster*. This is an important aspect of study, as the literature has neglected the effects of specific nutrients in growth media, and instead has focused on the effects of dietary restriction. Many studies have concluded that dietary restriction without malnutrition prolongs the lifespan of *D. melanogaster* (Bross *et al.* 2005, Lee *et al.* 2008, Min and Tatar 2006, Piper *et al.* 2005). More specifically, dietary restriction with a progressive dilution of food causes lifespan to reach a maximum, and then decrease due to starvation (Piper *et al.* 2005). However, little investigation has focused on how the process prolongs life spans.

The widely held belief that the life-extending effects of dietary restriction are due to caloric restriction has been challenged by hypotheses that focus on more subtle characteristics of diet. These hypotheses suggest that specific ratios of proteins, amino acids and sugar, rather than just calories alone, are responsible (Bross *et al.* 2005, Fujita and Tanimura 2011, Piper *et al.* 2005). Therefore, this experiment intends to explore the effects of sugar content, more specifically fructose and lactose, on the maturation time of *D. melanogaster*, rather than dietary restriction or caloric intake, in order to fill the gap found in experimental exploration of the maturation time of *D. melanogaster*.

H₀₁: The presence of fructose in growth medium will increase or have no effect on maturation time of *Drosophila melanogaster*.

H_{a1}: The presence of fructose in growth medium will decrease maturation time of *Drosophila melanogaster*.

H₀₂: The presence of lactose in growth medium will decrease or have no effect on maturation time of *Drosophila melanogaster*.

H_{a2}: The presence of lactose in growth medium will increase maturation time of *Drosophila melanogaster*.

Methods

At the beginning of our experiment, we extracted larvae from a dextrose-based cornmeal medium that contained wild-type *D. melanogaster* in all stages of maturation. We anaesthetized the adult flies with CO₂ and then we transferred them to the morgue solution, as we did not need them for our experiment. We extracted the larvae with a sterile loop from the original dextrose medium and transferred them into a petri dish half full of 18% sucrose solution (Figure 1). The solution served as a separating agent that removed the larvae from the medium without killing them. Before we transferred to the larvae into the treatment vials, we rinsed each larva in 18% solutions of the control and treatment sugars. We made these solutions from 5.4 g of the selected sugar and 30 mL of distilled water (Figure 2). After the rinse, we transferred the larvae into each replicate vial starting with dextrose first, followed by fructose and then lactose. This reduced cross-contamination in our replicates.

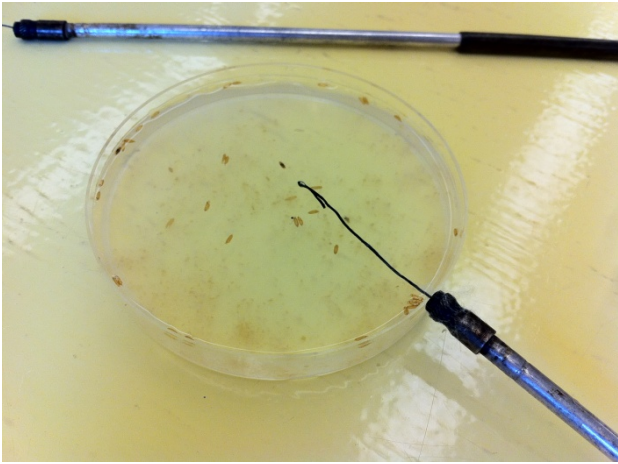


Figure 1. An example of the initial set up. Larvae were first extracted into an 18% sucrose solution for rinsing with a loop. The solution served as a separating agent that allowed larvae to float for easy collection



Figure 2. The three 18% treatment solutions for second rinse of the larvae. From left to right: Lactose Fructose and Dextrose, each made from 30 mL of distilled H₂O and 5.4 g of the selected sugar.

Our experiment consisted of 3 treatments with 5 replicates each, for a total of 15 replicates (Figure 3). Having 5 larvae in each replicate, we used a total number of 75 larvae. Dextrose acted as our control, and fructose and lactose were our treatments (Table 1). We then placed the replicate vials in a dark room with temperature varying between 22°C and 24°C.

We observed each replicate vial for a total of 12 days, excluding day 8 and day 9 due to restricted lab access. We counted the number of larvae, pupae and adults and noted their physical appearance and sex. We removed adults from the vials after each observation to avoid breeding. We then examined each adult under a dissecting microscope and photographed them using DinoXcope software. In addition, we used the field diameter and magnification to calculate the size of the

adults. We disposed of adult flies in the morgue solution once observations were complete.

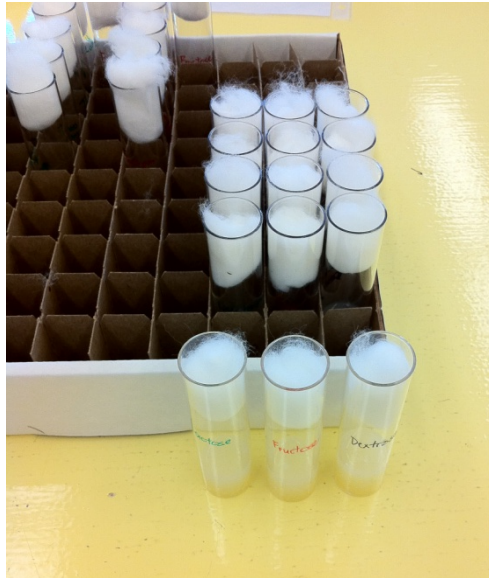


Figure 3. Set up of the replicate vials. We had 3 treatments and 5 replicates for each treatment, which gave a total of 15 replicate vials.

Table 1. Ingredients used to make the *Drosophila* cornmeal medium.

Lactose Medium	Dextrose Medium	Fructose Medium
Distilled H ₂ O 100 mL	Distilled H ₂ O 100 mL	Distilled H ₂ O 100 mL
Agar 1.9 g	Agar 1.9 g	Agar 1.9 g
Cornmeal 2.5 g	Cornmeal 2.5 g	Cornmeal 2.5 g
Lactose 0.5 g	Dextrose 0.5 g	Fructose 0.5 g

For each day, we calculated the average number of *D. melanogaster* adults in each treatment, based on sex. Then we totaled the number of adults in each treatment, for each day, and calculated 95% confidence intervals (C.I.). We then plotted the appearance of adults against time, in days, for both our sex-independent and sex-dependent data.

Results

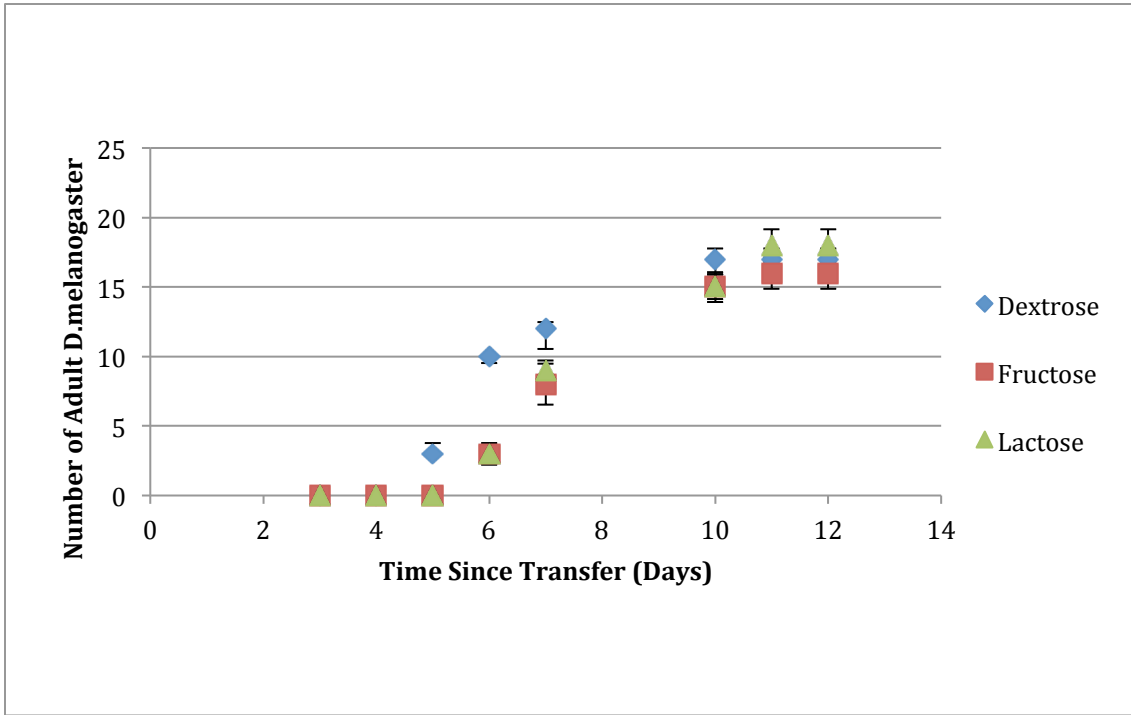


Figure 4. Appearance of adult *D. melanogaster* over the 12 days of experimentation. n=5 for each treatment. Bars represent 95% C.I. Temperature varied between 22°C and 24°C.

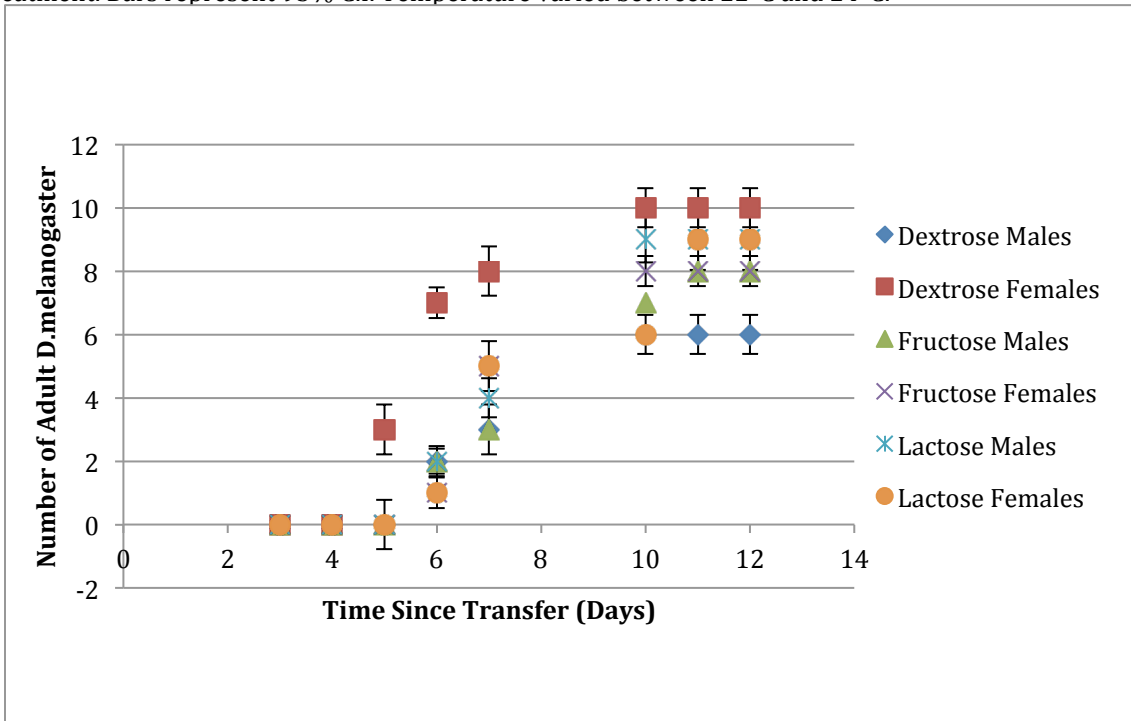


Figure 5. Appearance of male and female adult *D. melanogaster* over the 12 days of experimentation. n=5 for each treatment. Bars represent 95% C.I. Temperature varied between 22°C and 24°C.

The number of adults observed between day 5 and day 10 after transfer was significantly higher in our dextrose control than it was in either of our treatments (Figure 4). When comparing our two treatments to each other, we can see that the number of adults in each treatment are not significantly different on any days, and only deviate on three observed points (Figure 4). On day 7, one more adult was observed in lactose than in fructose and on days 11 and 12, 2 more adults were observed in lactose than in fructose. Overall, the results indicate that more adults appear sooner in a dextrose-based growth medium than in either treatment. Furthermore, the data indicate that more adults appear in a lactose-based growth media than in the fructose-based growth media, but not significantly more.

Although not directly related to the rejection of our null hypotheses, Figure 5 illustrates that females reared in dextrose-based media appear significantly faster than either sex in any other growth media between days 5 and 7 after transfer.

Sample Calculation (Day 7, dextrose):

Average:

$$\bar{X} = \frac{\sum x}{n}$$

$$\bar{X} = (2+3+3+2+2)/5$$

$$\bar{X} = 2.4$$

Standard Deviation:

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

$$S = \sqrt{[(2-2.4)^2 + (3-2.4)^2 + (3-2.4)^2 + (2-2.4)^2 + (2-2.4)^2] / 4}$$

$$S = 0.55$$

95% Confidence Interval:

$$C.I = \bar{x} \pm 1.96 \frac{s}{\sqrt{n}}$$

$$C.I. = 2.4 \pm 1.96(0.54772256/\sqrt{5})$$

$$C.I. = 2.4 \pm 0.5$$

Discussion

As our control treatment led to significantly lower maturation times than our fructose treatment, we failed to reject H_{01} : The presence of fructose in growth medium will increase or have no effect on maturation time. However, in regards to our lactose treatment H_{02} is rejected and support is provided for H_{a2} : The presence of lactose in growth medium will increase maturation time. As noted above, we observed that the maturation period for *D. melanogaster* growing on fructose and lactose was nearly identical, and the maturation period for *D. melanogaster* growing on dextrose was significantly shorter than both of the treatments.

Our findings are consistent with the literature to an extent. Our data show that fructose is sufficient to provide *D. melanogaster* with all essential nutrients to facilitate growth (Gospodaryov *et al.* 2011). However, it disagrees with Gospodaryov *et al.* (2011) as the observed maturation time on the fructose medium was not significantly lower in any regard. Similarly, our data show that lactose is sufficient for growth and maturation (Batista *et al.* 2008), even though observed maturation times were nearly identical to those observed on the fructose medium, which disagrees with Wigglesworth (1948), who suggested that lactose is far inferior to fructose as a source of energy and nutrition.

A possible reason for this discrepancy is that *D. melanogaster* has genes coding for the metabolic pathways required for the digestion and utilization of both lactose and fructose but do not actively transcribe them unless in the presence of the specific sugar (Dahanukar *et al.* 2007 and Brenner-Holzach 1979). This would explain why the flies in our treatments took longer to mature than in our control, even though a statistically equal number of flies were found on the last day. These data would suggest that *D. melanogaster* are capable of metabolizing all three sugars, which would be consistent with the literature, although it would not be able to explain why the results between lactose and fructose are identical.

Wigglesworth (1948) suggests another possible explanation of the results, stating that *D. melanogaster* is capable of storing sugar in the form of glycogen at a variety of different stages of its life cycle. It is possible that all flies stored energy from the original dextrose medium, which was sufficient for maturation. Our experiment would have simply forced the larvae in our two treatments to utilize these reserves. This would explain why the data on fructose and lactose were identical, as well as why development was significantly lower than dextrose until day 11.

Our qualitative observations of the adults help to bridge the gap between our quantitative data and the literature, as the adults found in the lactose medium were smaller, paler, and less active than those in fructose (Figure 6). First we thought this to have been a result of newly enclosed flies being observed; however, we quickly found that all adults in the lactose treatment had a similar appearance with the

exception of two males from day 3; replicate 1 and 5. All adults from the dextrose and fructose treatments appeared to have normal coloration, size and activity.

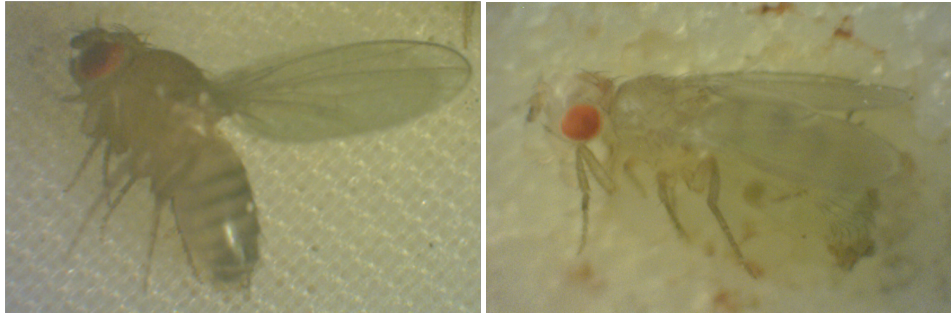


Figure 6. Healthy female from dextrose replicate #3, day 6, on the left (16X magnification, ~2.5mm) compared to pale and smaller female from lactose replicate #4, day 11, on the right (32X magnification, ~1.5mm)

There are a number of biological factors that may have led to a lack of significant difference in the means of our lactose and fructose treatments as well as any deviations from the literature. Firstly, although all of the *D. melanogaster* were in the larval stage when they were transferred into their respective treatments, it is possible that some were more mature than others, resulting in unexpectedly quick maturation. Amirtha *et al.* (2009) suggest that development from a larva to an adult should take about 12 days. As the appearance of our first adult occurred after only 5 days after transfer, there is a strong chance that our growth data was subject to this variation. Secondly, as our group was unable to visit the lab during the weekends, the *D. melanogaster* were left unattended during days 8 and 9. Being left unattended, any new adults could have interbred, resulting in new larvae and increased competition in the growth medium. Competition, could have possibly led to conditions similar to dietary restriction, which would have increased maturation time Partridge *et al.* (2005) and undoubtedly skewed our results. Partridge *et al.*

(2005) indicate that dietary restriction affect males and females differently, leading to longer development time in females. As we were unable to ensure that equal numbers of female and male larvae were put into each treatment, the slower development in our two treatments may have been due to a large female presence. Looking at Figure 5, we can see that the majority of flies that matured to adulthood were females, and therefore our data may have been affected by this source of error.

Conclusion

In conclusion, we were unable to reject H_{01} , as the presence of fructose in the growth medium led to a statistically longer maturation time in *D. melanogaster* than did our control, dextrose. We were able to reject H_{02} and provide support for our alternative hypothesis (H_{a2}), as our lactose treatment also led to longer maturation time in *D. melanogaster* than in our control, dextrose. These findings were not completely supported by the literature and ultimately were subject to large amounts of error. This error was exclusively biological – mainly our inability to ensure that all transferred larvae were of the same age, equal genders, and our inability to ensure no interbreeding occurred between day 7 and 10. Furthermore, we were unable to ensure that larvae did not store energy during their cultivation in the original dextrose medium.

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Literature Cited

- Amritha, A., Parvathi, D., and Paul, S. F. 2009. Wonder animal model for genetic studies – *Drosophila melanogaster* – its life cycle and breeding methods – a review. Sri Ramachandra Journal of Medicine, **2**(2): 33-38.
- Batista, F.R.X., Pereira, C.A., Mendonca, R.Z., and Moraes, A.M. 2008. Formulation of a protein-free medium based on IPL-41 for the sustained growth of *Drosophila melanogaster* S2 cells. Cytotechnology, **57**(1): 11-22.
- Bharucha, K. N. 2009. The epicurean fly: using *Drosophila melanogaster* to study metabolism. Pediatric Research, **65**(2): 132-137.
- Brenner-Holzach, O. 1979. Properties of fructose 1,6-diphosphate aldolase from *Drosophila melanogaster*, Archives of Biochemistry and Biophysics, **194**(2): 321-327.
- Bross, T. G., Rogina, B., and Helfand, S. L. 2005. Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. Aging Cell, **4**(6): 309-317.
- Dahanukar, A., Lei, Y., Kwon, J. Y., and Carlson, J. R. 2007. Two gr genes underlie sugar reception in *Drosophila*, Neuron, **3**(8): 503-516
- Fujita, M., and Tanimura, T. 2011. *Drosophila* evaluates and learns the nutritional value of sugars. Current Biology, **21**(9): 751-755.
- Gospodaryov, D. V., Lushchak O. V., Lushchak, V. I., and Rovenko, B. M. 2011. *Drosophila melanogaster* larvae fed by glucose and fructose demonstrate difference in oxidative stress markers and antioxidant enzymes of adult flies. Molecular & Integrative Physiology, **160**(1): 27-34.
- Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Soran, N., and Raubenheimer, D. 2008. Lifespan and reproduction in *Drosophila*: new insights form nutritional geometry. Proceedings of the National Academy of Sciences of the United States of America, **105**(7): 2498-2503.

Min, K., and Tatar, M. 2006. *Drosophila* diet restriction in practice: do flies consume fewer nutrients? *Mechanisms of Ageing and Development*, **127**(1): 93-96.

Partridge, L., Piper, M.D.W., Mair, W. 2005. Dietary restriction in *Drosophila*. *Mechanisms of Ageing and Development*, **126**: 938-950.

Piper, M. D., Skorupa, D., and Partridge, L. 2005. Diet, metabolism and lifespan in *Drosophila*. *Experimental Gerontology*, **40**(11): 857-862.

Wigglesworth, V.B. 1948. The utilization of reserve substances in *Drosophila* during flight. *Agricultural Research Council*, 150-153.