Measuring the effects of a low-nutrient diet versus a high-nutrient diet on developmental timing in *Drosophila melanogaster*

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Purpose

The purpose of this lab was to observe the effects of differences in nutrients in the diet on developmental timing of *Drosophila melanogaster. Drosophila* were given food either low in yeast concentration (1%) or high in yeast concentration (5%). Developmental timing was measured by the time it took for *Drosophila* to eclose. We expected that the *Drosophila* which was given food with higher yeast concentration, would enclose faster than those who were given the low-yeast concentration foods, suggesting a possible relationship between the developmental timing of *Drosophila* and diet.

Methods

Food and Mating Vial Preparation

Prior to the collection of *Drosophila* for this experiment, we prepared vials with 5% yeast concentrated food, or 1% yeast concentrated food. Food consisted of agar, sucrose, dextrose, cornmeal, propionic acid, Tegosept, and deionized water. Foods were prepared the same, except for the yeast concentration as the independent variable. Once food was prepared, we collected a total of 20 virgin female *Drosophila* and 20 male *Drosophila*.

Mating vials and egg plates were then prepared. Egg plates were made up of grape juice agar, of which a small dot of yeast and water paste was pipetted in the middle. Once the yeast paste was dried, three males and three virgin female *Drosophila* were placed into each mating vial, sealed with the egg plate at the base. Mating vials were then placed in the incubator at 25 degrees Celsius for 24 hours. The first round of mating vials was prepared on February 1st, and eggs laid in those vials were counted the next day (February 2nd). On the first day of egg collection, more mating vials were prepared with only two virgin female *Drosophila* and two male *Drosophila*, which were sealed with egg plates at the base. We prepared a total of eight mating vials over the course of two days.

Egg Collection and Measuring Eclosion

The next day, egg plates were removed, and eggs were carefully collected in groups of 30. Eggs were then placed in vials with low yeast concentrated food or high yeast concentrated food. We collected a total of 27 vials over the course of two days. On February 2nd, we collected enough eggs to be placed in eight 5% yeast vials and eight 1% yeast vials. On February 3rd, we collected more eggs to be placed in an additional five 5% yeast vials, and six 1% yeast vials. With the expectation of vial #17 (29 eggs) and vial #27 (22 eggs), all vials had 30 eggs.

After egg collection, vials were placed into the incubator at 25 degrees Celsius. For the next several days, vials were checked for dark pupa. If dark pupa were present in the vials, we would wait 24 hours for the pupa to eclose. Once vials had adult *Drosophila*, *Drosophila* were anesthetized in order to identify the sex of the *Drosophila*. *Drosophila* were counted each day except on February 20th and 21st. Once identified and recorded, *Drosophila* were disposed and vials were placed back into the incubator until there were no dark pupa present. Vials without any remaining eggs to be hatched were disposed.

Data Analysis

Once all flies were counted, data was entered into Excel. Average eclosion times were analyzed using a two sample t-test of unequal variances. Average flies per vial for both eggs that were harvested on 2/2/23 and 2/3/23 were plotted on a scatter plot. Finally, the average number of eggs to hatch and eclose was analyzed with a two sample t-test of unequal variances.

Results

The average eclosion time for *Drosophila* when given food with 1% yeast was 20.61 days post mating, while the average eclosion time for *Drosophila* which was given food with 5% yeast was 12.34 days post mating ($p<0.05$; $a=0.05$).

Eggs in vials 1-16 were collected on February 2nd, while vials 17-27 were collected on February 3rd. Because of the difference in post mating days, average flies per vial per day post mating were graphed separately to represent the difference in eclosion times (Figure 2). Here, there was a slight overlap between the end of *drosophila* eclosion from the group with food with 5% yeast and the start of the eclosion of the group with food with 1% yeast in both graphs from Figure 2 on the 16th day. The number of flies per vial differed between the two groups in terms of the timing of eclosion as well as the amount of flies present in each vial. It should be noted that *Drosophila* were not counted on days 17 and 18, so there is no data present.

At the end of the counting portion of the experiment, only one vial (vial #14) appeared to have all 30 eggs hatch and eclose. The rest of the 5% yeast vials had a higher average of 21.64 eggs hatch and eclose in comparison to the 1% yeast vials, which had an average of 6.21 eggs hatch and eclose (Figure 2; $p<0.05$; $a=0.05$). The highest number of eggs to hatch and eclose in the 1% yeast vials was 15 (vial #6).

Discussion

The results from our experiment support the hypothesis that *Drosophila* given food with a higher yeast content will have a faster eclosion rate and an overall higher amount of egg eclosion. In comparison with the low yeast concentrated food, the flies given the higher yeast concentrated food had an average eclosion time of 12.34 days while the flies given the lower yeast concentrated food had a significantly higher average eclosion time of 20.61 days (Figure 1). Additionally, more flies per vial had eclosed per day when given the higher-yeast concentrated food rather than the lower-yeast concentrated food (Figure 2). Overall, the average number of eggs that eclosed in the higher yeast concentrated food was higher than the lower yeast concentrated food flies (Figure 3). These results were expected, as flies given a lower nutrient rich diet have previously been reported to have slower eclosion rates as well as produce offspring that are smaller than usual (Tu and Tatar, 2003). While not the main focus of our experiment, the offspring in the lower yeast concentrated food did have flies that were much smaller than the offspring in the higher yeast concentrated food. In *Drosophila*, a nutrient rich diet is critical for metamorphosis, and a low nutrient diet slows down metamorphic development and results in slower eclosion rates (Reis, 2016; Quan and Eisen, 2018). While the 1% yeast food was lower in nutrients, the flies were still able to reach a critical mass at which they begin metamorphosis (Koyama et al. 2020). Our results suggest that lower nutrient availability impacts eclosion rates rather than eclosion itself.

While the results of our experiment were significant, there are errors to keep in mind. Due to the seasonal timing of this experiment, the food vials were consistently very dry. The lower-concentrated yeast was much drier than the higher-yeast concentrated food and required rehydration almost every day. It is possible that reduced water availability influenced the survival and eclosion of the *Drosophila*. Additionally, there were two days (day 17 and day 18) on which no one was able to count the flies that had eclosed on those days. Counting continued on day 19, but it is possible this disrupted the average number of flies per vial per day.

From this experiment, I would be curious to further investigate the life spans of the flies given the low nutrient food. The low nutrient food used in

this study had enough to sustain eclosion, but most of the flies that eclosed ended up being relatively smaller than the flies given the nutrient rich food. With lower nutrient availability, *Drosophila* are prone to infertility, diseases, and other external stressors (Klepsatel et al., 2019; Colinet and Renault, 2014). A next step to this experiment could be to study the fertility of the smaller female offspring and how changes in diet could have an impact. Additionally, with the smaller flies, I would be curious to observe the stress tolerance of the flies when exposed to external factors such as temperature changes, nutrient availability later in life, or exposure to infection, and how a poor diet during early development can ripple through an organism's life.

In understanding what is necessary for proper development in *Drosophila*, we can hopefully apply this knowledge to other organisms. For instance, just as nutrient intake is important for *Drosophila* larvae, nutrient intake is important for humans in utero. In addition, we can use this information to possibly identify what exactly is the most important nutrient in terms of development, and if timing has any relevance for when the nutrients are received.

Appendix

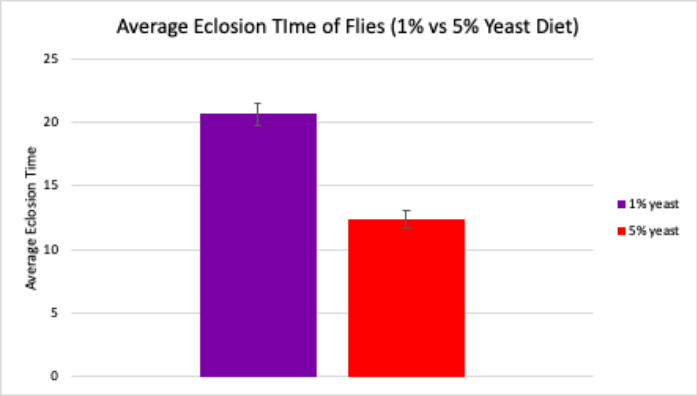

Figure 1. Figure 1 shows the average eclosion times of the *Drosophila* given either the 1% yeast food, or the 5% yeast food. p<0.05, a=0.05, t=27.7.

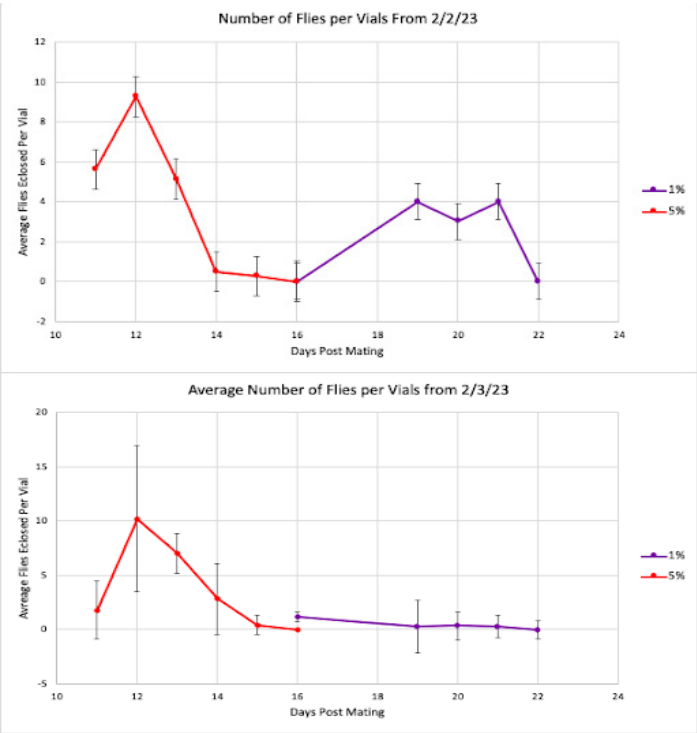

Figure 2. Figure 2 shows two scatterplots depicting the average number of flies per vial per day post-mating. The graph on the left plots the average number of flies per vial from eggs collected on 2/2/23. The graph on the right plots the average number of flies per vial from eggs collected on 2/3/23. Red represents the *Drosophila* given the 5% yeast food while the purple represents the *Drosophila* given the 1%.

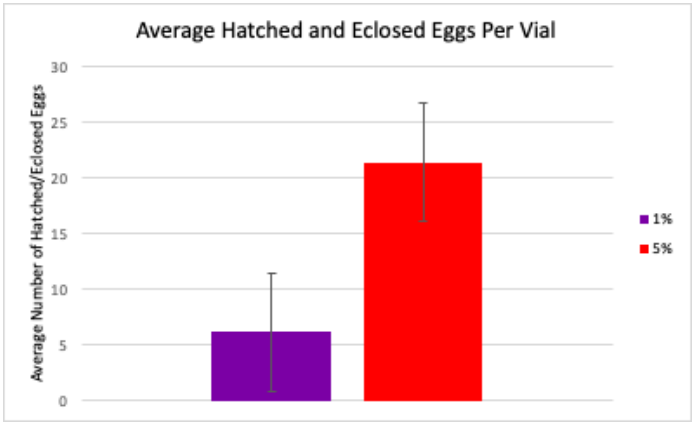

Figure 3. Figure 3 compares the average number of eggs that hatched and eclosed between the flies in the 1% yeast vials and 5% yeast vials. p<0.05, a=0.05, t=7.49.

References

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