Yeast-deprived diet extends *Drosophila melanogaster* larval period

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Purpose

The purpose of this experiment is to understand the effect on developmental timing when decreasing the percentage of yeast from 5% to 1% in a standard *Drosophila melanogaster* diet. This lab will specifically focus on the time to eclosion, which is the time it takes for eggs to mature into larvae, pupae, and finally, adult fruit flies. To answer this question, 30 eggs were transferred to multiple vials containing the standard diet for Drosophila melanogaster and either 1% or 5% yeast. Following roughly 10 days, the number of enclosed flies was counted and subsequently removed from each vial. Previous research has shown that a decrease in the nutritional value of food results in a longer period between the larval stage to pupariation (Shimada-Niwa & Niwa, 2014). This research suggests that the developmental timing of flies maturing in food with 1% food should be delayed compared to flies maturing in 5% yeast.

Methods

Preparation of fly diet

To prepare the customized diets for fly eggs to develop in, standard *Drosophila* diets were prepared identically except for yeast extract. In addition to either 1% or 5% w/v yeast extract, each diet contained agar (1% w/v), sucrose (2% w/v), dextrose (4% w/v), cornmeal (5% w/v), propionic acid (1% v/v), and Tegosept (0.16% v/v). To prepare food, agar, sucrose, dextrose, cornmeal and either 1% or 5% yeast were weighed out and mixed into ddH2O. After boiling the solution in a microwave, it was allowed to cool before adding propionic acid and Tegosept (microbial growth and mold inhibitors, respectively). Seven mL of prepared food was added to each fly vial and labeled respectively. All vials were allowed to cool completely before the addition of eggs.

Sample calculation for food preparation:
500ml total food x 0.05 cornmeal = 25g cornmeal
500ml total food x 0.01 propionic acid = 5 ml propionic acid

Experimental setup and data collection

Canton-S strain of *Drosophila melanogaster* was used for all experiments. Initial experimental setup consisted of collecting and isolating virgin females in vials (1/25). A few days later, egg lay plates were set up with four virgin females and three males (2/1). The next day (2/2), eggs were collected, and 30 eggs were transferred to each vial: 5 vials with 5% yeast food and 5 vials with 1% yeast food. While the eggs were developing, the vials were rehydrated with water twice. Beginning on the first day that flies eclosed (2/12), eclosed flies from each vial were counted, sexed, recorded, and then disposed of. This process was repeated until the final day of data collection on 2/24. All data was collected in the late afternoon or early evening of each day. *Note: no data was collected on 2/18.*

Data analysis

To compare the statistical significance between the time to eclosion for flies in 1% or 5% yeast, Microsoft Excel was used for all data analysis. Firstly, an F-test was used to determine if the variables being compared had equal or unequal variances. For variables where Fcritical > Fsample, a t-test assuming equal variances was used. Likewise, when Fcritical < Fsample, a t-test assuming unequal variances was used. In either case, T-tests were used to compare the statistical significance between the time to eclosion for 1% and 5% food. In each figure, standard deviation is shown for each data point/ bar using error bars. When applicable, a horizontal line over two bars represents a t-test was conducted; t-tests where P < 0.05 is denoted with a "" symbol and t-tests where P > 0.05 is denoted as "ns" for not-significant.*

Results

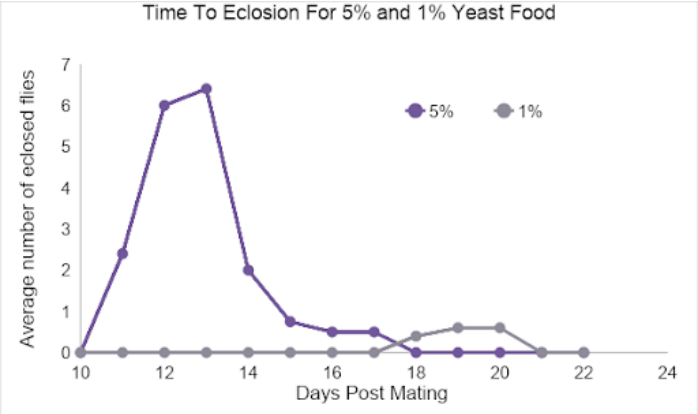

Figure 1. General trend of time to eclosion shows shorter time to eclosion for majority of flies from 5% yeast food compared to flies from 1% yeast food.

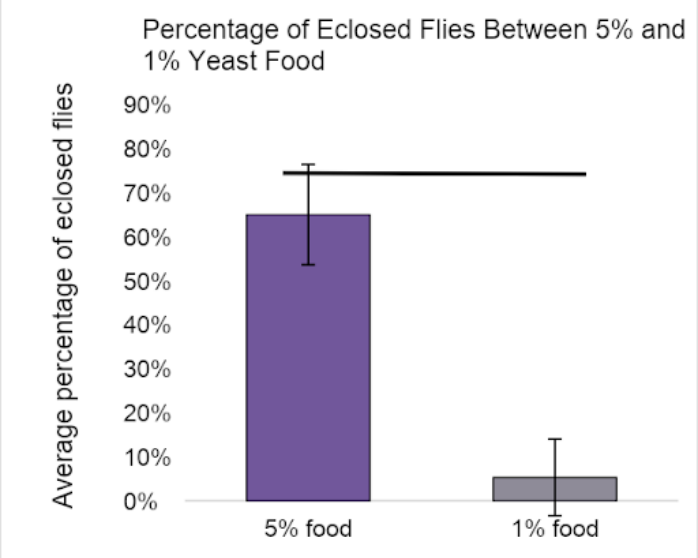

Figure 2. Higher average value of eclosed flies from flies in 5% yeast food compared to 1% yeast food (P < 0.05).

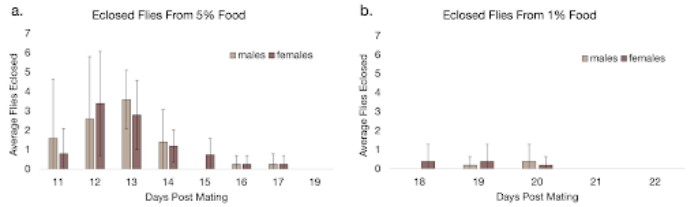

Figure 3. Examination of sex differences between time to eclosion for flies from 5% yeast food and 1% yeast food. (a) Comparison of the average number of male and female flies eclosed from 5% yeast food. (b) Comparison of the average number of male and female flies eclosed from 1% yeast food.

Figure 3 examines the differences between the number of female and males flies that eclose over time and between 1% and 5% yeast food. Figure 3a compares sex differences from 5% yeast food while figure 3b compares the sex differences from 1% yeast food. This figure shows that the trend for eclosion time for males and females is similar.

Discussion

In the present study, Figure 1 illustrates that the trend in developmental timing of flies is affected by the percentage of yeast in their food. Specifically, flies in 1% yeast food took multiple days longer on average than flies in 5% yeast food. Figure 2 shows that fewer

total flies in 1% yeast food eclosed compared to 5% yeast food. Finally, Figure 3 illustrates the difference in eclosion time between males and females from 5% and 1% yeast food. Overall, there does not seem to be sex differences between males and females in either condition.

Overall, these results are consistent with previous research and scientific literature. For example, previous research has determined that differences in food quality and nutrition based on percentage of yeast extract did have an effect on the development of flies (Shimada-Niwa & Niwa, 2014). One possible molecular explanation of this is that neurons from flies in low yeast have a more difficult time sending their signals to the prothoracic gland which controls sending the signals for pupariation from larva to adult flies (Shimada-Niwa & Niwa, 2014). This observation of delayed developmental timing based on malnutrition is a result consistent across a variety of model organisms including rats, mice, and even humans (Soliman et al., 2014). Evolutionarily speaking, it makes sense that development is delayed when nutrition in diet is restricted because it would not make sense for an organism to enter a growth phase when there is inadequate accessibility to the nutrients required to grow.

In terms of sex differences, previous literature has observed a sexual dimorphism between the developmental timing of males and females in nutritionally restricted diets which is inconsistent with the results from this study (Sánchez-Garrido et al., 2013). While this study used a different model organism, it did identify a delay of approximately 2 days between female and male rats reaching puberty when postnatally malnourished (Sánchez-Garrido et al., 2013). Notably, one major reason it is possible there was not a difference between males and females observed in this study is because the scale of development is so much faster in fruit flies compared to rats. It is plausible that a dimorphism between male and female flies existed in the current study, but it was a difference of hours and not days which is too short of a window to be observed in the 24 hours between data points in this study.

An alternative explanation to the result that suggests fewer flies eclose from 1% yeast food is that the flies may not require yeast for their nutritional value but are rather involved in a symbiotic relationship. While yeast do provide some nutritional value to the developing larva, some yeast spores survive digestion by fruit flies and can then be dispersed by the mobile Drosophila larva (Hoang et al., 2015;Tina Hesman Saey, 2014). Additionally, the yeast provides some protection to the developing fly against fungal infections (Hoang et al., 2015). To better understand if the yeast is nutritionally important for developing Drosophila or for the tasks they offer the developing Drosophila, dead yeast could be used in the fly food instead of live yeast.

To better understand sex differences between development in poor nutrition diets, this experiment could be repeated where data points are collected every 8 or 12 hours instead of approximately 24. Additionally, more stages of development, including the molting of larva, transition to pupa form, and time to eclosion could be recorded to better understand which specific stages of development are delayed. A greater sample size could also be used to reduce the margin of error. This may enable the use of a t-test between males and females eclosed at each time point to understand if there are statistically significant sex differences in development.

Another future direction for this study could be to alter other components of the fly food to see if they have a similar effect on developmental timing. For example, it could be interesting to decrease all components of their diet instead of just yeast extract. Although, this would make it difficult to determine which nutrient specifically is responsible for any changes observed in developmental timing.

A possible source of error from this experiment is that when eggs were transferred from egg lay plates to their vials some eggs may have been damaged or killed in this process. This may be why the highest percentage of total flies that eclosed was only 65%. It is also possible that there were very slight differences between the base of the fly diet between the two conditions (the parts of the food that were not yeast) because they were prepared by two different groups.

Broadly speaking, it is important to understand the developmental effects and mechanisms of malnutrition due to the unfortunately large number of people suffering from malnutrition worldwide. The World Health Organization reported that in 2021, over 800 million people were affected by hunger (WHO, 2022). To learn how to prevent and treat malnutrition, it is important to understand the underlying mechanism that causes delayed development and specific nutrients that are responsible.

References

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