

Malnourishment in *Drosophila* lengthens developmental timing and decreases offspring viability

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Purpose

The purpose of this experiment is to address the following research questions:

1. Does nutrition have an impact on developmental timing?
2. Does nutrition have an impact on sex determination?
3. Does nutrition have an impact on viability?

The techniques to measure this include creating two different concentrations of yeast, collecting female virgins, sexing flies, and utilizing mating cages. Excel F-tests and T-tests were run to measure statistical significance.

It has been well established that developmental timing is extended during dietary stress, and that lower nutritional availability decreases offspring viability (Güler, 2015). The purpose of this experiment is to test these pre-established findings by replicating a similar model of testing the influence of two different yeast concentrations. It is expected that there will be a delay in developmental timing in eggs placed in the lower yeast concentration. It is not expected that there will be a sex determination difference with different nutritional availability. Additionally, it is also expected that there will be a lower viability in the eggs in the lower yeast concentration.

Methods

General fly care

All flies were provided with the same amount of food with similar food moisture levels, kept in the same incubator, had the same density of flies per vial/mating cage, and were kept on the same day/night cycle.

Collecting female virgins and males

Twenty virgin female flies were collected to control for a potential confounding maternal fertility and/or fecundity differences; females can store sperm and fertilizing eggs after mating. Additionally, males were collected. This was done using a stereomicroscope. Flies were sedated using carbon dioxide and placed under the microscope. Two paintbrushes were used to manipulate the flies. Female virgins and males were grouped in separate vials. All flies were used 7 days after collection.

Making high and low yeast food

The yeast concentration was manipulated to test the time to eclosion. This was achieved through making two different food batches to add to vials: 1% concentration and 5% concentration. The following table lists the recipes that provide the number of grams of materials used; a standard precision scale was used. These ingredients were all combined in a 500 mL flask to make a total of 350 mL. 7 mL was used per vial in both concentrations.

Ingredient	1%	5%
1% agar (g)	5	5
2% sucrose (g)	10	10
4% dextrose (g)	20	20
5% cornmeal (g)	25	25
yeast (g)	5	25
HEATED		
1% propionic acid (mL)	5	5
0.16% tegosept (mL)	8 mL of 10% tego	8 mL of 10% tego

Table 1: Grams of materials used for each concentration recipe. For both concentration

recipes, the food was heated in a conventional microwave until boiling after the yeast extract was added and before 1% propionic acid was added.

Setting egg lay plates

Yeast was mixed with distilled water in a plastic weigh boat until a pancake batter consistency was reached. Egg lay plates were created by placing a thin 0.5-inch diameter circle of yeast paste on a 30 mm x 15 mm grape juice agar petri dish. This was given 20 minutes to dry completely to avoid flies getting stuck in the yeast paste. Holes were poked with a needle through a plastic conical flask. Previously collected flies were sedated with carbon dioxide again, and three males and three virgin females were placed in each flask. The petri dish was placed in the mouth of the flask and lab taped; once the flies woke up, these mating vials were flipped and placed in the incubator for 24 hours. Five mating vials were set up.

Egg collection and transfer

The egg lay plates were removed from the mating cages after 24 hours, and the parent flies were properly disposed of. The egg lay plates were placed under the stereomicroscope and a brush wetted with distilled water was used to transfer 30 eggs onto the high or low yeast food vials by investigating the yeast circle in the middle of the plate as well as the edges. Eight high yeast food and eight low yeast food vials were set up in total; these were placed back in the incubator and checked daily for eclosions. Eclosion began three days after these eggs were transferred.

Counting eclosed flies

Once flies began to eclose, vials were checked every 24± 2 hours for 11 days. Any food that appeared dry was given distilled water; these were typically the low concentration vials. If flies had eclosed, they were sedated and placed under a stereomicroscope. Males and females were documented and the total number of flies per vial was accounted for; eclosed flies were properly disposed of, and the vials were resealed and placed back into the incubator.

Statistical measurements

All statistical tests and figures were created in Microsoft Excel. Between the two diet conditions, number of days until eclosion, male to female eclosions, and viability between the two diet conditions were compared. For all three variables, an F-test was run comparing the two concentrations or the male to female ratio. The data analysis toolkit in Excel was utilized to create a T-test chart that includes means, variances, observations, degrees of freedom, t stat, and p-value. The standard used when checking for significance within the data was 0.05.

Results

The two diet conditions produced results consistent with past findings. Developmental timing, male to female ratio, and viability was measured in the context of a high yeast and low yeast concentration (Table 2).

Day	10	11	12	13	14	15	16	17	18	19	20	21
1% ♂	0	0	0	0	0	0	0	0	0	0	0	0
1% ♀	0	0	0	0	0	0	0	0	0	4	15	2
1% total	0	0	0	0	0	0	0	0	0	12	29	4
5% ♂	7	17	43	12	5	1	0	0	0	0	0	0
5% ♀	2	12	29	19	3	2	0	0	0	0	0	0
5% total	9	29	72	31	8	3	0	0	0	0	0	0

Table 2: Raw data. Grey indicates the days until eclosion. Orange indicates the 1% yeast concentration food condition, and blue indicates the 5% yeast concentration food condition.

Both conditions include the sex eclosed and the total amount eclosed for each diet condition per day.

The high yeast concentration diet condition was expected to yield a faster eclosion time compared to the low yeast concentration diet condition (Güler, 2015). This would be reflected in the number of days it takes for the flies to eclose. Faster eclosion indicates faster development, and, likewise, slower eclosion indicates slower development (Figure 1).

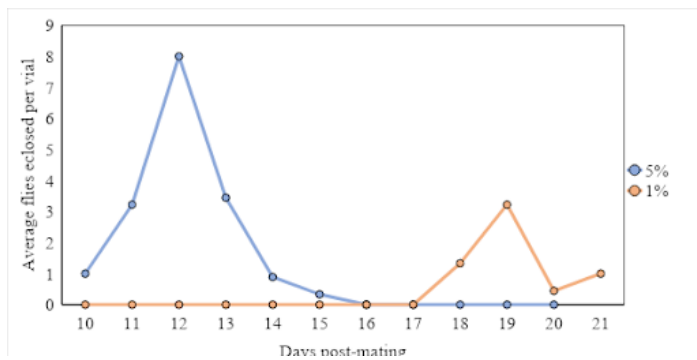


Figure 1: Days until eclosion in a high and low nutrient environment. Orange indicates the 1% yeast concentration diet condition, and blue indicates the 5% yeast concentration diet conditions. $P = 5.42088E-11$

High yeast concentration diet condition flies began eclosing ten days post-mating and peaked on day 12; the peak number of flies eclosed was eight (Figure 1). Eclosions declined steeply on day 13 and slowly tapered off until there were zero eclosions from days 16-21 (Figure 1). Low yeast concentration diet condition flies began eclosing eighteen days post-mating and peaked on day 18; the peak number of flies eclosed was 3.2 (Figure 1). Eclosions declined steeply on day 20 and went slightly up on day 21 (Figure 1). There is a 6-day difference before eclosion between the high and low concentration conditions, with eclosion taking longer in a low nutrient environment (Figure 1). There was a statistically significant difference between the days until eclosion ($P = 5.42e-11$) (Figure 1). Additionally, qualitatively viewing these results, the peak number of eclosions at the low concentration closely matches with both the slight increase and decrease in the high concentration (Figure 1).

The average number of flies eclosed between the two conditions over the 11-day time span was measured (Figure 2).

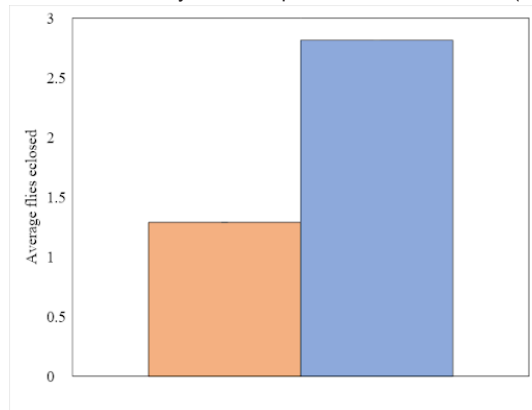


Figure 2: Average number of flies eclosed over an 11-day period. Orange indicates the 1% yeast concentration diet condition, and blue indicates the 5% yeast concentration diet conditions. $P = 0.01895009$

The average number of flies eclosed at different concentrations varied between the low and high yeast concentration diet conditions (Figure 2). Qualitatively, there is a visual distance between the bars; quantitatively, the 1% concentration yielded an average of 1.29 flies over an 11-day period, and the 5% concentration yielded an average of 2.81 flies over an 11-day period (Figure 2). There was a statistically significant difference between the average flies eclosed ($P = 0.01895009$) (Figure 2).

During the data collection, a potential sex difference during developmental timing was observed. To investigate this observation, the day, average number of eclosions, and male to female ratio was compared within each diet condition (Figure 3a and b).

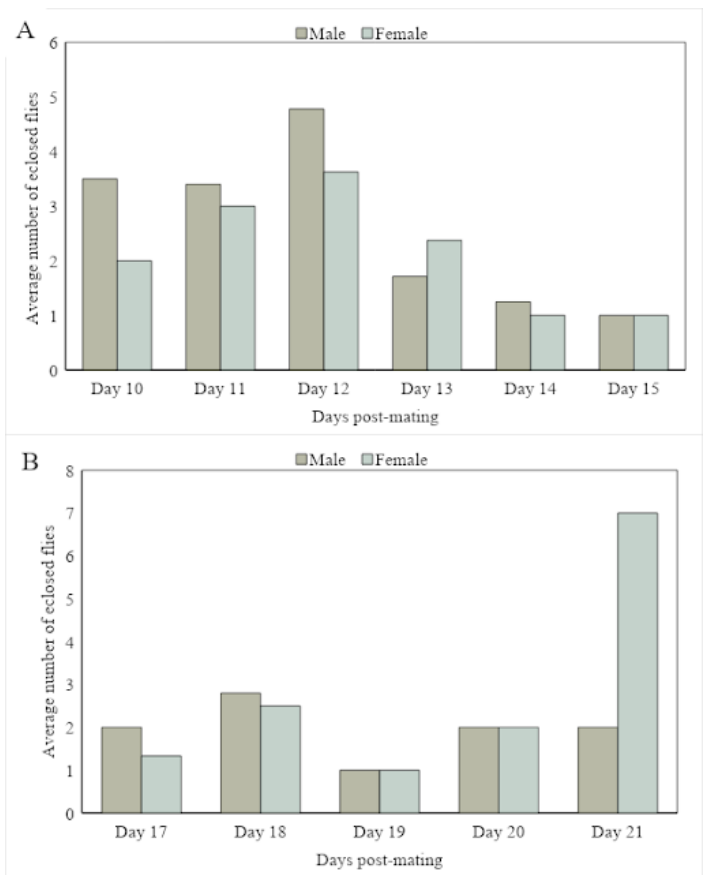


Figure 3: Daily ratio of male to female eclosions between different nutritional conditions (a) 5% yeast concentration condition had eclosions between days 10-15 post-mating, with males in green and females in blue ($P = 0.57124513$). (b) 1% yeast concentration condition had eclosions between days 17-21 post-mating, with males in green and females in blue ($P = 0.050604214$).

In both treatments, there was no statistically significant difference between the number of males and females that eclose with regards to both developmental timing and viability (Figure 3a and b). Qualitatively, in the 5% yeast concentration condition, there is a drop-off in the male eclosions between day 12 and 13; day 14 and 15 also have low numbers of both males and females (Figure 3a). Both males and females follow a small jump, then decrease in eclosions; however, the male population appears larger in the earlier days (Figure 3a). In the 1% yeast concentration condition, there is a low amount of both males and females until day 21, when there is a sudden spike in females but a small number of males still (Figure 3b). The males and females appear more consistently similar in the 1% treatment compared to the 5% treatment (Figure 3a and b).

Discussion

In *Drosophila*, days until eclosion were significantly different between a low nutrient condition and a high nutrient condition, with low nutrient flies taking longer to eclose compared to high nutrient flies. Additionally, the viability of the offspring was significantly different between the treatments; the low nutrient condition had a lower number of eclosions compared to the high nutrient condition. There was no significant difference with sex differentiation at different times in development or between the two treatments. These results corroborate years of nutritional research that has been studied extensively in many organisms.

Maturation into adult form and eclosion can only happen when the juvenile has attained the nutrients needed to grow, reproduce, and maintain its body (Danielson, 2013). In a low-nutrient diet condition, the developmental period was extended because maturation took longer due to insufficient nutrient availability (Danielson, 2013). The same can be said for viability: because less nutrients were

available, fewer flies were able to fully mature and eclose. Additionally, in a high-nutrient diet condition, the developmental period was shortened due to an enhanced juvenile growth phase (Danielson, 2013). Again, the same can be said for viability: more flies eclosed because there were more available nutrients for growth and maturation. The basis of fitness relies on the pressure of a carrying capacity due to resources, space, and competition. In lower nutrient environments, there may be a lower viability rate due to low resource availability compared to higher nutrient environments. Perhaps a reason all the high-nutrient diet condition flies did not eclose is because the space in the vial was infringing on all thirty eggs per vial fully developing.

Alternatively, there may have been unaccounted differences in the food consistency, including the low-nutrient diet condition vials needing significantly more distilled water added over multiple days to increase the moisture of the food compared to the high-nutrient diet condition vials needing a few drops total over the whole experimental period. Dryer vials have the potential to slow down the developmental period due to larvae being physically limited from moving. This may have been a potential error due to improper food preparation or may just be the nature of having a low amount of yeast in the food recipe. This potential physical limitation placed on the low-nutrient treatment offspring may account for why they took longer to eclose compared to the high-nutrient treatment. Potentially tweaking the recipe to account for this consistent food dryness may decrease alternative explanations for the results and help verify and build on the results currently found. Other potential sources of error include not accounting for the age or fertility of the males. Potentially, males that have mated prior to the experiment may have a difference in their sperm compared to males that have not previously mated. Furthermore, the recipe for the egg lay plates was not exact, and the larvae may have been exposed to different levels of yeast at the very beginning of their development. This may have contributed to different developmental timing events and offspring viability.

Additionally, adding more diet conditions with different percentages and/or quality of yeast may build on the current results. This may include using desiccated yeast, live yeast, different brands of yeast, or different ingredients with the yeast and adding a 2%, 3%, and 4% concentration to see if the extent of the relationship between diet and developmental timing. Furthermore, altering the size of the vials while maintaining the same amount of food may account for the limitation of physical space on the developing larvae and specifically test diet.

Nutrition is a topic that is not completely understood, and this research has far-reaching implications. In humans, the influence of maternal nutrition during development of a fetus is understudied. Studying *Drosophila* provides the ability to manipulate the nutrition directly in the vial and see the effects without the maternal metabolism influencing the nutrient availability. Additionally, the access of *Drosophila* is much easier ethically compared to human subjects. Even in primates, which are closer in evolutionary lineage to humans compared to fruit flies, there is a large influence of nutrient restriction during pregnancy on brain development (Antonow-Shloke, 2011).

Moderately restricting maternal nutrition during baboon pregnancy results in cerebral developmental disturbances including cell proliferation and apoptosis imbalance, impaired glial and neuronal process formation, and many more mechanistic issues that have life-long implications for the fetal baboon (Antonow-Shloke, 2011). This provides further reasoning as to why research on nutritional influences on development needs more attention. Additionally, this provides scientific footing that socio-economic disparities, poverty, food deserts, and many more diet-based issues facing America and other countries may potentially have a lasting generational impact; there is an intersectional overlap between nutritional quality, economic viability, and environmental sustainability (Herforth, 2014). This overlap needs to be documented through data collection to monitor and comprehend the inequities occurring in groups experiencing potential nutritional harm (Herforth, 2014). These broader issues have implications in the world of scientific discovery and should be at the forefront of developmental research.

Acknowledgements

Djurdjina Jovanovic and Jesus Figueroa were my lab partners helping to conduct this experiment and contributed significantly to the completing the methodology stated above.

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