Effects of Diet on Developmental Timing in *Drosophila melanogaster*

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Purpose

Nutrient availability is a major factor that influences key characteristics of development, such duration of the development period. Here, we aim to address whether the concentration of dietary protein influences the time it takes for *Drosophila melanogaster* to develop from eggs to adults. To test this, we reared Drosophila melanogaster in food vials that contained either 1% yeast or 5% yeast concentration and measured the length of the development period to reach eclosion and percent viability of each nutrient concentration. We expect flies reared on 1% yeast food to develop slower (and to have a lower viability) than flies reared on the 5% yeast food to demonstrate that nutrient availability influences development time.

Methods

Flies

Thirty-two virgin males and thirty-two virgin females of the Canton S strain of *D. melanogaster* were collected shortly after eclosion. Females and males were each transferred to new food vials twice a week for one week. At one week of age, flies were transferred to a mating vial with a plate for egg collection. Each mating vial contained 2 females paired with 2 males. The grape agar plate contained a layer of yeast paste to encourage clustered egg laying behavior. Eggs were collected from plates and 30 eggs were transferred to the surface of each food containing vial (see SI). Eight vials contained 1% yeast concentrated food and eight vials contained 5% yeast concentrated food. The number of eclosed male and female flies were counted every 24 hours for 5 days after the start of eclosion for each condition of yeast concentration. All flies were maintained at 25°C and 60% air humidity.

Statistics

All data analysis was performed in Microsoft Excel and SPSS. The α level was set to 0.05 unless otherwise specified. Two sample T-tests assuming equal variances were used, unless equal variance was violated. To calculate developmental viability, we divided the number of eggs that eclosed in each vial by 30, since each vial contained 30 eggs, to obtain the percent of developmental viability.

Results

Sex Distribution

After the fly eggs were transferred to the food-containing vials, the total number of males and females that eclosed after post-mating were recorded each day after eclosion began for the group that received 1% yeast food (Fig. 1a) and for the group that received 5% yeast food (Fig 1b). We sought to determine whether there was difference in the number of males and females that eclosed within each yeast concentration, and between the yeast concentrations. To test whether there was a difference in average number of eclosed male and female flies given 1% yeast (M=7, SD=6.44; M=7.4, SD=6.77) or 5% yeast (M=17.6, SD=18.45; M=15.6, SD=12.32), a Repeated Measures ANOVA was performed. The RM ANOVA revealed no significant difference between the average number of males and females (no significant main effect; F1 =0.176, p>0.05), nor a significant difference for the average number of males and females between 1% yeast and 5% yeast (no significant interaction; F1 =0.396, p>0.05).

Yeast Concentration on Development

After determining the distribution of the sex of eclosed flies, we sought to determine the distribution of daily average of total (male + female) flies that eclosed per vial post-mating for flies that received 1% or 5% yeast food (Fig 2a). An independent samples t-test revealed no significant difference in the daily average of total eclosed flies between the 1% yeast (M=1.8, SD=0.71) and 5% yeast conditions (M=4.15, SD=3.8) (t(4.5)=-1.276, p>0.05). After the all the flies were finished eclosing, which was determined by zero eclosed flies in all vials for each yeast condition, we sought to determine whether there was a difference in the time it took for all the flies to eclose for each of the yeast concentrations (Fig. 2b). To test this, we first performed a two sample F-test, which revealed that the equal variance assumption was not violated for the average number of days of fly eclosion between the two yeast concentrations (F6=2.52, p=0.14). Then we performed a two-sample t-test, which revealed a significant difference between the average number of days of fly eclosion between the 1% yeast condition (M=20.33, SD=0.92) and the 5% yeast condition (M=12.14, SD=0.67) (t(14)=20.31, p<0.001).

Yeast Concentration on Viability

After determining the average eclosion time between the yeast conditions, we were interested in whether there was a difference in developmental viability between the yeast conditions (Fig 3). A two sample F-test revealed that the equal variance assumption was not violated for the average percent viability between the two yeast concentrations (F7=0.76, p=0.36). Then we performed a two-sample t-test, which revealed a significant difference between the average percent viability between the 1% yeast condition (M=30%, SD=16%) and the 5% yeast condition (M=69%, SD=018%) (t(14)= -4.6, p<0.001).

Discussion

In this study we sought to understand whether nutrient availability in the larval diet would affect different characteristics of development in *Drosophila melanogaster*, such as the distribution of sex in the adult flies, length of time to reach eclosion (transition to adult), and viability of the eggs. We found that the concentration of yeast did not affect the distribution of males and females between larvae reared on food containing 1% or 5% yeast. These results were expected, since sex determination in *Drosophilais* dependent on the number of X chromosomes inherited. Specifically, if a fly inherited two X chromosomes, it would be females, whereas if it inherited one X chromosome, it would be male (Bridges, 1925). Thus, it is unlikely that yeast concentration would affect sex determination. We also found that yeast concentration did not significantly affect the average number of total flies (sum of males and females) that would eclose on a given day. This result is contrary to what we predicted, since we hypothesized that more flies would eclose on average when the larvae are given a higher concentration of yeast in their food. We based this prediction on a previous finding that larvae that are only fed sucrose die more often and fail to growth compared to larvae given a protein-rich diet, demonstrating that nutrient restriction halts development (Danielsen et al., 2013). In this study, generally, more flies reached adulthood in the 5% yeast condition than the 1% yeast condition in this sample, but the trend was stronger in the sample of flies collected for a replication of this study (SI Table 1). Thus, the non-significant results are likely due to sampling variability.

Additionally, we found that the average time to reach adult eclosion was significantly shorter when the larvae were fed a higher concentration of yeast, than flies fed a lower concentration of yeast. This result was expected, since we predicted a longer development period in larvae that were fed a lower yeast concentration. This prediction was based on a previous study that shows that nutritional deficiencies decrease the speed of development, growth rate, and body mass (Gebhardt & Stearns, 1988). The link between yeast concentration and length of development period may be explained by a molecular pathway that couples nutrition and developmental timing. Canonically, the insulin/IGS system controls the growth rate in Drosophila by regulating ecdysone biosynthesis and Ras/Raf signaling (Brogiolo et al., 2001). Specifically, reaching a certain body size threshold triggers the biosynthesis of ecdysone, which allows for the transition from larva to pupa. The study found that TOR signaling in the prothoracic gland (a nutrient sensor), is required for the production and accumulation of ecdysone, and thus development (Layelle et al., 2008). Therefore, in this study, low nutrient availability in the 1% yeast condition should delay TOR signaling and the buildup ecdysone needed to continue through the stages of development to eventually reach adulthood. However, one limitation to this study

is the lack of evidence that these molecular mechanisms caused the delay in development. Future studies should expand upon the connection between TOR signaling and adult eclosion as a function of protein availability.

Lastly, we also found that larvae reared on a higher concentration of yeast had a higher viability than larvae reared on a low concentration of yeast. This result was expected, since as mentioned earlier, nutritional deficiencies halt development and led to a higher larval mortality rate (Danielsen et al., 2013). Therefore, the flies in this study could not develop into adults if they died during an earlier development stage.

Future research is still needed to determine the molecular mechanisms that lead to larval death under nutrient deficient conditions, as well as determining whether ecdysone accumulation explains the developmental delay in this experimental paradigm. Future studies should also determine whether the length of the development period is sexually dimorphic when larvae are fed food with concentrations of yeast. Future studies should also be done to explore whether different larval diets result in change in lifespan and fertility to demonstrate the importance of larval diets on later life characteristics and survival.

Overall, the results of this study demonstrate the importance of a rich protein diet for larvae to increase development speed and viability in Drosophila melanogaster. Furthermore, many genes and molecular mechanisms are conserved between flies and humans. Hence, these results in combination with findings in the scientific literature, invite further research to explore the connection between developmental and nutrient-dependent mechanisms in mammals.

Figures

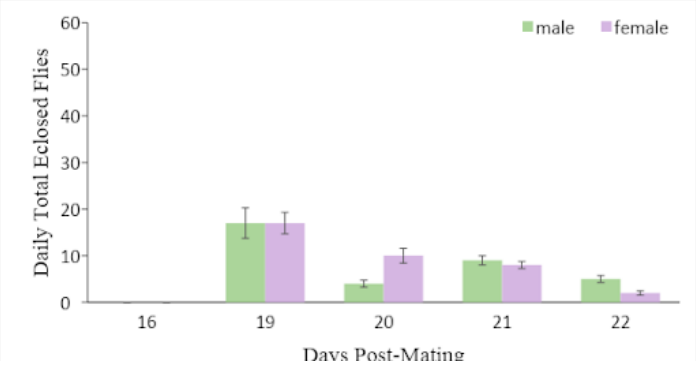

Figure 1a. Total number of male and female flies that eclosed each day (16, 19, 20, 21, and 22) post-mating when raised on food medium containing 1% concentration of yeast. Error bars indicate standard deviation.

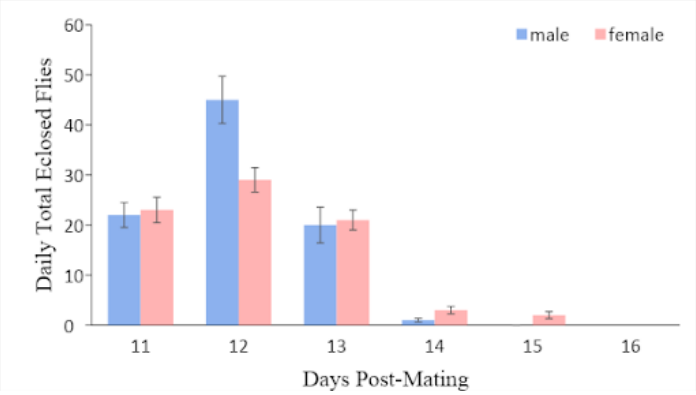

Figure 1b. Total number of male and female flies that eclosed each day (16, 19, 20, 21, and 22) post-mating when raised on food medium containing 5% concentration of yeast. Error bars indicate standard deviation.

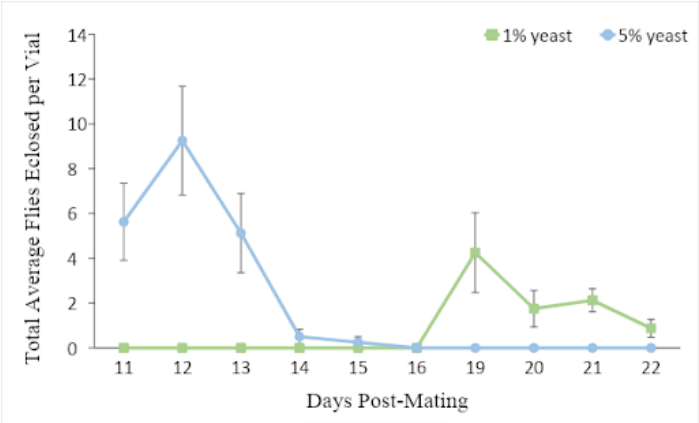

Figure 2a. Total average of flies (males + females) that eclosed each day after post-mating for flies raised on food medium containing 1% or 5% concentration of yeast. Error bars indicate standard deviation.

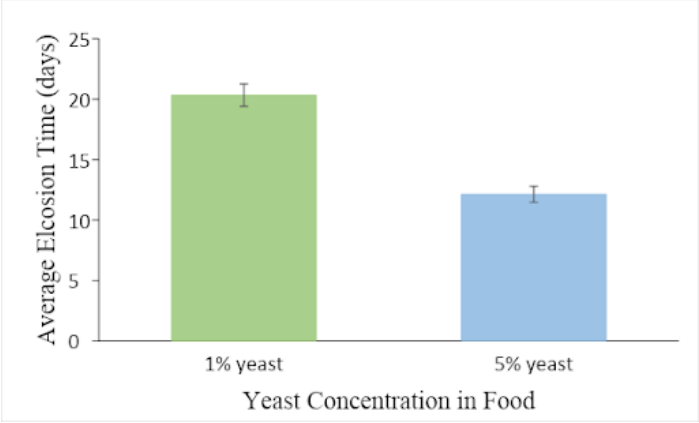

Figure 2b. Average eclosion time in days as a function of yeast concentration in fly food medium (1% yeast and 5% yeast). Error bars indicate SEM.

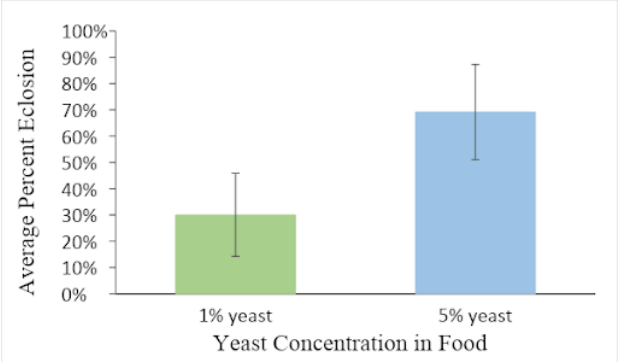

Figure 2c. Average percent of flies that eclosed when raised on food medium containing 1% or 5% concentration of yeast. Error bars indicate standard deviation.

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Supplemental Information

Fly food

Flies were raised on fly food containing: 1% agar, 2% sucrose, 4% dextrose, 5% cornmeal, 1% propionic acid, 0.16% Tegosept, 1% or 5% yeast, and water for the remaining volume. Percentages are based on the final volume of food.

	First replicate	Second replicate (BOGO)
1% yeast	72	30
5% yeast	166	111

Table 1. Sum of flies collected for the first and second (BOGO) experimental replication.