Effects of nutrients from bananas on maturation time of *Drosophila melanogaster:* preference of *Drosophila melanogaster* for bananas from different sources.

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Abstract

The objective of our research was to investigate whether the addition of nutrients affects the maturation time and the substrate preference of *Drosophila melanogaster* larvae. The nutrient sources used in our research were bananas, either conventionally or organically grown. Two experiments were designed to conduct the study. In the development experiment, three treatments were used to determine maturation time: dextrose medium plus organically grown bananas, dextrose medium plus conventionally grown bananas and dextrose medium with no added bananas. In the choice experiment, three choices of medium were given to determine substrate preference: organic bananas, conventional bananas and dextrose medium. Developmentally, the control showed a faster maturation time, however, this was not significant. There was no maturation to adult flies in the medium with added organic banana. This was possibly due to fungus growth inhibiting the larvae growth in the organic and conventional banana treatments. As well, there was no significant preference in substrate type because the apparatus failed to allow the larvae to make a distinct and definite choice. Overall, the addition of nutrients does not significantly affect *Drosophila melanogaster* larvae in terms of maturation time and preference of substrate type.

Introduction

The organism of our study is *Drosophila melanogaster,* a species that belongs to the family Drosophilidae, which is commonly known as a fruit fly or vinegar fly (Chhabra *et al.* 2013). For our research experiment, we used the larvae of wild-type fruit flies. The common wild-type fruit flies are brown in colour with red eyes, and have black horizontal stripes on the abdomen. They have three pairs of legs, a set of wings, and bristles along their bodies (Ng and Kopp 2008).

The objective of our research was to investigate whether or not the addition of nutrients affects the maturation time and the substrate preference of *D. melanogaster* larvae. The nutrients

used in our research were represented by bananas, either conventionally or organically grown. The conventional methods for growing produce include applying chemical fertilizers to promote plant growth, spraying synthetic insecticides to reduce pests, and using herbicides to manage weeds (Mayo Clinic 2012). In contrast, organic methods include applying natural fertilizers to feed soil and plants, applying pesticides from natural sources, and using methods such as crop rotation to protect from weeds (Mayo Clinic 2012). Organically grown food is a fast growing market since it is considered to be nutritionally superior to conventional food. Organic food reportedly has noticeably more vitamins, carotenoids, unsaturated fatty acids, and polyphenols (Chhabra *et al.* 2013).

D. melanogaster has been widely used for biological research; from previous experiments, flies subjected to a nutrient-rich organic produce diet had greater fertility and longevity compared to flies subjected to a conventional produce diet (Chhabra *et al.* 2013). Through our research, we attempted to determine if *D. melanogaster* larvae preferred the addition of nutrients in organic bananas and if these nutrients affected their maturation time.

In order to do so, we designed two experiments. The purpose of the first experiment was to see if the addition of nutrients affected the maturation time of *D. melanogaster* larvae. The hypotheses for our first experiment were as follows:

H_{o1}: Addition of nutrients increases or does not affect the maturation time of *D. melanogaster*.

H_{a1}: Addition of nutrients decreases the maturation time of *D. melanogaster*.

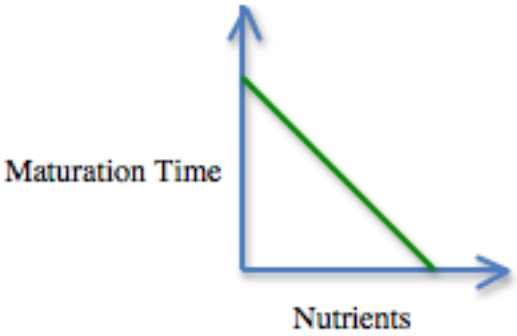

Figure 1. A model for how increased nutrients would affect maturation time of *D. melanogaster* larvae.

Figure 1 suggests that the maturation time should decrease with respect to an increase in nutrient value. For instance, the maturation time of larvae in a medium with bananas was expected to be less than the maturation time of larvae in a medium without bananas.

Our second experiment was designed to see if *D. melanogaster* larvae preferred a particular type of substrate when subjected to conventional bananas, organic bananas, and dextrose medium in a Petri dish. The hypotheses for this experiment were as follows:

H_{o2}: Larvae of *D. melanogaster* do not prefer media with additional nutrients for development.

H_{a2}: Larvae of *D. melanogaster* prefer media with additional nutrients for development.

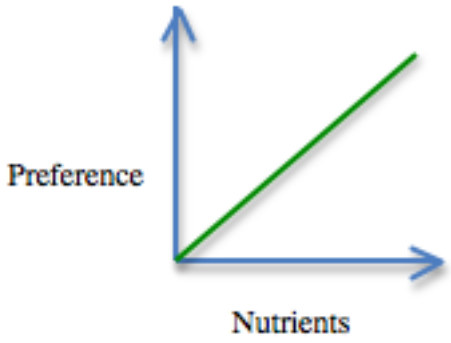

Figure 2. A model for how increased nutrients would affect the substrate preference of *D. melanogaster* larvae.

Figure 2 suggests that preference for a medium should increase with an increase in its nutrient value. For instance, the larvae would prefer to mature in a medium with bananas to a dextrose medium without bananas.

The results obtained through our experiments might give some insight on whether buying certain bananas will attract and produce more *D. melanogaster* or not.

Methods

Experiment 1: Development Experiment

In this experiment, we tested larvae's maturation time on organic and conventional banana substrates. There were two treatments and a control, each with five replicates. The control consisted of a dextrose medium with agar, yeast extract and mood inhibitor. The organic treatment consisted of mashed organic banana added on top of the control medium. Similarly, the conventional treatment had mashed conventional banana added on top of the control medium. First, we used two mortar and pestles, one for each type of banana to mash the bananas until they were well mixed (Figure 3). We added 5.0 g of the different bananas into appropriate vials. We repeated this for the remaining five replicates in each treatment and set them aside.

Figure 3. The appearance of fully mashed bananas

In order to collect *D. melanogaster* larvae, we took the vials containing wild-type *D. melanogaster* at all maturation stages and anesthetized the adult flies with CO_2 gas. In order to collect live larvae, we took them from the original dextrose medium using sterile loops and placed them into petri dishes containing 18% sucrose solution (Figure 4). This allowed the larvae to be taken from the medium without killing them. We placed five larvae into each vial until they were all inside their respective treatments. In total, there were 75 larvae in 15 replicates (Figure 5). We stored the vials in a dark room at an average temperature of 23.6 °C. The optimal temperature growth is around 25.0°C (Pandey and Nichols 2011).

Figure 4. Removing larvae from 18% sucrose solution. A larva is seen in the loop.

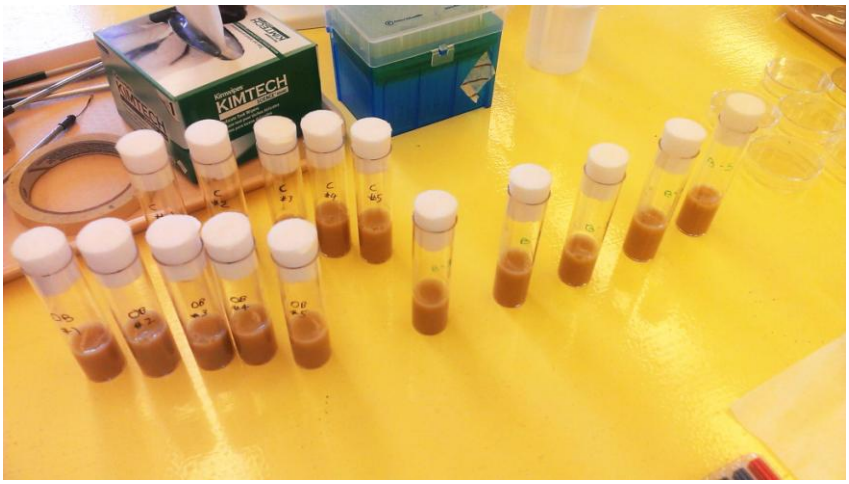

Figure 5. The set up of three treatments (control, organic banana, conventional banana) with five replicates each.

The experiment ran for 12 days with data collected on days 1, 4, 5, 6, 7, 8, and 12. Data collections consisted of quantitative data and qualitative observations. Quantitative data included the number of adult flies observed in a vial and qualitative observations included larval size, changes in growth stage, colour of the larvae as well as the state of the medium. We then took the means for each treatment and calculated their respective 95% confidence intervals. This analysis determined if there were significant differences among the means of the treatments: conventional bananas, organic bananas, and the control medium.

Experiment 2: Choice Experiment

In the second experiment, we observed which medium larvae preferred, through their first choice and final choice. There were 20 replicates; each larva was placed in a 100 mm diameter petri dish with three choices offered: conventional banana, organic banana and dextrose medium. We prepared each type of banana by mashing them in separate mortar and pestles. We divided the petri dish into three spaces using a divider for each choice and labeled the areas accordingly

so that each replicate has the same medium placement. We used a sterile spatula to spread a thin layer of each choice into the appropriate areas (Figure 6). Each nutrient source was spread without contamination, as each end of the spatula was associated with only one nutrient.

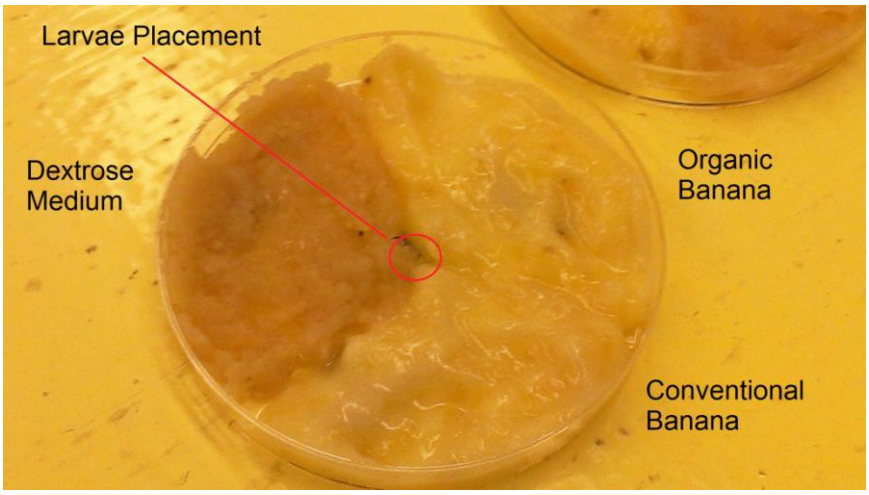

Figure 6. The set up for the three choices: dextrose medium, organic banana and conventional banana. The placement of larvae is indicated above.

Similar to the development experiment, we anesthetized and transferred adult flies to the morgue to isolate larvae. We placed the larvae into an 18% sucrose solution in a petri dish to separate them from the medium. We used a sterile loop to take a larva out and placed it into the centre of the three choices. We categorized the larva's first choice as the first substrate it moved to. We repeated this process for 20 replicates. After 20 minutes, we observed all replicates again and considered this their final choice. We documented observations on larvae for each replicate. After the experiment, we tallied each treatment and analyzed the final total values through the chi-square test.

Results

The maturation time appears to be shortest in the control, while the longest is the organic banana treatment. Observed on day five, there were four mature flies over all control replicates and in the conventional banana treatment, there was only one. However, the control maturation time was not significantly different from all other treatments (conventional and organic bananas).

Figure 5 shows that conventional banana treatment and control are not significantly different from each other because their confidence intervals overlapped. The only exception is at day eight, where the mean of the number of control adult flies (3.4 flies) was more than the mean of the number of conventional banana adult flies (1.2 flies) and the confidence intervals did not overlap. Since the organic banana treatment showed no maturation in all five replicates, there were no adult flies or variance measured.

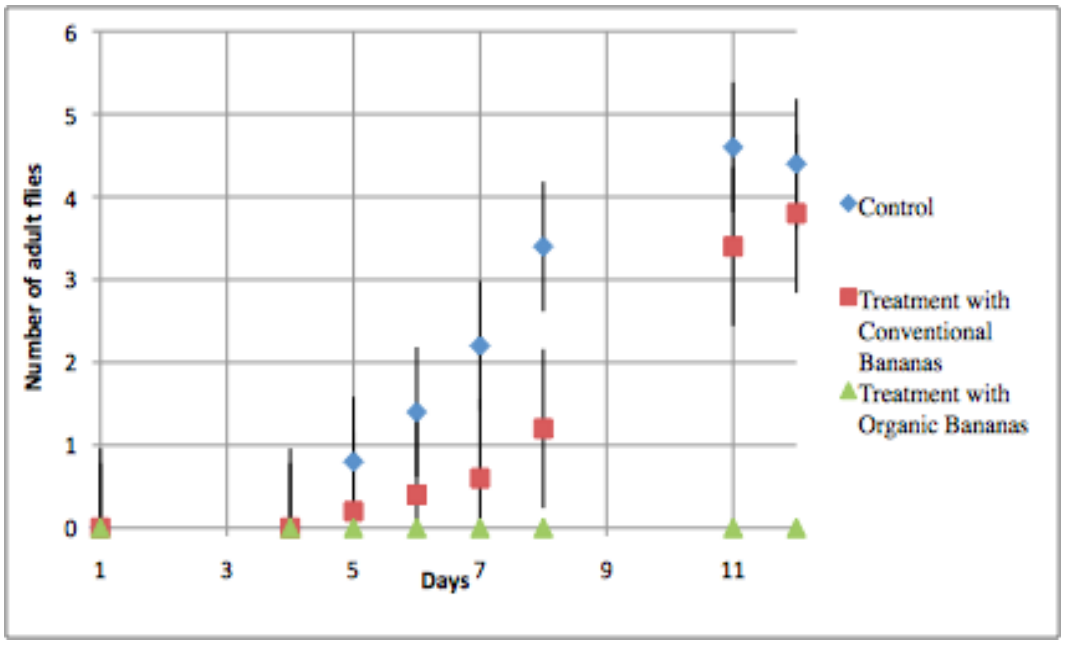

Figure 7. The effect of nutrients on the maturation time of *D. melanogaster* from larval stage to adult flies when placed on organic or conventional bananas and control medium. Mean numbers of adult *D. melanogaster* flies are observed over 12 days. Bars represent 95% confidence intervals, n = 3.

The control, conventional banana treatment, and organic banana treatment have confidence intervals of ± 0.8, ± 1.0, and ± 0, respectively. The conventional banana treatment has the greatest variance, while organic banana has no variance (Figure 7).

Qualitative observations noticed throughout our experiment included the stages of maturation of the larvae. The media were also observed; fungal growth developed more quickly and was more apparent in the organic banana treatment. In addition, the adult flies in the conventional banana treatment appear to be more energetic and lively than the flies in the control as shown through their movement within the treatment vials.

In our second experiment, there was no significant preference to which substrate type larvae were attracted to when subjected to dextrose medium, conventional bananas and organic bananas (Figures 8 and 9). With the expected choice of organic bananas, we analyzed larvae's first and final choice using the chi-square test (See sample calculation of chi-square test). This analysis shows if there is a preference or if it is a random choice between selections. For three treatments, the critical chi-square (χ^2) value with two degrees of freedom is 5.99.

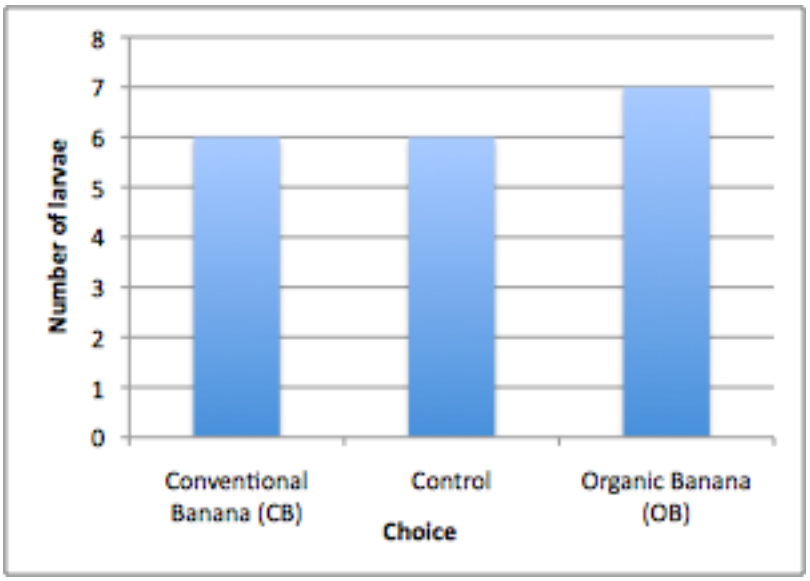

Figure 8. The number of *D. melanogaster* larvae that first chose a specific substrate. n = 20, p = 0.05, χ^2 = 0.16.

With regards to the larvae's first choice, there is no distinct trend towards one substrate type (Figure 8). However, after 20 minutes, larvae appear to stray away from conventional bananas (3 larvae) and migrate to either control (9 larvae) or organic banana (8 larvae) as shown in Figure 9. The calculated chi-square value after 20 minutes (3.10) is still less than the critical chi-square value (5.99), thus indicating there is no significant preference for a type of substrate.

Figure 8 shows that the chi-square value for the first choice data is very small (0.16). Similar results are seen in for the final choice data at 3.10 (Figure 9). In both the first choice and final choice, the calculated chi-square value was less than the critical chi-square value. This is shown by the calculated χ^2 value for the first choice (0.16) being smaller than the calculated χ^2 for the final choice (3.10).

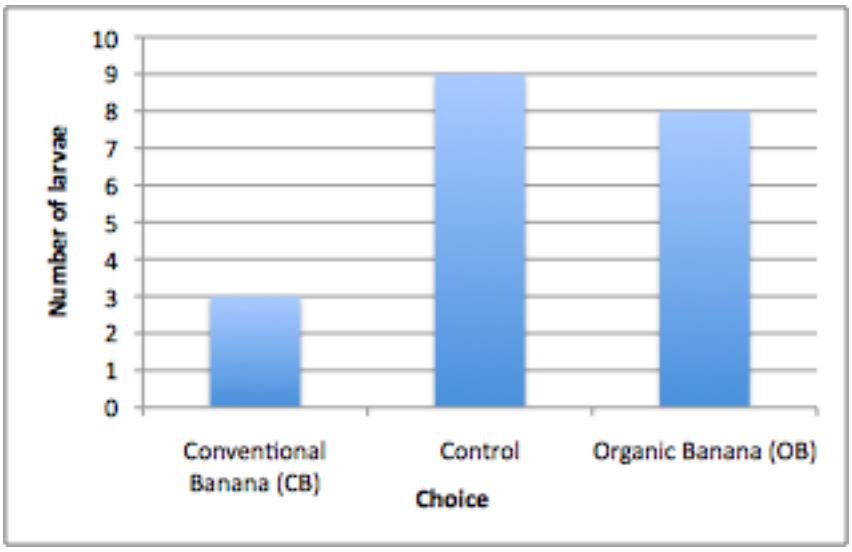

Figure 9. The number of *D. melanogaster* larvae that made a final choice on a specific substrate after 20 minutes. n= 20, p= 0.05, χ^2 =3.1.

Sample Calculation of Chi-square (χ^2) test

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}} \qquad \text{(eq. 1)}$$

Using the equation above (eq.1), one can calculate the chi-square value for the experiment.

Below are step-by-step calculations for the above equation (eq.1).

1. Calculate expected values based on Ho.

Number expected = $\frac{\text{total number of replicates}}{\text{number of items from which to choose}}$

Number expected = 20/3 = 6.67

2. Calculate $\frac{(\text{observed-expected})^2}{\text{expected}}$ for each choice.

For control, $(\text{obs.} - \text{exp.})^2/\text{exp.}$ is:

$(6.00 - 6.67)^2/(6.67) = 0.07$

Repeat for conventional and organic banana treatments.

Table 1. Observed and expected results for substrates by *D. melanogaster* larvae.

Choice	Observed	Expected	$(obs.-exp.)^2$	$\frac{(obs.-exp.)2}{exp.}$
Control	6.00	6.67	0.44	0.07
Conventional Banana	6.00	6.67	0.44	0.07
Organic Banana	7.00	6.67	0.11	0.02

3. Sum the $\frac{(obs.-exp.)^2}{exp.}$ values for all classes.

$$\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}} = 0.07 + 0.07 + 0.02 = 0.16.$$

Discussion

Analysis of Results

First, we analyzed the results of the first experiment showing the effect of nutrients on the maturation time of *D. melanogaster.* As seen from Figure 7, there was no significant trend in the data, as the confidence intervals overlapped. Therefore, we failed to reject the null hypothesis that the addition of nutrients would increase or have no effect on the maturation time of *D. melanogaster*.

Next, we analyzed the results of our second experiment. This experiment determined the number of *D. melanogaster* larvae on given substrates after making their first choice as well as their final choice. In regards to both the first choice and the final choice of substrate, we obtained

very small chi-square values. This suggests that the data are random; thus, we fail to reject the null hypothesis and do not support the alternate hypothesis that the addition of nutrients to a substrate would increase the tendency for larvae to choose that substrate. Overall, it was decided that the addition of nutrients did not significantly affect the maturation time of *D. melanogaster* larvae.

Biological Explanation

There were some limitations of our study that may have been the downfall of obtaining any significant data. On day six of the first (developmental) experiment, we noticed fungi growing on the top of the organic banana and a much smaller amount on the conventional banana. Over the next few days, this fungus continued to grow heavily especially in the organic banana replicates. It is possible that this is the reason as to why no larvae matured into flies in this treatment, as the fungus inhibited larval growth instead of the actual type of banana. Another possibility is that the fungus on the banana created a thick layer that did not allow the larvae to burrow through the substrate in order to grow. Additionally, it has been found that fruit with mold or fungus on it tend to have a complete loss of sugars such as glucose and fructose (Adisa and Okey 1987). This means that the nutrients needed for larval growth were not available, which led to the starvation and then the eventual death of the larvae. Finding no growth in the organic banana substrate was surprising; in previous studies, it was found that organically grown foods promoted good health in *D. melanogaster* (Chhabra *et al.* 2013). Despite our results, organic crops are known to have more vitamin C, iron, magnesium and phosphorus (Worthington 2001). These benefits should have helped promote *D. melanogaster* growth as larval growth rates depend on nitrogen and phosphorus-rich environments (Markow and O'Grady 2008). Overall, it seemed as though the fungus growth on both the organic and conventional banana was inhibitory

to the growth of the larvae into mature adult flies. This could be the reason why we failed to reject our null hypothesis and why our results were contrary to the literature.

The choice experiment was then performed to see if there was a preference for a substrate, in which larvae would prefer to grow on. However, the experiment results were random and thus did not provide much feedback. We expected that the larvae would make a first choice of moving onto a substrate that was nutrient-rich such as organic bananas. The larvae make these choices by using their antennomaxillary complex and ventral organs to process sensory information, which may aid them in sensing nutrient rich areas (Helmbeck *et al.* 1999). Despite this; we found that the larvae had the tendency to make random choices. This disagrees with Figure 2 where larvae made a first choice onto a medium-rich in nutrients. Other experiments may have been more successful in their results, as one researcher used an apparatus that contained enclosed funnels to five separate chambers each containing a choice of a different fruit (Ware 2009). The funnels ensured that when the *D. melanogaster* made a choice, they could not leave the chamber and then after 24 hours they were counted (Ware 2009).

When collecting data for the maturation time of the *D. melanogaster*, the number of mature flies seemed to increase over time (other than for the organic banana substrate). At closer inspection, it was found that one of the flies died just after we collected data for the day eleven replicate in the control group. We suspect that same fly was the one observed a few days prior stuck in a small burrow in the substrate, where it could not fly out. It most likely died from the lack of nutrients and the fact that *D. melanogaster* do not thrive behaviorally in confined spaces due to the claustrophobic effect. The claustrophobic effect is prevalent in flies as they show

hesitant and reluctant behaviour when faced with small spaces, as they can sense the sides of the space through visual stimuli (Ewing 1963).

Sources of error and variation

During the experiment, we tried to minimize error as much as possible. However, there were actions that could have led to variation in our results. Firstly, the greatest source of error happened when placing the banana into the vials. We believe that too much banana was added into the vials as it completely covered the medium below. As mentioned previously, this caused fungus to grow and cover the banana, thus not allowing the larvae to burrow into or out of the banana or to the media below. In the end, this may have led to no larval growth in the organic banana vial and a low number of larvae in the conventional banana vial. Another source of error was placing the larvae into the various vials with the metal loop. Since larvae are very fragile, we cannot be sure that when we transferred the larvae from the sucrose solution to the vial that they were all alive. It is possible that some of them were suffocated or squashed when pushed into the substrate with the metal loop. This would affect our results, as fewer larvae would be able to mature into adult flies in certain vials.

In terms of the choice experiment, we spread an excessive amount of each substrate on the petri dish, making the layers too thick. This led to the larvae burrowing to the bottom of the petri dish in the middle and sometimes getting lost or stuck. This would affect our results as we wanted to test which substrate the larvae would chose to move towards, not burrow down into and stay stationary in. Thus, we saw a lot of larvae near the middle of the Petri dish making no apparent choice.

Conclusion

Our results from the first experiment have shown that we failed to reject the null hypothesis that additional nutrients increases or does not affect the maturation time of *D. melanogaster* and thus could not support our alternate hypothesis that the addition of nutrients would decrease the maturation time of *D. melanogaster*. From our second experiment, our results have shown that we failed to reject the null hypothesis and did not support the alternate hypothesis that the addition of nutrients to a substrate would increase the tendency for larvae to choose that substrate. Due to these results, additional nutrients might not be an important factor for *D. melanogaster* larvae.

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