

# May the Fastest Fruit Fly Larva Win: Inquiry-based Evolution and Food Preference Experiments

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An experiment was conducted by students in the St. Francis College Biological Evolution course in which they “raced” fruit fly larvae on agar Petri plates to determine which were the fastest. The “FAST” and “SLOW” larvae were placed in separate vials, and were allowed to breed. The larvae in the next generation were then raced again in an attempt to see if speed could be selected for. Subsequent student experiments included varying the type of food provided. In this mini-workshop, participants “race” wild-type fruit fly larvae on a Petri dish to see which type of sugar or sugar substitute they prefer.

**Keywords:** fruit fly larvae, evolution, inquiry-based experiments, selection, food preferences

## Introduction

The idea for this inquiry-based laboratory project came about by mistake. I (KN) asked Russell Burke, a colleague at Hofstra University, what kind of experiments his students do for evolution labs. He said that they have been “racing” nematodes on agar Petri plates, saving the fast ones, breeding these, and then selecting for speed again. In effect, he is trying to create a “speedier” nematode. When I (KN) taught the course, I brought some soil into the lab (after the nematodes were mistakenly placed in the freezer, or was it the 37°C incubator)? We had prepared plates the day before by streaking *E. coli* onto a semi-circle of the plate that was 7 cm from one side. The plates were incubated overnight at 37°C. We found nematodes by examining the soil under a dissecting scope, and placed them, two at a time, on a Petri plate for our races. We did find this a bit difficult to do as the nematodes are so small and hard to handle because of their small size. We used forceps to pick up the nematodes. We also videotaped the nematodes moving across the plates. We had fun, and decided to continue the experiments the next week. “Dr. Nolan!” they exclaimed. “Our nematodes grew!” What the students saw on their plates the following week, however, were not nematodes, but fruit fly larvae that had gotten into the plates through contamination. (Those pesky adult fruit flies---who do they think they are going around and laying eggs on our plates when we had them open the week before??)

So we decided to race and select for fast and slow fruit fly larvae. This time we used dried fly food as a “lure”. We then placed ten “FAST” fruit fly larvae in a vial and labeled it (“FAST” was a speed greater than 3 cm/minute) and then placed ten “SLOW” fruit fly larvae in a vial. We let them

mate and produce another generation of larvae (it takes around two weeks) and repeated the experiment. After the next generations, there was no significant difference in speed between the “FAST” and the “SLOW” larvae; however, the average speed of randomly picked larvae from the “FAST” vial was slightly faster than that for the “SLOW” larvae (see Selected Results).

This is where the experiment gets interesting, and where I heard many ideas and received numerous suggestions in the ABLE mini-workshop. I gave each participant a nutrient agar Petri plate, so that they could each experience actually doing the lab. I suggested that each person do a different treatment, and provided white sugar, raw sugar, Splenda™, Sweet and Low™, Equal™ and Miller Ice Beer™ as the “lures”. To hold to stricter scientific standards, students should weigh each item before placing it on the Petri plate. Other suggestions included Stevia™, agave, honey, carrot seed oil, and other fruit juices or alcohols. Next, two fruit flies should be raced on a plate at a time. (Another variable to explore would be varying the number of larvae placed in the plate---is there an interaction effect?) Various mutants or even species could be tested.

ABLE participants suggested different ways of actually conducting the experiment. Suggestions included: putting a contrasting dye in the Petri plate so that the tracks could be observed, photographed, and measured with software such as Image J. The larvae do not always go to the food, but this would be a way of measuring movement. Since we are timing the movement, the distance in cm per minute could be calculated.

If one wanted to do the speed selection experiment, the

two variables, speed and food source could be tested. An evolution or genetics class could do a multi-generational study, whereas non-majors might opt for the food preference experiments. The beauty of the model is that it is simple, cheap, and easy to generate data, easy for students to do (once taught how) unsupervised, and it can lead to inquiry-based experiments.

Upon perusing the literature, I found MANY references to experiments with fruit fly larvae, so students should be able to conduct a literature search to find out a variety of applications for this type of experiment. Greenspan and Kretzman (2008) give a nice overview of medical research with fruit flies, and how we share many genes in common. Ryuda *et al.* (2008) also attempted to tease out a genetic component for food preference of fruit fly larvae. There were many agricultural references in which scientists would like to track larval attraction to crops, and find ways to deter pests. Olfactometers (which can be quite complex or as simple as a Y tube) were used to place odors in the proximity of larvae and study preference. One such article by Silvia *et al.* (2007) studied the response of parasitoid (of fruit fly larvae) species to volatiles of guavas infested (or not infested) with fruit fly larvae. The authors measured the time that the parasitoid spent in the arm of the olfactometer with the particular volatile in question. Another article that might interest the students is by Rothenfluh and Heberlein (2008) who studied the affects of compounds such as ethanol on the flies.

Min and Condron (2005) placed a yeast suspension in the middle of a Petri plate and used a half-time for a measurement of larval interest under different environmental conditions (the time it took one-half of a group of larvae to reach the yeast suspension). Crawling motion and speed was also studied and videotaped by several research groups (on several Diptera groups) (Berrigan and Pepin, 1995; Charabidze *et al.* (2008). The larvae do not always move in a straight line, as was also noticed by many ABLE participants. Charabidze *et al.* (2008) were able to apply this information to forensic science, by being able to determine more accurately time of death based on temperature and the time it takes certain fly larvae to leave a corpse. Glider (2011) presented a major workshop in which participants also experimented with flesh fly larvae and their movements in response to light---which was another forensic approach.

Another article that I (KN) remember reading last year (but I was unfortunately unable to find this reference) starved the larvae for a period before the food preference experiments. This could be another variable for student experiments. Another fun variation is to use the Sense of Smell kit produced by Carolina Biological Company. A bead from the various "smell" vials can be placed in the center of the Petri plate and fly larvae can be placed at the periphery and "raced" to the odor source.

## Student Outline

### Materials and Methods

Fruit fly larvae at the crawling, three-instar stage (*Drosophila melanogaster* or other)

Cotton-tipped applicators

Petri plates with agar (plain is preferred), or nutrient agar—1.5%)

rulers

markers

food source (fly food, sugar, artificial sugars)

watches, timers, or stop watches

beaker of water (you may add soap, bleach, or Lysol to make a Fly Morgue)

1. Draw a line (chord) with a marking pen and ruler across the bottom of a plain (or nutrient agar) Petri dish approximately 7 cm from one edge (Figure 1.) (Alternatively, draw a circle the size of a half-dollar in the center of your plate. Then draw a cross through this circle to make four quadrants (Figure 2.)

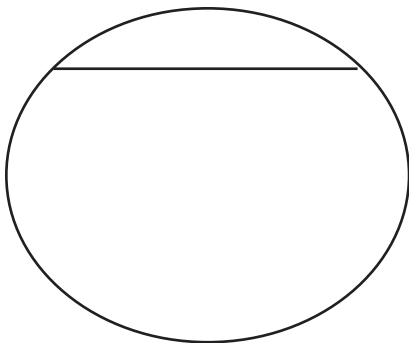


Figure 1. 7 cm chord

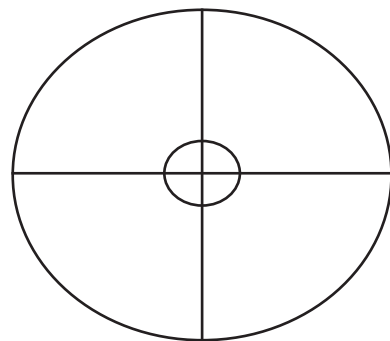


Figure 2. Four quadrants

2. Turn the plate over, open it, and place your food of choice in this semi-circle (or center circle).
3. “Drown” the adult flies in a vial of fruit flies. Lightly push the plug of the vial to the side while running a stream of water into the vial. Wait a few seconds; this will kill most of the adults, but leave most of the larvae alive. Pour off the adults into a beaker of soapy water (the Fly Morgue).
4. Take a cotton-tipped applicator stick and remove two-four larvae from the vial and place the larvae on the side(s) of the plate opposite the food. (To be more precise, one may weigh the amount of food used).
5. Record the time it takes the larvae to reach the food. From the time it takes them to reach the food, you will be able to discern the rate in distance per minute, and then come up with arbitrary times for “fast” and “slow” larvae. Record your data in Table 1.
6. For example, you may decide that a rate of 3 cm per minute is “fast”, and that anything less than 3 minutes per centimeter is “slow”. Divide the larvae into “fast” and “slow” larvae.
6. Place the larvae into “FAST” and “SLOW” vials.
7. Repeat the races with new larvae ten times. Place the winners and the losers into the “FAST” or “SLOW” vials.
8. Your instructor will open up an Excel spreadsheet and label three columns “Name”, “FAST” and “SLOW”. Type your names and the rate of movement of your larvae into this spreadsheet which will be displayed on the board (Table 2). The raw data is preferred as you can tally it and do rate averages for each column at the end.
9. For students conducting evolution experiments, save these vials and repeat the experiments every two weeks (so you will have new larvae.) Start out with at least ten larvae in each vial. Count the adults that emerge in each generation and note any differences between “FAST” and “SLOW” vials. Sex the adults. Look at your averages over time. Are the “FAST” larvae faster, on average, over time than the “SLOW” larva. Are you able to select for speed??

One can do a Chi-square analysis on the results, as you would expect to get the same number of fast and slow larvae each time. However, remember that you will not be able to count all of the larvae, only a sub-set. Are your “fast” larvae getting faster? Are your “slow” larvae getting slower?

**Table 1.** Raw data for each individual group.

<b>Time to travel 7 cm (or measure to the center)</b>	<b>Distance (cm) /minute (speed)</b>
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

**Table 2.** Data from the class to be projected onto the board.

<b>Name</b>	<b>FAST Distance/minute (&gt; 3 cm/minute)</b>	<b>SLOW Distance/minute (&lt; 3 cm/minute)</b>
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

## Notes for the Instructor

This model really makes for a fun, inquiry based lab. Experiments can be conducted with food preferences just once, or over several generations. We tried to see if we could select for speed as the larvae “raced” to a food source. In our initial experiment, we used the powdered fly food that can be obtained from Carolina Biological.

Before beginning the experiment, see if the students can tell you how they calculate the rate of movement. Give them a simple problem such as, “If it took me five seconds to walk ten feet, then how many feet did I walk per second?” It should then dawn on them that they take the total distance traveled (10 feet) and divide it by the time it took to travel the distance (5 seconds) to come up with the answer of 2 feet/second. The students should then use this logic to take 7 cm and divide by the total time it takes the larva to crawl that distance to get the cm/minute rate needed. We arbitrarily picked greater than or equal to 3 cm/minute as a fast rate. A speed less than this was considered “slow”. Students might become confused by this and forget to take the distance traveled and divide it by the time, so that they will “mix up” their data by saying that if a larva traveled the distance in over 3 minutes, they must be faster than a larva that covered the distance in 2 minutes, because “3” is a bigger number than 2. This is the point at which you can introduce the fact that the time it takes to travel the distance is inversely proportional to the speed of the animal.

### Selected Results from the 2009 Biology Evolution Course

From a vial of wild-type fruit flies, larvae were raced for three trials each. From the class results, ten slow and ten fast larvae were placed in vials labeled “SLOW” and “FAST”. The second generation was then raced. From the slow vial, 18 fast and 26 slow larvae were recovered. From the fast vial, 19 slow and 25 fast flies were recovered. There was no significant variation in the speed of the larvae from each vial. Using a Chi-square analysis, we were unable to reject our hypothesis that there would be no difference in speed in the offspring of slow versus fast larvae. Even so, there was a slight difference in the average speed of offspring of the two sets of flies—the offspring of the slower larvae were slower overall (3.5 cm/minute) than the offspring of the fast larvae (4.14 cm/min.).

## Acknowledgements

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