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Using Foraging Behavior of Fruit Flies to Introduce Undergraduates to Research

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This mini-workshop introduces methods to study foraging behavior of fruit flies (*Drosophila melanogaster*) as an ideal area of research for undergraduates in both beginning and advanced biology courses. The methodology discussed is cheap and requires only a basic background in biology. The questions that students can ask using these methods include ecology, behavior, physiology, genetics, and evolution. The types of experiments lend themselves to formulate clear hypotheses and predictions and to learn statistical analysis. Instructors can use these methods in stand-alone short experiments in various biology courses, or to start long-term research projects.

Keywords: fruit flies, behavior, foraging

Introduction

Rationale

Experiments on feeding and foraging behavior of fruit flies can be used as stand-alone exercises in the following courses: Physiology, Behavior, Ecology, Genetics, Zoology, Entomology, Invertebrate Biology, Research Methods, and Introductory Biology classes for majors and non-majors. The methods discussed here can also be used to start a low budget, serious undergraduate research project for all levels of undergraduate students. Although the assays discussed here are directed toward experimentation at the organismal level, they can also complement molecular research. In my laboratory, we are currently cooperating on one of our projects with a neurophysiologist who generates mutants that we can test with our behavioral assays.

Some of the advantages of the methodology of this workshop include: low cost, fast learning curve, and high level of engagement of the students. The most expensive piece of equipment needed is a dissecting microscope (see Materials). The fruit fly colonies can be obtained easily in three different ways depending on the topic to be tested: collected in the field, ordered from biological supply companies, and acquired from various genetics laboratories (when particular mutants are needed). The behaviors can be tested in the laboratory in small testing arenas (large Petri dishes) and can be observed in real time, filmed, or recorded at the end of the test.

The various skills my students have learned over the years from these types of experiments include: basic experimental design, planning and organization, formulation of hypotheses and predictions, preparation of solutions, statistics, oral and written presentation of results, and literature search. I have successfully used these methods in my research laboratory but also as stand-alone three- hour laboratory exercises in Research Methods, Zoology, Entomology, and Physiology courses.

General Theoretical Background

Foraging

Fruit flies (*Drosophila melanogaster*) feed on bacteria and fungi that grow on rotting fruit. They localize the food by distant chemoreception during flight and land close to the source of the smell. Then they proceed to search for the food on the ground. Foraging behavior in fruit flies on the ground depends on contact chemoreception; the flies have chemoreceptors in their front legs that allow them to taste the substratum as they walk on it. When they detect a suitable source of food, they stop, extend their proboscis, and feed. If the food source is a small droplet that does not satiate them, the flies finish the droplet and engage in "local search". This is a walking pattern characterized by walking paths that have a high turning rate and a low locomotory rate and it helps the flies remain in a patch of food. A patch is defined as an area that has higher density of food than the surrounding area (Bell, 1991). As time progresses, the walking path becomes increasingly straighter and walking becomes faster, which increases the probability of leaving the patch. If the flies, however, encounter another droplet of food, local search is reset and the walking path becomes again convoluted, allowing the flies to remain longer in the patch and find more food sources.

Walking speed and turning rate can be quantified and show a normal distribution in populations of wild type flies. Flies that tend to turn a lot and walk slow are called "sit-

ters" and flies that walk fast and straight are called "rovers". Most wild type flies are intermediate. The rover/sitter adult phenotypes were selected from a wild type population in the Bell lab and the *for* (foraging) gene was also characterized in the Sokolowski lab in Toronto. Molecular mapping placed *for* mutations in the *dg2* gene, which encodes a cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG). Rovers have higher PKG activity than sitters and natural variation in PKG activity seems to be the cause of the behavioral polymorphism in wild type flies.

Feeding

In the laboratory, fruit flies that have been deprived of food for 24 hours with access to water readily feed on various sugar solutions. A 0.25 µL drop of 250 mM sucrose will release local search, but other types of sugars are also ingested at different levels of preference. These feeding preferences can easily be determined by two-choice tests. When the flies are tested in semi-natural patches of food and are free to forage, sucrose is preferred over glucose and fructose, both of which are preferred over mannose and lactose (Aracena, 1996). The flies prefer sucrose solutions between 100 and 500 mM. High concentrations are too viscous and probably difficult to ingest, whereas lower concentrations have lower excitatory effects. Excitatory and inhibitory effects modulate the flies feeding choices. Quinine is a powerful deterrent, and hunger is an excellent excitatory stimulus. Very hungry flies (about 48 hours of food deprivation) will feed on solutions of sugar containing levels of quinine that are unacceptable to satiated flies. High concentrations of salt (NaCl) can also be used as a deterrent (Cochran and Aracena, in prep.). Other stimuli can also affect feeding choices, such as light, visual stimuli, smells, temperature, competition, predation, and mates (Bell, 1991).

Basic Description of Experiments

The foraging and feeding behavior of fruit flies can be observed directly without a microscope. This is a useful exercise at the beginning of a stand-alone laboratory exercise but especially when starting a long-term research project. A large Petri dish with a small hole on the top can be used as a testing arena. A feeding tray is taped to the bottom center part of the dish (see Figure 1).

The sugar solutions are colored with food coloring either red or blue, depending on the solutions tested. For example, if the choice is between two different concentrations of sugar, solution A could be red 250mM sucrose and solution B could be blue 125mM sucrose solution. A certain number of hungry flies are allowed to enter the dish from the hole above and then they are allowed to feed for 45 minutes. The flies are then frozen (if a CO_2 source is available, they can be anesthetized instead) and then they are scored under a dissecting microscope. The food color of the solution ingested is easily visible through the abdominal walls, allowing the students to easily score the numbers and proportions of flies that ingested the red solution, the blue solution, both solutions, or neither solution (Figure 2 below). Preference is then determined using a Chi-square test.

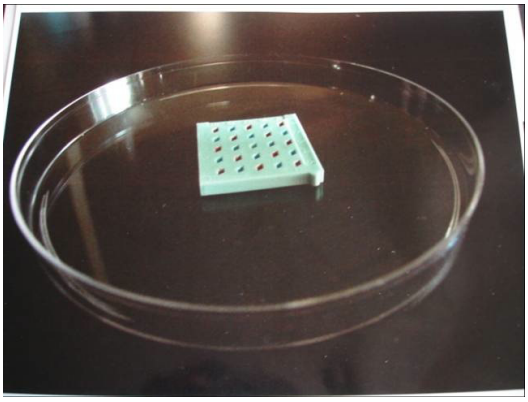

Figure 1. Testing arena bottom part (large Petri dish) with feeding tray taped at the center. The feeding tray has 25 wells that contain the red and blue sugar solutions.

The same assay can also be filmed and the foraging behavior of individuals can be analyzed later from the film. However, the easiest assay does not involve observation of the flies. We can test large numbers of flies and simply count the flies at the end of the 45-minute feeding.

Figure 2: Dissecting microscope view of frozen fruit flies that ingested colored sugar solutions the color shows through their abdominal walls. The fly with the purple abdomen has ingested both red and blue solutions.

Student Outline

I prefer not to provide student instructions here because they will depend on the level of the class, the specific topic chosen by the instructors, and the time dedicated to the lab. I suggest the students use the following type of table to count flies of different colors (score the results):

Table 1. Scoring table for feeding preference.

	Male	Female	Total
Red (Solution a)			
Blue (Solution b)			
Purple (Mixed)			
No color (Not fed)			
Total			

Notes for the Instructor

Materials for a stand-alone, three hour laboratory (for 10 pairs of students):

For the whole lab:

- Regular refrigerator with freezer
- 500 g sucrose or regular sugar cane granulated sugar
- Desktop balance (0.1 g accuracy enough) and weighing paper or boats
- Mixing plate and magnets
- 2 x 250 ml volumetric flasks with caps
- Food coloring (red and blue)
- Quinine
- Hole puncher

To be distributed among the tables of students:

- Fruit flies *Drosophila melanogaster* apterous (10 small colonies with > 50 adults each)
- 10 dissecting microscopes
- 10 x 50 ml beakers (glass or plastic)
- 10 micropipetters (10 – 100 mL) or (1 cc plastic syringes)
- 100 non-sterile yellow tips (or small needles for the syringes)
- 10 small calculators (optional)
- 20 syringes (10 cc) with needles
- 20 plastic Petri dishes (20 x 2 cm) with holes *
- 100 #00 size corks
- 20 cardboard caps (3 cm diameter)*
- 20 50 mL plastic fly vials and 20 sponge caps
- Box of 10 cm diameter filter paper
- 20 feeding trays (electron microscope sample grids) *
- 20 1-2 drum glass vials with caps for solutions
- Labeling tape
- Sharpies
- Stopwatches
- 10 small brushes
- Scoring tables
- 300 2 mL plastic sample vials with caps *

See specialty materials below

Specialty materials:

1) Electron microscope grid boxes to be used as **feeding trays**:

   www.tedpella.com/grids_html/gridbox.htm#anchor1280885

   PELCO® TEM Grid Storage Box, Product # 160. Each box costs approximately $5.00. I cut the trays into two equal halves of 25 wells each, but full trays of 50 wells can also be used.

2) Sample cups to be used as **vials for transferring and viewing individual flies**:

   From Fisher Scientific:

   Catalog # 02-544-17: Case of 1000 2 mL Dynalon sample cups **and** Catalog # NC9563861 Case of 1000 Push-on sample cup stoppers, Dynalon Item # 202044.

   Total price for 1000 cups and stoppers: approximately $50.00.

3) Large Petri dishes for fly **testing arenas** can be ordered from many catalogs. I used this one.

   From Carolina Biological Supply Co.:

   Catalog # 19-9279: 25 x 150 mm deep and wide Petri dishes, 12 per pack (about $40.00).

4) Cardboard caps (3 cm diameter):

   This is the only item I can no longer find to buy online. You can use thin cardboard and cut circles. The purpose is to make a hole with the hole puncher on the cap and plug it with the #00 cork. Then you can tape this cap on top of the fly vials and let the flies walk one by one into the 2 mL sample vials through the small hole-punched hole.

Methods

a. Long before the lab:

- Order the specialty materials above.
- Establish colonies of fruit flies. The easiest way is to order 10 vials from Carolina Biological Supply Co. at least two weeks before the lab.
- If you are adventurous, try regular wild type Oregon R flies, but beginners might want to start with apterous flies that won't fly away.
- Use a file or a hot nail to make a small round hole in the middle of the top part of each Petri dish. The hole should be smooth enough and of a size that can be perfectly plugged by a cork # 00.
- Cut the feeding trays (electron microscope grid boxes) in half, if you wish. You need someone with a machine workshop because the plastic is very hard.

b. 24 hours before the lab:

- Put the flies in vials with no food. Fold a 10 cm filter paper in four, completely soak in tap water and press it against the wall of a clean 50 mL fly plastic vial.
- Let about 50 flies walk in and cap the vial with a sponge cap.
- Repeat with the nine other vials. These flies are now being deprived of food for the experiment tomorrow and should be hungry.

c. Solution preparation: (To be done before or during the lab)

- You may do this yourself or let your students prepare the solutions themselves depending on time constraints. If time permits, it is a good learning experience for students of all levels.
- Prepare 500 mL of 250 mM sucrose solution.
- Aliquot the solution into ten 50 mL beakers: one for each pair of students.
- The students can then use the 10 mL syringes to prepare appropriate dilutions.
- If you want to test quinine, you can prepare 5 mM quinine in 250 mM sucrose. This solution is completely rejected by hungry flies. Dilutions of 0.05 mM quinine in 250 mM sucrose still decrease acceptance in hungry flies.
- The students can decide the concentrations of solutions to be tested in two-choice experiments, or you can suggest solutions, depending on the purpose of the lab and the level of the students.
- The students can then color two solutions to be compared. One drop of food coloring usually works well for 5 mL of solution. Blue tends to be more intense than red and you may need to adjust the number of drops needed for red vs. blue.

d. During the laboratory the students will do the following:

- Tape the feeding trays to the Petri dishes and use micropipetters or small syringes to fill the wells of the feeding trays. For a feeding two-choice test, I recommend that they intercalate colors to form a checkerboard pattern of red and blue, so that the flies have higher chances of finding both solutions.
- Close the Petri dish; tape the top securely to the bottom of the dish so the flies cannot escape.
- Plug the top hole with a #00 cork.
- Tape the cardboard caps to the top of the food deprived fly vials, then take the cork plug off and let individual flies climb into the small 2mL sample cups.
- Cap these cups and count and sex the flies.
- Then uncap the small vials and place them one by one on top of the Petri dish hole and let the flies walk in. Place at least 10 flies but no more than 50 flies into the arena (Petri dish) and start the stopwatch.
- Either observe the behavior or place the arena in a dark box for 45 minutes while the flies feed.
- At the end of this time period, anesthetize the flies and freeze them.
- Count the flies under a dissecting scope.
- Use a Chi-square to determine preference for solution A vs. solution B.

Final suggestions for the instructor:

- Practice placing individual flies into sample cups and into the Petri dish before the lab. The flies may need to be tapped into the Petri dish.
- Anesthetizing the flies with CO_2 and then freezing them reduces feeding after the experimental time. It is better to freeze them than just to keep them asleep, unless they are needed live for further testing.
- Apterous flies and other mutants tend not to do well when deprived of food. 24 hours is the longest I would try on a first run. Wild types collected from the field are much hardier and probably will not be very hungry at 24 hours of food deprivation. 48 hours may be better for those flies.
- To see clear differences in preference, at least 4 replicates of 50 flies each are necessary. In a three-hour lab, however, differences can be seen even with 20 flies.
- Females tend to survive starvation much better than males and their feeding preferences can also be different.
- When 50 flies are tested at a time, 0 to 30% of the flies do not feed in 60 minutes when tested in the dark. Larger percentages of flies not feeding probably mean that the solution tested is not very stimulating, or that the flies are not hungry enough, or that they were heavily disturbed during feeding
- There is usually a chemical effect from the food coloring that results in a bias (preference for one of the colors) even when testing in the dark. Therefore, controls must include switching colors between the solutions tested in two-choice tests.

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