*Tested Studies for Laboratory Teaching*
*Proceedings of the Association for Biology Laboratory Education* Volume 39, Article 34, 2018

# Flying 101: Introducing *Drosophila* in Undergraduate Lab Courses

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*Drosophila melanogaster* is a versatile model system that can be used to illustrate genetic and developmental biology concepts. However, many undergraduate students are not given the opportunity to work with fruit flies in developmental biology courses. We utilize a simple and cost-effective two-choice plate assay to introduce undergraduate students to *Drosophila* genetics and life cycle. In addition, this module is flexible and can be adapted to study diverse aspects of fruit fly gustatory behavior, such as correlation of RNA-seq data with stage-related nutritional preferences and observation of taste-related genetic dimorphisms. We have incorporated this module into an upper division undergraduate developmental laboratory. Students used the two-choice plate assay to test *Drosophila* sugar substitute taste preferences. Quantitative results obtained are presented as the preference index as well as percent participation.

**Keywords**: *Drosophila melanogaster*, developmental biology, taste preference assay, undergraduate, development, genetics, two choice plate assay, Flybase

## Introduction

*Drosophila melanogaster* is a versatile model organism that can be used to illustrate genetic and developmental biology concepts. Flies are a convenient model system for undergraduates to study, due to their short life cycle, well-characterized behaviors and development, and low cost of maintenance. However, many undergraduate students are not given the opportunity to work with fruit flies in developmental biology lab courses. The two-choice plate protocol is a versatile assay that uses readily available and inexpensive reagents. We utilized this assay to introduce undergraduate students to *Drosophila* life cycle and genetics. This laboratory exercise is flexible and easily adapted to study taste receptor mutants, correlate RNA-seq data with stage-related nutritional preferences using Flybase, observe taste-related genetic dimorphisms and demonstrate the evolutionary conservation of taste.

We have successfully incorporated this module in an upper division undergraduate developmental biology laboratory. This module can be completed within a four-hour lab period paired with a two-hour lecture. The assay can be easily modified for use at different levels in an undergraduate curriculum.

## Applications

### *Sugar Substitute Preferences*

In this lab, students test *Drosophila* sugar substitute taste preferences using the two-choice plate assay. The preference for sucrose, the main dietary sugar of fruit flies, is compared to commercial sugar substitutes containing sucralose, aspartame, erythritol or stevia as the main ingredient. Quantitative results obtained are presented in the form of a preference index (PI) and percent participation (%P). Students should obtain quantitative results that demonstrate *Drosophila* prefer sucrose over sugar substitutes because *Drosophila* should prefer calorie-rich food after starvation (Dus, 2011).

### *Broader Applications*

The plate assay was first described in a publication by Tanimura, to study the genetic differences in trehalose taste sensitivity in *Drosophila* (Tanimura, 1982). The assay has been used to isolate mutations that alter taste modality (Arora, 1987), the molecular and cellular basis of bitter taste (Weiss, 2011), the effect of nutritional state on amino acid intake (Toshima, 2012) and genetic variation in sugar sensitivity (Uchizono, 2017). The module can be adapted to different model organisms, and developmental stages and genotypes.

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# Student Outline

## Objectives

Explain the advantages and disadvantages of using *Drosophila* as a model system
Analyze and interpret quantitative data from a behavioral assay
Form hypotheses based on literature
Design experiments to control for variables

## Introduction

*Drosophila melanogaster* is a widely-used model organism for genetic studies. The short life cycle of fruit flies (approximately 10 days from eggs to mature adults) makes them easy to rear and use in a laboratory setting. Sophisticated genetic tools have made it an attractive organism for studying the molecular genetic basis of development, neurobiology and behavior, among others. For example, since these insects undergo metamorphosis it is a useful model for understanding hormonal signaling and stage progression from an immature form to a sexually mature human. Importantly, *Drosophila* have functional homologs of approximately 75% of the genes that cause disease in humans, but lack the functional redundancy that exists in the human genome. Humans have multiple genes participating in a common role, while *Drosophila* have a single gene that has a unique function, making it much easier to study the function of each gene. In addition, fruit flies have well-characterized behaviors, including circadian rhythm, mating rituals, flight and feeding habits. Extensive mutant screens have identified mutations that disrupt these behaviors that although seemingly insignificant, provide basic models for more complex human diseases. One such example is a shaking leg phenotype in flies, caused by mutations in *Shaker* and *ether-a-go-go*. Studies of these mutations in flies revealed defects in potassium channel function, which in humans, results in cardiac arrhythmias, epilepsy and deafness. Recent studies aim to use high throughput assays in *Drosophila* for drug discovery and efficacy in combating human diseases (Pandey, 2011) (Sonoshita et al., 2017).

One question that is of fundamental significance to all organisms is how we detect nutrients in our environment and respond to gustatory stimuli, and how we avoid toxic or deleterious compounds. In order to understand how neurons respond to and differentiate between different taste stimuli in *Drosophila*, scientists have developed a two-choice plate assay. This simple assay utilizes the feeding habits of fruit flies and is a quantitative behavioral assay that tests the response of large numbers of flies simultaneously. It was first published by Tanimura (1982) who used it to identify the gene responsible for trehalose sensitivity in different *Drosophila* laboratory strains. Since then, this assay has been used extensively to identify mutations in genes that effect gustation in fruit flies, and to investigate the molecular genetic basis underlying gustatory behavior, for example, the response to salt and sugar modalities (Arora, 1987), the basis of bitter taste (Weiss, 2011), the effect of nutritional state on amino acid intake (Toshima, 2012), and the genetic variation of sugar sensitivity (Uchizono, 2017), among others. You will be performing this assay on *Drosophila* strains to test whether fruit flies prefer commercial sugar substitutes compared to sucrose, the main dietary sugar of fruit flies. Dus (2010) used this assay to show that adult *Drosophila* flies, mutant for the sugar receptors Gr5a and Gr64a, were nevertheless able to preferentially select calorie-rich food over nonnutritive substitutes, thus identifying a novel detection mechanism for nutritive food. However, a paper from Gordesky-Gold (2008), analyzing functional similarities between fly and mammalian taste receptors, found that *Drosophila* and humans share sweetness preferences, and that wildtype fruit flies prefer compounds considered sweet by humans over less sweet substitutes. In your assay, you will be comparing a sugar substitute with sucrose. What results do you expect to get?

## Methods and Data Collection

### *Part A: Setting up the Assay*

1) Get two solutions from the instructor:

| Solution A: | Solution B: |
|---|---|
| 980 μL purified water | 980 μL purified water |
| 20 μL red dye (2%) | 20 μL blue dye (2%) |
| 20 mM Sucrose | 20 mM Sugar Substitute |
| 1% Agar | 1% Agar |

2) Keep these solutions molten using a temp block (at 60°C) or microwave.

3) Get a 60 micro-well plate with a lid from the instructor. Use a micropipetter or plastic Pasteur pipette, to fill alternating wells with your red solution.

 

a. Wells should be filled past the top of the well, creating a convex bubble-like surface for the flies to feed on.
b. You can fill up the micropipetter (p1000) and aliquot into each well efficiently to make sure the agar solution stays molten. **DO NOT exceed 750 µL on a p1000 micropipetter.**
c. It may take some practice to fill each well consistently. It is best to practice with 1% Agar.

4) After you fill wells with solution of one color, fill the remaining empty wells with the other solution as indicated in Figure 1. The goal is to create a pattern that is unbiased in distribution of the two food choices.

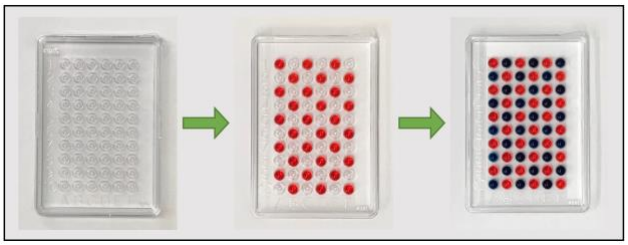

**Fig. 1.** Setting up the assay. Once the lid is removed, Solution A is filled in alternating wells. Once Solution A is solidified, fill the other wells with Solution B.

*Part B: Preparing Drosophila for the Assay*

1) Get a vial containing the appropriate fly strain from the instructor.
   a. Count your *Drosophila* that have been starved for 24 hours
   b. Observe if they have any defects that vary from wildtype, shown in Figure 2A.
   c. If you notice any defects or issues, ask the instructor if they are supposed to be there.

2) Place your vial of flies in dry ice to *anesthetize* them (you do not want to kill them!).
*If you leave your flies in dry ice for too long it **will kill them** (do so for 2 minutes and periodically check the appearance of flies until they appear asleep, dead flies will stick their wings out).*

3) Label the **plate and lid** with your group name and experimental conditions

4) Remove the lid of the plate and shake the anesthetized flies out of the vial onto the plate. Put the lid firmly back on the plate immediately.

5) Put the plate in a dark location at room temperature for 0.5-2 hours. A good location to store the flies during the assay is in a cabinet that is free from strong odors and light. *Do not check on your flies until the full time is up.*

6) Transfer your entire plate to the dry ice kill the flies, this will take at least 5-10 minutes, or more, depending on the temperature.

7) Shake out your flies out of the plate onto a slide and use a microscope to examine the color of food in the abdomens of each fly to determine which food was ingested. See Figure 2B, C and D for reference.

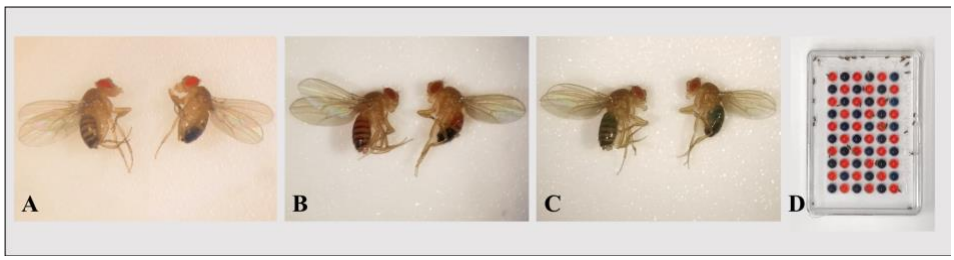


**Fig. 2.** Preparing Drosophila for the assay A) *wildtype* female (left) and male (right) *Drosophila* before feeding. B) *wildtype* female (left) and male (right) *Drosophila* that have ingested a solution containing red dye C) *wildtype* female (left) and male (right) *Drosophila* that have ingested a solution containing blue dye D) Drosophila in the two-choice plate assay (with lid on).

8) Record data for each trial, making note of the color of the abdomens, the genotype of flies, solutions tested and trial number. Note that in some cases, abdomen may be purple if flies have ingested both red and blue choices.

Data Analysis

Preference Index = (Nblue + 0.5 Npurple)/(Nred + Npurple + Nblue), where Nred, Nblue and Npurple represent the number of flies with red, blue and purple abdomens. Control experiments show that the dyes do not affect preference. Based on this equation, a P.I. of 1.0 indicates a complete preference for the blue solution, while a P.I. of 0 indicates a complete preference for the red solution.

% Participation = $N_{colored}/(N_{colored}+N_{uncolored})x100$

## Discussion

*Drosophila melanogaster* is a simple model organism that allows researchers to understand fundamental science concepts and study the genetic basis for human disease. The advantages of this model organism include low functional redundancy in the *Drosophila* genome, a short life cycle with four distinct developmental stages, and well-characterized mutations that allow us to better understand human diseases. The two-choice plate assay described here allows the quantification of response to gustatory stimuli and can be paired with mutational analysis to identify genes that are involved in the chemosensory response to molecules in the environment and understand the neuronal basis of taste modality. These studies have contributed to the identification of analogous systems, and a better understanding of chemosensory behavior in other organisms including mammals. Additionally, fruit flies are useful for studying genetic changes that occur during sexual maturation due to their distinct life cycle, consisting of egg, larva, pupa and adult stages. Differential expression of taste receptor genes are correlated with the nutritional requirement of each life stage (Depetris-Chauvin, 2015). In this laboratory exercise, you have tested if adult fruit flies prefer sugar substitutes over sucrose. If you were to repeat this experiment on *Drosophila* larva, the results could vary due to changes in taste-related gene expression. On http://flybase.org/, use the RNA-Seq Profile to select candidate genes that may be responsible for the difference in results between larva and adult trials. Next, design an experiment using the two-choice plate assay that will test whether your candidate genes are responsible for the changes in taste preferences. Be sure to include control experiments to eliminate any uncontrolled variables. Finally, in the literature, search for human homologs of your candidate genes and analyze their temporal expression patterns in humans.

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Toshima N, Tanimura T. 2012. Taste preference for amino acids is dependent on internal nutritional state in *Drosophila*

*melanogaster*. Journal of Experimental Biol. 215(16):2827-2832.

Uchizono S, Tanimura T. Genetic variation in taste sensitivity to sugars in *Drosophila melanogaster*. 2017. Chemical Senses. 42(4):287-294.

Weiss LA, Dahanukar A, Kwon JY, Banerjee D, Carlson JR. 2011. The molecular and cellular basis of bitter taste in *Drosophila*. Neuron. 69(2):258-272.

## Materials

Materials and methods for the instructor are included in APPENDIX A. Vendors and care for *Drosophila melanogaster* are included in APPENDIX B. Students are grouped in pairs to work on the assay and will require a computer with internet access to use http://flybase.org/ for discussion activities.

## Notes for the Instructor

Students in our developmental biology lab course are 4th year undergraduates and have a prerequisite of scientific writing, safety and ethics for research and cell or developmental biology. However, this assay can be taught or modified for undergraduates at more junior levels. We have students work in pairs, but write their lab reports and discussion individually. One of the major challenges of this assay is to ensure that students do no kill the flies while anesthetizing them, by leaving the flies in dry ice for too long. It is also important to emphasize that the flies are to be kept in a dark, secluded space during the assay, to prevent external stimuli such as light or odors from influencing their decision-making process.

Few undergraduates understand the significance of *Drosophila* as a model system. This assay is designed to emphasize the advantages of the fruit fly. However, these students should also identify the disadvantages of using *Drosophila*, when compared to other model organisms. This two-choice plate assay allows students to see the powerful data that can be obtained with a simple assay, when all possible variables are controlled for. Experimental design is another emphasis of this lab, especially in the discussion section. We have found that undergraduates have difficulty understanding the basic concepts of negative and positive controls, until they design their own experiments. Generally, control experiments show no preference between red and blue choices, and experimental conditions indicate that sucrose is preferred over sugar substitutes.

As stated above, this experiment can be paired with many other assays and exercises depending on which developmental concepts are being taught. We have chosen to pair our sugar substitute assay with genomic data from Flybase, to demonstrate how changes in gene expression correlate with behavior, as well as emphasize the unique life cycle of *Drosophila melanogaster*. Included in Appendix B, are citations for papers that have used the two-choice plate assay, if you wish to expand the applications of this assay.

## Cited References

Arora K, Rodrigues V, Joshi S, Shanbhag S and Siddiqi O. 1987. A gene affecting the specificity of the chemosensory neurons of Drosophila. Nature. 330(6143):62-63.

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Toshima N, Tanimura T. 2012. Taste preference for amino acids is dependent on internal nutritional state in *Drosophila melanogaster*. Journal of Experimental Biol. 215(16):2827-2832.

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## Acknowledgments

Special thanks to the UCI Graduate Assistance in Areas of National Need (GAANN) for funding this project. Thank you to all the teaching assistants and undergraduate students of D111L for their help in improving this module. Thank you for all the feedback from the UCI GAANN fellows.

## About the Authors

Kristina Lackey is a PhD Candidate at University of California Irvine, where she plans to get her PhD in 2020, and is studying signaling pathways that impact stage progression and metamorphosis in *Drosophila.* She has served as a teaching assistant in a Developmental and Cell Biology Laboratory course for a quarter, during which she created and implemented this module into the course. She was funded by a GAANN grant to UCI.

Kavita Arora is a Professor of Developmental and Cell Biology at the University of California Irvine. Her favorite model organism is the fruit fly Drosophila, which she first encountered as a graduate student studying the genetic basis of chemosensory behavior. She enjoys teaching both undergraduate and graduate level courses in developmental biology, and her research interests include cell-signaling pathways that regulate Drosophila development, growth and metabolism.

Debra Mauzy-Melitz is an assistant teaching professor in the Department of Developmental and Cell Biology, at the University of California, Irvine. Debra teaches Scientific Writing and Developmental and Cell Biology laboratory. She received her degree in Developmental Molecular Genetics from Marquette University, Milwaukee, WI. 2013-2014.

## APPENDIX A
## Materials and Methods for Instructor

**Materials:**
Stock Vials for *Drosophila* stocks - Varying genotypes based on assay (per group)
Vials with Drosophila media for fly rearing
30-50 *Drosophila* per experiment (3-5 days old) - total number will depend on number of trials per group
Cotton balls
Dry ice, Flynap, $CO_2$ or Freezer (alternative methods for anesthetizing flies)
Microwell® Mini Trays with lids (60 wells) (Nalgene Nunc Intl.)
Agar (Powder)
Sucrose (make 20mM stock solution)
Sugar substitutes or other taste testants (make 20mM stock solutions)
Graduated Cylinder
Beaker
Hotplate/Microwave/Temp Block
spatula/scoopula
Weigh boats
Balance or scale
Purified Water
Micropipetter or plastic Pasteur pipettes
Dissecting Microscope
Two colors of commercial food dye (red, blue) (available at grocery stores)
Device with internet access (preferably laptop or desktop)

**Setup:**
1) At least a month before your experiment, obtain *Drosophila* stocks for your experiment from the Bloomington Drosophila Stock Center.
    a. To make the experiment more manageable, we recommend using the adult viable *wingless* mutation,($wg^1$) as their inability to fly could make them easier to work with.
2) Once you have obtained *Drosophila*, propagate the stocks by serially transferring adults every 5 days into vials with fresh media, to ensure that you will have enough progeny to work with. Once progeny begin to hatch from the seeded vials, use for experiments or continue to transfer flies to new food vials until you have sufficient flies. Always save one batch of stock flies.
    a. Need at least 30 flies/genotype/student that are 3-5 days old.
3) 20-24 hours before the assay, prepare vials containing cotton balls moistened with water. Transfer 30-50 (3-5 day old) *Drosophila* into each vial. The purpose is to starve these flies.
   Be sure to write the genotype/sex on side of vial.
    a. Depending on the experiment, you may need to segregate flies by sex and/or genotype.
    b. It is not *necessary* to starve flies, but best results are obtained when food is limited prior to the assay.

**Controls Methods:**
This control experiment is used to control for the food dye and make sure that the concentrations of dye used are not affecting experimental results.

1) In both solution A and B, add 20 mM sucrose to all solutions, NO sugar substitutes.
    a. If there is no specific preference for either red or blue colored agar containing sucrose, this indicates that the color of the dye is not affecting response of the flies.
    b. If your results display a color preference, this indicates a need to optimize concentration of the dyes. Repeat the assay with varying concentrations of dye, instead of 2%, to determine a concentration that results in a PI of 0.5 for each color.
    c. If a specific dye appears to be problematic, switch to another color or source.

You can control for the age of the flies by collecting flies that eclose (emerge as adults) on a single day, and age by a fixed number of days prior to use in your experiments.

## APPENDIX B
## *Drosophila* Frequently Asked Questions

Why use flies in research?
http://modencode.sciencemag.org/drosophila/introduction
Pandey UB, Nichols CD. 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol Rev. 63(2):411-436.

Sonoshita M, Cagan R. Modeling human cancers in *Drosophila*. Current Topics in Developmental Biology Fly Models of Human Diseases. 2017:287-309.

How do you maintain flies?
http://www.unc.edu/depts/our/hhmi/hhmi-ft_learning_modules/fruitflymodule/

Where can I get flies?
http://fly.bio.indiana.edu/
http://stockcenter.vdrc.at/control/main
https://shigen.nig.ac.jp/fly/nigfly/
https://kyotofly.kit.jp/cgi-bin/stocks/index.cgi

Flybase link:
http://flybase.org/

RNA-Seq Profile Database:
http://flybase.org/static_pages/rna-seq/rna-seq_profile_search.html

**Papers that use two-choice plate assay:**

Arora K, Rodrigues V, Joshi S, Shanbhag S and Siddiqi O. 1987. A gene affecting the specificity of the chemosensory neurons of Drosophila. Nature. 330(6143):62-63.

Charlu S, Wisotsky Z, Medina A, Dahanukar A. 2013. Acid sensing by sweet and bitter taste neurons in *Drosophila melanogaster*. Nature Comm. 4(2042).

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## Citing This Article

Lackey K, Arora K, Mauzy-Melitz D. 2018. Flying 101: Introducing *Drosophila* in Undergraduate Lab Courses. Article 34 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 39. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). **http://www.ableweb.org/volumes/vol-39/?art=1**