INVESTIGATION 12

FRUIT FLY BEHAVIOR

What environmental factors trigger a fruit fly response?

BACKGROUND

Drosophila melanogaster is an organism that has been studied in the scientific community for more than a century. Thomas Hunt Morgan began using *Drosophila melanogaster* for genetic studies in 1907. The common fruit fly lives throughout the world and feeds on the fungi of rotting fruit. It is a small fly, and one could question why so much time and effort have been directed to this organism. It is about the size of President Roosevelt's nose on a dime, but despite its small size, the fly is packed with interesting physical and behavioral characteristics. Its genome has been sequenced, its physical characteristics have been charted and mutated, its meiotic processes and development have been investigated, and its behavior has been the source of many experiments. Because of its scientific usefulness, *Drosophila* is a model research organism. Its name is based on observations about the fly; the fly follows circadian rhythms that include sleeping during the dark and emerging as an adult from a pupa in the early morning. This latter behavior gave rise to the *Drosophila* genus name, which means "lover of dew." The explanation for the species name *melanogaster* should be clear after observing the fly's physical features. It has a black stomach. No doubt the dew-loving, black-bellied fly will continue to make contributions to the scientific community and to student projects.

These investigations explore the environmental choices that fruit flies make. A choice chamber is designed to give fruit flies two choices during any one test, although students could also think about how to build an apparatus that would give fruit flies more than two choices. Adult fruit flies are attracted to substances that offer food or an environment in which to lay eggs and develop larvae. Typically those environments are rotting or fermenting fruit. Adult fruit flies are attracted to bright light, and their larvae move away from bright light. Adult fruit flies also demonstrate a negative geotaxis; they climb up in their chambers or vials against gravity. Movement toward a substance is a positive taxis. Consistent movement or orientation away from a substance is a negative taxis. In most cases, the experiments done in the choice chamber will be chemotactic experiments, as indicated by the number of flies that collect on one end of the chamber or another in response to a chemical stimulus. At some point, students may wish to investigate if the chemotactic response is greater than a geotactic or phototactic response. The flies could also exhibit a behavior that is not oriented toward or away from the stimulus; rather, the stimulus elicits a random response. Such behavior would be considered a kinesis.

As students investigate the choices of fruit flies, it will be important for them to identify the ingredients in the household materials they are testing. Can they discover a pattern to the behavior of the flies? Are there substances that can attract fruit flies down in a vertically held chamber? If fruit flies are attracted to a picnic table or a counter in the kitchen, what attracts them? Are they attracted to the capers in a salad or the fruit on the counter? Are the fruit flies attracted more to light than to the picnic items? The substances to which fruit flies are attracted, such as capers or mustard, typically share one ingredient, vinegar. However, it is important NOT to tell students about vinegar or alcohol before they complete the lab. The investigation will allow them to discover a pattern in fruit fly behavior, so help them look for patterns in the data that they collect using the choice chambers. Which of the three responses — geotaxis, chemotaxis, or phototaxis — is the strongest for the flies that the students are using?

Although some activities in this investigation are open inquiry, the components in the beginning are structured inquiry, allowing for students to learn and practice key skills before they move on to more independent research. In structured inquiry, you provide a general procedure, but the expected outcome is unknown; once students are comfortable with techniques and the construction of the choice chambers, they can design and conduct their own investigation based on questions they have raised. Structured inquiry is particularly suited for introducing scientifically naïve students to inquiry.

PREPARATION

Materials and Equipment

- Fruit fly cultures (approximately 30–40 fruit flies per lab group each day)
- Choice chambers constructed from two matching plastic water bottles and caps per lab group
- Extra caps to fit water bottles (at least six extra caps per lab group)
- Cotton balls with tape to secure the cotton balls (20 per lab group)
- A variety of materials to test on Day 2 of the experiment. Use household substances, including condiments like capers, mayonnaise, mustard, ketchup, salad dressing, jelly or jam, peanut butter, and yeast; fruits like bananas, melons, apples, etc.; and lab chemicals like ethanol, ammonia, and distilled water. Small quantities (20 mLs) are needed.
- Safety goggles or glasses
- Timers (one for each lab group)
- Clear plastic packing tape (one roll)
- Droppers for each substance (20 or more)
- Dissecting microscopes (one per team)

Timing and Length of Lab

This investigation can be conducted during the study of many topics throughout the year. It would also be an excellent final lab activity to draw together many of the concepts and big ideas and science practices from the AP Biology Curriculum Framework. The investigation pulls together topics such as genetics, animal behavior, development, plant and animal structures from cells to organs, cell communication, fermentation, and evolution.

This lab requires two to four 50-minute class periods. The basic part of the lab can be completed in two class periods. This would include the initial experiment with geotaxis and the basic construction and experimental setup with the choice chamber and food item taxis. The student-directed investigation could take up to two additional 50-minute class periods for the completion of the experiments and collaboration with classmates. Be flexible in planning the lab because some students groups will take more time than others.

It is important to allow enough class time for students to complete several trials of their experiments. Additional time outside of class would be required to complete data tables and a chi-square analysis of data collected. Set aside class time for presentations of results by students.

Safety and Housekeeping

- You should monitor the use of chemicals that students use. They should not have access to stock bottles of any chemical, such as an acid or a base, including vinegar, ammonia, or bleach. As students request materials, the chemicals should be allocated to dropper bottles or other small bottles.
- All chemicals, including household materials, should have a clear label and be disposed of properly.
- Students should wear safety goggles or glasses when working with liquid chemicals.
- Students should not eat any of the food items.
- Have students bring clear bottles and caps to class. Remind them that a softer plastic bottle, such as a water bottle, is easier to cut than a harder plastic bottle, such as a Gatorade bottle. Choice chamber bottles should be 12–16 oz. in size for the initial experiments, but students may want to bring smaller or bigger bottles for their independent work.
- Remind students to use small amounts of liquid. (Five–10 drops should be enough.) More liquid can cause overflow into the chamber. The substance must remain on the cotton balls in the cap to ensure an accurate test of choice.
- In one class session, several choice experiments can be done. Small pieces of fruit could also be used, but students should be sure to firmly adhere the fruit to the cotton. It is important to change (or clean and dry) the cap after each choice unless the same substance is used again.
- New flies should be added if the experiment continues beyond the first day. However, if the flies are still active at the end of the first day, students can return them to a culture vial by tapping the chamber into a funnel placed in the culture vial.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to interactions (big idea 4) or to cellular processes (big idea 2), specifically the capture, use, and storage of free energy. In addition, some of the questions are likely to connect to big idea 1 if students explore the evolution of observed behaviors. As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 2D1: Reinforce the concept that all biological systems from cells and organisms to populations, communities, and ecosystems are affected by complex biotic and abiotic interactions involving exchange of matter and free energy.
- 2E3: Timing and coordination of behavior are regulated by various mechanisms and are important in natural selection.
- 4A6: Interactions among living systems and with their environment result in the movement of matter and energy.
- 4B4: Interactions between and within populations influence patterns of species distribution and abundance.

Learning Objectives

- The student is able to refine scientific models and questions about the effect of complex biotic and abiotic interactions on all biological systems from cells and organisms to populations, communities, and ecosystems (2D1 & SP 1.3, SP 3.2).
- The student is able to design a plan for collecting data to show that all biological systems (cells, organisms, populations, communities, and ecosystems) are affected by complex biotic and abiotic interactions (2D1 & SP 4.2, SP 7.2).
- The student is able to analyze data to identify possible patterns and relationships between a biotic or an abiotic factor and a biological system (cells, organisms, populations, communities, or ecosystems) (2D1 & SP 5.1).
- The student is able to analyze data to support the claim that response to information and communication of information affect natural selection (2E3 & SP 5.1).
- The student is able to justify claims, using evidence, to describe how timing and coordination of behavioral events in organisms are regulated by several mechanisms (2E3 & SP 6.1).
- The student is able to connect concepts in and across domain(s) to predict how environmental factors affect response to information and change behavior (2E3 & SP 7.2).
- The student is able to apply mathematical routines to quantities that describe interactions among living systems and their environment that result in the movement of matter and energy (4A6 & SP 2.2).

- The student is able to use visual representations to analyze situations or solve problems qualitatively to illustrate how interactions among living systems and with their environments result in the movement of matter and energy (4A6 & SP 1.4).
- The student is able to predict the effects of a change of matter or energy availability on communities (4A6 & SP 6.4).
- The student is able to use data analysis to refine observations and measurements regarding the effect of population interactions on patterns of species distribution and development (4B4 & SP 5.2).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

Before students attempt this investigation, they should be able to demonstrate understanding of the following concepts. The concepts may have been taught at different times of the year, but they give a conceptual understanding that could help students formulate questions related to the behavior of *Drosophila*.

- An insect life cycle that includes a complete metamorphosis (egg, larva, pupa, and adult)
- The role of apoptosis in metamorphosis
- The genetic basis of behavior and role of a taxis
- The types of environmental factors that trigger a behavior
- The generalized structure of sensory organs and neurons

Skills Development

Students will develop the following skills and reinforce their observation skills. They may have worked with fruit flies in previous investigations, so they may be familiar with some of these skills. If the students are new to working with fruit flies (or other research animals), care must be taken to ensure the proper care and handling of living organisms.

- Determining the sex of fruit flies: While the investigation can be completed without knowing the sex of the fruit flies, sex identification does give students a more complete understanding of the organism. Use clear visuals (not diagrams) and dissecting microscopes when instructing students how to determine the sex of the flies. You can show students Figure 1 to help them determine the sex of fruit flies. The sexing of the flies can be completed at any time in the curriculum.

Figure 1. Determining the Sex of Fruit Flies

- Preparation of solutions: All solutions should be used from labeled containers and have some connection to household use. It is interesting to have students make choice chambers containing mustard, capers, and other condiments. Lab chemicals such as HCl or NaOH should be no more concentrated than 0.1 M, and care should be given when these solutions are made and used.
- Construction of choice chamber: For eight lab groups, collect at least 16 plastic water bottles with caps. Soft plastic bottles are best because they are easy to cut. Obtain extra caps in order to change the conditions for the choice chamber. While all of the bottles do not need to be the same size, collect pairs of the same type so that each team has two matching bottles. The choice chambers can vary in size from team to team. Students should cut off the bottom of the bottle using scissors, rinse out the bottle, and dry the bottle with towels if there is *any* liquid remaining. Then they match two bottles end-to-end on the cut side, use clear plastic packing tape to tape the bottles together, and label one side "A" and the other "B" (see Figure 2). They can remove the caps to add cotton balls with the testing substances.

Figure 2. Choice Chamber

- Use of different-aged fruit flies: Some experiments could be designed using fruit flies of different ages. To set up those cultures, be sure to have flies arrive at least two weeks before the experiments. Isolate any adult flies into a new vial of food. These flies will then be two weeks old for your tests. Young flies will not emerge until about two weeks later in the new culture. The original culture can then be used as young flies if the adults are let out of the culture the day or two before the experiments.
- Use of larvae: Students could design experiments using fruit fly larvae. The third instar larvae leave the moist food in a culture and climb up into the dryer environment of the vial to get ready for pupation. Students should place larvae on a glass slide to test for chemotaxis or phototaxis. The larvae will move more easily if a layer of non-nutrient agar is placed on the slide. Students make the 2% agar by mixing agar powder and water (2 g and 100 mL water) and heating it. Then they place it with an eyedropper in a puddle on the slide. They need to wait for the agar to harden to use as a platform for any experiments using larvae. They could also use Knox gelatin to make a smooth surface by following the recipe to make gelatin with one package of Knox gelatin to 1 cup of water.

Potential Challenges

Students must design a controlled experiment for the choice chamber. They must be sure to test the chamber with distilled water to determine if there are any variables, such as light or the angle of the chamber, that could affect the experiment. They should be advised to make observations and then turn the chamber to see if the fruit flies respond differently.

Although it is difficult to count fruit flies accurately, the students should work as a team to count as accurately as possible. One technique that works is to divide the chamber into quadrants that are counted separately by four different students. Vestigial wing flies take longer to arrive at the ends of a choice chamber, but they may be easier to transfer and count because they do not fly.

Students should be warned that fruit flies are escape artists, and care should be taken to keep the flies contained. Some “lost” flies can be recaptured by making a fly trap with a small amount of vinegar or piece of fruit such as a banana or banana peel placed in a container with a funnel. Be sure that there is space at the bottom of the funnel so that the flies can enter the beaker to explore the vinegar or fruit. They typically do not escape back up through the funnel.

Moving flies from one container to another can be challenging. Flies are tossed by using their tendency to move up (their negative geotaxis) to help control their transfer. To toss flies, students should gently tap a culture of flies on a table to force them down to the bottom of the culture. They must quickly remove the plug from the culture and place an empty vial on top of the fly culture. Some flies will walk up into the empty vial, or students could turn the empty vial to the bottom and tap flies into the empty vial. Students must immediately plug the two vials. To toss flies into a choice chamber, they use the same technique to tap flies into the bottom of a culture and then quickly invert the culture into a funnel that is placed into one end of a choice chamber, making sure that the cap is on the other end of the chamber. Students then tap the culture to place at least 30–40 flies into the chamber, lift up the culture vial, and immediately plug the vial and cap the chamber. If a culture vial is upside down even with flies in it, very few flies will escape before it is plugged again.

An additional technique to avoid the problem of flies escaping is to refrigerate them before the transfer. If vials are chilled for at least 15–30 minutes before tossing, they are easier to transfer. Be sure that the chilling does not add moisture to the culture, as moisture can make the flies stick to the vials.

Students may have the misconception that fruit flies are attracted to fruit, but they will determine that it is not the fruit but rather the *rotting* fruit and the accompanying chemotaxis to various products, such as vinegar or alcohol, of this decomposition process that prompts the fly's behavior. How can you help students identify the difference without telling them? Is the chemotaxis a strong taxis? Does a geotaxis or phototaxis override the chemotaxis?

THE INVESTIGATIONS

Getting Started: Prelab Assessment

You may assign the following as a think, pair/group, share activity, in which pairs or small groups of students brainstorm ideas and then share them with other groups, or as a whole-class discussion to assess students' understanding of key concepts pertaining to fruit flies:

Day 1

1. During this discussion, you can assess if your students know what fruit flies are and when and where they have seen them. The class should make a list of when and where they notice fruit flies. They should generate a list that may include a bowl of fruit, a picnic dinner, and someone's glass of wine. Students should also view pictures of fruit flies to recall previous experiments with these model research organisms used in genetics or population studies.

2. Have the students make observations about fruit fly behavior by conducting the following very simple geotactic experiment. Students can work in small groups.

   a. Using fruit fly cultures, toss at least 10 flies into an empty vial. Do not anesthetize the flies before this or any of the behavior experiments.

   b. Observe the position of the flies in an upright vial sitting in a test tube rack on the lab table. Do not touch the vial while making observations.

   c. Invert the vial and observe the position of the flies after 15 seconds and after 30 seconds. Make a list of observed behaviors.

   d. Observations should generate questions, including *What was the flies' response? Was there an orientation movement? If so, what was the stimulus? Could this be considered a taxis?* Explain your answers.

Designing and Conducting Independent Investigations

Day 2

When developing their own investigations, students should choose substances to test that are interesting to them. They may have experiences with fruit flies in their home and can think about what attracts flies. They also may want to find a substance that would repel a fly. They can bring substances from home to test, but make sure they obtain your permission to use the substances before they conduct their tests. The students should work in groups to determine the chemotactic response to various food items. They should share and graphically illustrate their results.

Days 3–4+

The following are suggestions for the student-directed lab activities based on questions students ask during their preliminary study of the fruit flies. Their questions might include the following: *Does the age of a fruit fly affect the speed of their negative geotactic response? What wavelengths of light stimulate a phototactic response in fruit flies?*

Possible investigations generated from students' observations and questions include the following. However, it is suggested that students generate their own questions to explore.

- From an ingredient list, select substances (such as vinegar) that students think might be affecting fly behavior. Isolate the materials and give the flies a choice.
- Determine if the sex of the fly makes a difference in their choice. (An F1 population of flies with white-eyed males and red-eyed females could be made available.)
- Determine if the sight of the material makes a difference by covering up the cotton ball in parafilm.
- Find the effect of light by changing the light source at different ends of the chamber or by studying how flies make choices in different colors of light. (Different ends of the chambers could be wrapped in transparent colored films or acetate.)
- Determine if the ripeness of the fruit makes a difference. For example, ripe bananas could be compared to green bananas.
- Determine if fruit flies are attracted to or repelled by carbon dioxide by placing pieces of Alka-Seltzer in moist cotton balls.
- Determine the effect of age or the developmental stage of the fruit fly on choice by using newly emerged flies in the chamber and/or the third instar larva on a glass slide.
- Work with different mutants of fruit flies to determine if vestigial or white-eyed flies (or other mutants) make the same choices. Determine if the Adh-negative mutant affects the flies' response to alcohol. Determine if mutant eye colors (white, cinnabar, brown) affect the flies' response to light.
- Are there other organisms that respond like fruit flies? Can you think of any organisms that respond differently?

Students should verify the results of their experiment by conducting several trials and changing the position of the substances at the ends of the chamber.

Summative Assessment

This section describes suggestions for assessing students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment. Each student (or group of students) should present data from a repeated, controlled experiment with graphic representation and quantitative analysis of fruit fly choices.

1. Have students record their experimental design, procedures, data, results, and conclusions in a lab notebook, or have them construct a mini-poster to share with their classmates.

2. Revisit the learning objectives for the investigation and develop strategies (e.g., questions or activities) that can help determine whether or not the learning objective has been met. For example, one learning objective is *The student is able to analyze data to identify possible patterns and relationships between a biotic or abiotic factor and a biological system* (2D1 and 5.1). The student might be asked to complete a data grid of choice for fruit flies, analyze the data, and draw conclusions. Table 1 is an example of a data table that students could construct for themselves as they use the choice chamber (x = number of flies at one of the two ends of the chamber). The students may need to adjust the table to indicate multiple trials.

Table 1. Fruit Fly Choices

Substance	1		2		3		4		5		6		7	
A	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	X	X												
2	X		X	X										
3	X	X	X		X	X								
4		X	X		X	X	X	X						
5	X		X	X	X	X		X	X	X				
6	X	X	X		X		X			X	X	X		
7	X	X	X	X	X		X		X		X		X	X

3. Complete a chi-square analysis of the results to determine if the distribution of the flies is significant.

4. Released AP Exams have several multiple-choice and free-response (essay) questions based on the concepts studied in this investigation. One good example is the free-response question about *Bursatella leachii* (sea slug) from the 1997 AP Biology Exam.These questions could be used to assess students' understanding.

Where Can Students Go from Here?

One possible extension for this investigation is to ask students to identify another organism that behaves similarly to the fruit fly and one that they expect would behave differently. For example, students could substitute ladybugs, houseflies, or mealworms for fruit flies and construct choice chambers using other substances that they think might be attractive to these organisms.

SUPPLEMENTAL RESOURCES

www.fruitfly.org The Berkeley *Drosophila* Genome Project includes links to images and other resources for teachers and students. Teachers should consider looking through this website before beginning the lab to appreciate the breadth of information about *Drosophila*.

Flagg, Raymond. *The Carolina Drosophila Manual.* Burlington, NC: Carolina Biological Supply Company, 1988.

This is a useful manual that comes with each *Drosophila* culture order from Carolina. It may be ordered separately (order number 45-2620 from Carolina Biological Supply Company). Excellent photographs help teachers and students identify different mutants and give guidance for sexing the flies.

http://www.Flybase.org This is a classic database of *Drosophila* genes and genomes used extensively by researchers and educators. This resource is cited on many other sites, including the Berkeley *Drosophila* Genome Project. It is a general database that could be used by teachers or students who would like to have more information about any particular mutant.

Gargano, Julia Warner, Ian Martin, Poonam Bhandari, and Michael S. Grotewiel. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila. Experimental Gerontology* 40, no. 5 (May 2005): 386–395.

Fruit flies' negative geotaxis response declines with age. This resource would be helpful for teachers or students who have designed experiments that use the age of the fruit fly as a variable.

http://www.hhmi.org The Howard Hughes Medical Center includes multiple resources for teachers about fruit flies. This is a general resource that could lead to ideas for experiments or general information about fruit flies before the class begins the experiments. It includes links to other resources for general information and the scope of research about *Drosophila*. This resource includes very accessible material that would be helpful as a teacher begins the experiments.

www.ncbi.nlm.nih.gov The National Center for Biotechnology Information website offers access to biomedical and genomic information. The database could be used as extensions for students or teachers interested in some of the specific sequences of DNA or proteins that are characteristic of some of the different *Drosophila* mutants.

Raman, Baranidharan, Iori Ito, and Mark Stopfer. Bilateral olfaction: two is better than one for navigation. *Genome Biology* 9, no. 3 (2008): 212.

Fruit fly larvae can localize odor sources using unilateral inputs from a single functional sensory neuron. This resource documents the techniques and results of chemotaxis experiments with *Drosophila* larvae. It would be useful for teachers or students interested in designing a lab using the larvae rather than the adult flies.

http://www.ceolas.org/fly This is a general resource about fruit flies that has links to many other resources. Teachers or students interested in finding more information about a particular mutant or images of fruit flies or understanding the scope of research about *Drosophila* will find this resource to be useful.

Weiner, Jonathan. *Time, Love, Memory: A Great Biologist and His Quest for the Origins of Behavior.* New York: Alfred A. Knopf, Inc., 1999.

This book is a classic read about the behavior of fruit flies as investigated by Seymour Benzer, one of the great biologists of the 20th century. This text is a very good summary of research completed in classical experiments in physiology and behavior. It sets the stage for a teacher interested in working with fruit flies. It would be an excellent book for students to read after completing the experiments because of the engaging connections made between *Drosophila* and the researchers.

INVESTIGATION 12

FRUIT FLY BEHAVIOR

What environmental factors trigger a fruit fly response?

BACKGROUND

Drosophila melanogaster, the common fruit fly, is an organism that has been studied in the scientific community for more than a century. Thomas Hunt Morgan began using it for genetic studies in 1907. The common fruit fly lives throughout the world and feeds on fruit and the fungi growing on rotting fruit. It is a small fly, and one could question why scientists have spent so much time and effort on this tiny insect. It is about the size of President Roosevelt's nose on a dime, but despite its small size, the fly is packed with many interesting physical and behavioral characteristics. Its genome has been sequenced, its physical characteristics have been charted and mutated, its meiotic processes and development have been investigated, and its behavior has been the source of many experiments. Because of its scientific usefulness, *Drosophila* is a model research organism. Its name is based on observations about the fly; the fly follows circadian rhythms that include sleeping during the dark and emerging as an adult from a pupa in the early morning. This latter behavior gave rise to the name *Drosophila*, which means "lover of dew." The explanation for the species name *melanogaster* should be clear after observing the fly's physical features. It has a black "stomach," or abdomen. No doubt the dew-loving, black-bellied fly will continue to make contributions to the scientific community and to student projects.

We begin our investigation with a few simple questions. What do you know about fruit flies? Have you seen fruit flies outside the lab and, if so, where? Describe where and when you have noted fruit flies.

Learning Objectives

- To investigate the relationship between a model organism, *Drosophila*, and its response to different environmental conditions
- To design a controlled experiment to explore environmental factors that either attract or repel *Drosophila* in the laboratory setting
- To analyze data collected in an experiment in order to identify possible patterns and relationships between environmental factors and a living organism
- To work collaboratively with others in the design and analysis of a controlled experiment
- To connect and apply concepts (With the fruit fly as the focal organism, your investigation could pull together many topics, such as genetics, animal behavior, development, plant and animal structures from cells to organs, cell communication, fruit ripening, fermentation, and evolution.)

General Safety Precautions

- Do not add substances to the choice chamber unless your teacher has approved them.
- If the substance you add is flammable, such as ethanol, use precaution and do not conduct your experiment near a heat source or flame.
- Many of the substances used in this experiment are food items, but you should not consume any of them.
- Fruit flies are living organisms that should not be released to the environment. After all the investigations are complete, flies should be tapped into a “morgue” through a funnel. The morgue typically is a 150-mL beaker that contains about 50 mL of salad oil or 70% alcohol.

THE INVESTIGATIONS

Getting Started

This procedure is designed to help you understand how to work with fruit flies. You may start with general information about how to determine the sex of a fruit fly. How do you tell the difference between male and female flies? Is the sex of the fly important to your investigations? Look at the female and male fruit flies in Figure 1. Then look at the fruit flies in Figure 2. Can you identify which ones are female and which ones are male? Focus on the abdomen of the flies to note differences.

Figure 1. Determining the Sex of Fruit Flies

Figure 2. Fruit Flies

Step 1 Using fruit fly cultures, carefully toss 10 to 20 living flies into an empty vial. Be sure to plug the vial as soon as you add the flies. Do not anesthetize the flies before this or any of the behavior experiments.

Step 2 When flies are tossed, they are tapped into an empty vial. Tap a culture vial (push the vial down on a solid surface several times) on the table to move the flies to the bottom of the vial. Quickly remove the foam or cotton top and invert an empty vial over the top of the culture vial. Invert the vials so that the culture vial is on the top and the empty vial is on the bottom, and tap the flies into the empty container by tapping it on a solid surface several times. Be sure to hold the vials tightly to keep them together. You must then separate the vials and cap each separately. Do not try to isolate every fly from the original culture. It is difficult to separate flies, and you may lose a fly or two in the process.

Step 3 After your lab group has the flies in a vial without food, observe the position of the flies in your upright vial.

Step 4 Invert the vial, and observe the position of the flies after 15 seconds and after 30 seconds.

Step 5 What was the flies' response? Did most/all of the flies move in the same general direction? If so, this might be an "orientation movement," which is a movement that is in response to some stimulus. Based on how you manipulated the vial, to what stimulus might the flies be responding? Do you think that they were responding to some chemical change in the vial? Did your observations generate other questions? Explain your answers.

Procedure

Animals move in response to many different stimuli. A chemotaxis is a movement in response to the presence of a chemical stimulus. The organism may move toward or away from the chemical stimulus. What benefit would an organism gain by responding to chemicals in their environment? A phototactic response is a movement in response to light. A geotactic response is a movement in response to gravity.

You will investigate fruit fly movement using a choice chamber that exposes the flies to different substances that you insert into the chamber. Because flies are very common in households (in fact, fruit flies live almost everywhere that humans live), think about using foods or condiments that might result in a positive or a negative chemotactic response from the flies. What foods or condiments do you think would attract or repel flies? Why? Do fruit flies exhibit a response to light or to gravity? How can you alter the chamber to investigate those variables?

Step 1 Prepare a choice chamber by labeling both ends with a marker — one end "A" and the other "B" (see Figure 3). Cut the bottom of the bottles, dry the interior thoroughly, and tape them together. Remove any paper labels.

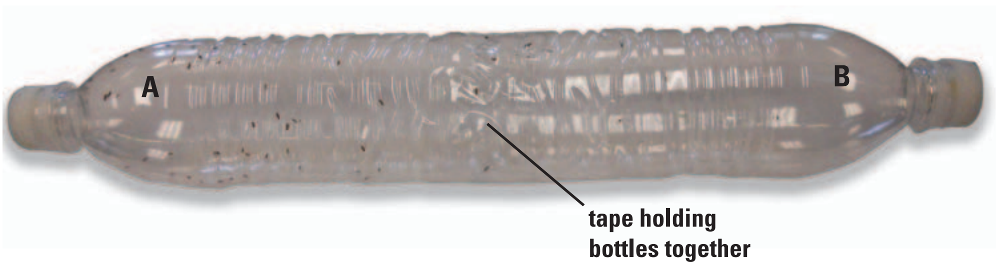

Figure 3. Choice Chamber

Place a cap on one end of a chamber before adding flies. Insert a small funnel in the open end of the chamber and place the chamber upright on the capped end. Tap 20–30 fruit flies into the choice chamber using the funnel.

Step 2 After transfer, quickly cap the other end of the chamber.

Step 3 Begin your study of the choice of flies by placing a few (5–10) drops of distilled water on two cotton balls, and adhere one moist cotton ball to each end of the chamber. (Do not add too much of any chemical to the cotton; too much liquid will drip down into the chamber and affect the experiment by sticking flies to the bottle.) What is the importance of using distilled water at both ends of the chamber?

Step 4 Lay the chamber down on a white surface or on white paper.

Step 5 Give the flies at least 5 minutes of undisturbed time, and then count (or closely approximate) the number of flies at each end of the chamber. Create a table to record the number of flies you find at each end (A and B) of the chamber.

Step 6 List all of the substances that you will be testing, and predict what you think the flies will prefer based on your knowledge of fruit flies.

Step 7 Begin to test each substance you are including in your investigation. Place a few drops of one substance on a cotton ball. Remove cap A, place the cotton ball in the cap, and replace the cap on the chamber. Place a cotton ball with distilled water on the other end. How might you determine which of the substances stimulate a negative chemotaxis and which stimulate a positive chemotaxis?

Step 8 Lay the chamber down on a light colored surface (or on white paper) and observe the flies.

Step 9 Give the flies at least 5 minutes of undisturbed time, and then count the number of flies at each end of the chamber.

Step 10 Change the caps, and give the fruit flies another substance.

Step 11 Gather data for at least four different substances. Which substances do fruit flies prefer? Which do they avoid?

Step 12 Quantify the results and express them graphically. Complete a chi-square analysis of your results. Using data from the entire class, construct a preference table. Were your hypotheses about the preferences of fruit flies supported or not? Did the flies demonstrate a chemotaxis in relation to any of the substances you chose? Can you think of any reasons for their preferences?

Designing and Conducting Your Investigation

Now that you have discovered the preferences for individual substances, design an experiment using the choice chamber to compare the preferences of fruit flies to all test substances or the chemotactic responses of your flies. Create a table that includes the results comparing all of the substances you tested.

The following are questions that you could investigate; however, as you worked through the beginning of this lab, you should have developed your own question and an investigation to answer that question:

- Are all substances equally attractive or repellant to the fruit flies?
- Which substances do fruit flies prefer the most?
- Which substances do fruit flies prefer the least?
- Do preferred substances have any characteristic in common?
- What other factors might affect whether or not the fruit flies moved from one part of your choice chamber to another?
- Do you think that it is the fruit itself that attracts the flies? Should they be called *fruit flies* or something else?
- Some experiments could be designed using fruit fly larvae. Do larvae respond the same way that adults respond? Are there other factors in the environment that affect the choice?
- What factors must be controlled in an experiment about environmental variables and behavior?
- What is the difference among phototaxis, chemotaxis, and geotaxis? Do fruit flies demonstrate all of them?
- Does a phototactic response override a chemotactic response?
- Does the age of the fruit fly change its geotactic response?
- Are there other organisms that respond the same as fruit flies? Are there other organisms that respond differently from fruit flies?

Analyzing Results

Look for patterns in fly behavior based on the number and ratio of fruit flies on different ends of your choice chamber. How will you determine which of the substances stimulate the greatest negative chemotactic response and positive chemotactic response? Do you see any patterns about materials or forces to which fruit flies are attracted?

Develop a method for sharing your results and conclusions to classmates — and then share them!

Evaluating Results

1. Is there anything that was shared by all of the environmental factors to which the flies were attracted?
2. Is there anything that was shared by all of the environmental factors to which the flies were repelled?
3. How do you explain the behavior of fruit flies in someone's kitchen or in nature based on the information you collected? Do your data explain all fruit fly movements? Explain your answers.

Where Can You Go from Here?

One possible extension for this investigation is to identify another organism that behaves similarly to the fruit fly and one that you expect would behave differently. For example, you could substitute ladybugs, houseflies, or mealworms for fruit flies and construct choice chambers using other substances that you think might be attractive to these organisms.

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