EDVOTEK®

AP BIOLOGY

BIG IDEA 4
INVESTIGATION
12

EDVO-Kit: AP12

Fruit Fly Behavior

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to the concept of distribution of common fruit fly, *Drosophila melanogaster*, in a resource gradient. Student will investigate Fruit fly responses to gravity (geotaxis), chemicals (chemotaxis), and light (phototaxis).

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Experiment Components

- Transfer pipets
- Cotton balls
- Edvotek® Instant *Drosophila* Growth Media
- *Drosophila* vials
- Vial plugs

Store the entire experiment at room temperature.

This experiment is designed for 10 lab groups.

Requirements

- Wild-type *Drosophila**
- Plastic water bottles (2 per group and extra caps)
- Any combination of the following substances
  - Household condiments (mayonnaise, mustard)
  - Fruits (banana, melons)
  - Lab chemicals (ethanol, HCl, NaOH)
- Laboratory notebook
- Dissecting microscopes
- Color pens (for graphing)
- Transparent colored film (for wrapping chamber)
- Clear Tape
- Goggles
- Funnel
- Timer
- Water

* *Drosophila* must be requested 2-3 weeks prior to experiment. Use enclosed card to order the Drosophila.

Background Information

In order to survive, organisms must adapt their behavioral responses to meet the challenges presented by the environment. One way of designing a nervous system is to employ information gathered by successful ancestors. Genes carry the basic parameters of nervous systems which enabled past generations to survive and produce offspring. This would be sufficient if the environment never changed and each generation faced exactly the same situations, but the environment is always changing and offspring rarely face the same challenges as their parents. Therefore, in order to survive, it is important to have some flexibility built into the program.

Another way to design a nervous system is to let a young animal experience its world and then adapt its behavioral repertoire to the conditions it encounters. In most cases adult behavior is believed to be a combination of inherited genetic information and experience where genes describe the parameters in which the system operates and experience sets the scale.

Early experience is known to affect the brain and behavior of many animals, including humans. Beginning life in a stimulus-deprived environment typically leads to diminished performance in adults, while growing up in a stimulating environment should allow the nervous system to develop to its full potential. The field of behavioral biology is replete with examples: rats raised in enriched environments score better on learning tests than those raised in standard laboratory cages and cats reared in darkness are visually retarded. Exposure to appropriate stimuli early in life is often necessary for appropriate behavior in adults. For example, young male passerine birds must be exposed to the courtship songs of conspecific males early in development or else they will be unable to sing normally when they mature.

Ethology is the study of animal behavior which is both **learned** and **innate**. **Orientation behaviors**, such as taxis and kinesis, allow the animal to move into its most favorable environment. The animal moves either toward or away from the stimulus. A stimulus, such as moisture, chemicals, light, sound, or heat, which results in orientation to the stimulus, is **taxis.** For example, an organism that moves away from a dry area into a moist area is demonstrating taxis. Movement that is random in all directions with respect to a stimulus, such as moisture, is **kinesis.**

Agnostic behavior and mating behavior are two behavioral responses of animals in relation to other animals. Animals respond to each other by **agnostic behavior**. For example, an aggressive or threatening display by one animal may result in a submissive display by the other animal. **Mating behavior**, such as courtship and mating with a member of the same species, involves complex interactions unique to that species.

Until recently, developmental plasticity was thought to be a unique characteristic of the mammalian brain, while the nervous systems of simpler animals were supposed to be genetically predetermined bundles of instinctive responses. In the last twenty years however, research has indicated that early experience is also an important factor in determining the adult behaviors of lower vertebrates and even invertebrates.

The fruit fly *Drosophila melanogaster* (**Figure 1**) is a popular model system for biological studies due to its short generation time and ease of culture. It is best known as a genetic workhorse for its contributions in the field of development. It has also been shown to perform dozens of complex behaviors which can be quantified and explored.

Background Information

The life cycle (from egg to adult) takes about 10 days at room temperature. Eggs are laid and hatch into first instar larvae. These larvae feed voraciously on the culture medium provided. These first instar larvae go through several instar stages and eventually the third instar larvae crawl up the sides of the bottle away from the culture medium. There they stop and their larval cuticle hardens forming a dark brown pupa. Metamorphosis takes place during the pupal stage. Larvae tissues degenerate and reorganize forming an adult fly inside the pupal case. When metamorphosis is complete, the adult fly emerges from the pupal case. After the fly emerges, the wings expand and dry, the abdomen becomes more rotund, and the color of the body darkens.

Sexing flies: Male and female fruit flies can be distinguished from each other in three ways:

1. Only males have a sex comb, a fringe of black bristles on the forelegs.
2. The tip of the abdomen is elongated and somewhat pointed in females and more rounded in males.
3. The abdomen of the female has seven segments, whereas that of the male has only five segments.

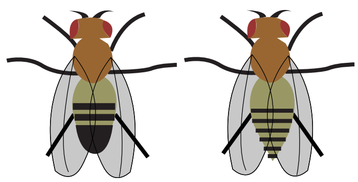


Figure 1: Male (left) and Female (Right) Fruit fly

Geotaxis – Effects of Gravity on Behavior

It is known that some fruit flies respond to gravity by choosing to fly into either high or low level tubes when given choices in glass mazes. The flies are respectively described as having negative (high) or positive (low) geotaxis. A geotactic response is a movement in response to gravity.

Chemotaxis – Effects of Chemicals on Behavior

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.

Phototaxis – Effect of Light on Behavior

Phototaxis has been studied in Drosophila since 1905. Phototaxis begins when the stimulus impinges on the light receptor and culminates with the locomotion, or lack of locomotion, of the animals. Therefore, phototactic response is a movement in response to light.

In this experiment, students will study the response of fruit flies to different stimuli. Movement toward a substance is a **positive taxis**. Consistent movement or orientation away from a substance is a **negative taxis**.

Experiment Overview and General Instructions

EXPERIMENT OBJECTIVE

The objective of this experiment is to introduce students to the concept of distribution of common fruit fly, *Drosophila melanogaster*, in a resource gradient. Students will investigate Fruit fly responses to gravity (geotaxis), chemicals (chemotaxis), and light (phototaxis).

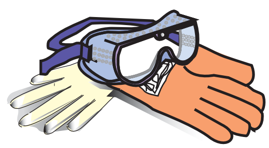
EXPERIMENT OVERVIEW

In this experiment, students will construct a choice chamber to study fruit fly behavior and investigate what environmental factors trigger a response by observing behavior of fruit fly as students present different options in the choice chamber.

WORKING HYPOTHESIS

If an organism is placed in an unfavorable environment, then the organism will exhibit an orientation behavior that allows the organism to move away from that environment into a more favorable one.

LABORATORY SAFETY



1. Gloves and safety goggles should be worn routinely as good laboratory practice.
2. Do not consume any of the substances used in this experiment even though they are food items.
3. Do not release fruit flies into the environment after the completion of the experiment. They should be tapped into a "morgue' through a funnel. Refer to Pre-lab Preparations for instruction of how to create a "morgue."

Experimental Procedure

Before starting the investigation, it is advised that teachers help students determine the sex of fruit flies. While the experiment can be completed without knowing the sex of the fruit flies, sex identification gives students a better understanding of the organism.

1. Use clear visuals (not diagrams), dissecting microscopes, and Figure 1 as a reference, to determine the sex of fruit flies.

2. Make a detailed sketch of a male and female fly and summarize your observations of their behavior.

Question: Can you identify which ones are female and which ones are male? (Hint: Focus on the abdomen of the flies to note differences.)

Note: When working with Fruit fly, do not anesthetize the flies before the following procedure or any of the behavior investigations.

Investigation I: Construction of the Choice Chamber

1. Prepare a choice by chamber cutting 2 plastic water bottles at the bottom. Be sure to remove the labels from the bottles.
2. Remove and discard the bottom and dry the interior of the bottles thoroughly.
3. Tape the bottles together.
4. Label both ends with a permanent marker - one end "A," and the other "B" (see Figure below).

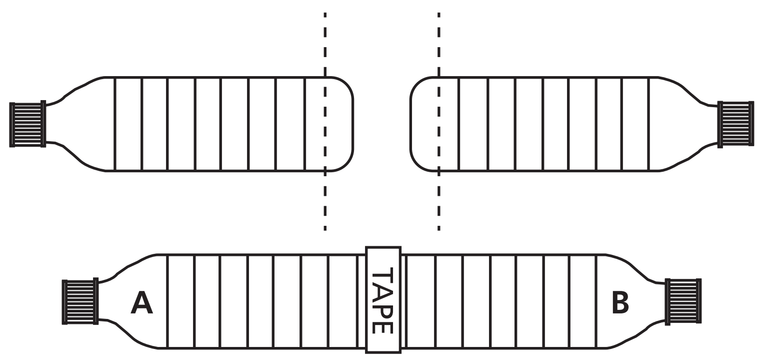


Figure 2 - Use scissors to cut the bottom of the bottles. Then, tape the bottles together using clear tape.

Investigation II: Geotactic Responses in Fruit fly

1. Place a cap on one end of the chamber before adding flies. Insert a small funnel in the open end of the chamber and place the chamber upright on the capped end.
2. Tap 10-20 fruit flies into the chamber using the funnel. Be sure to close the opening of the chamber as soon as you add the flies.
3. Tap the chamber on the lab bench to collect the flies to the bottom of the chamber.
4. Invert the chamber so that the end containing the flies is on top.
5. Tap the flies into the empty end by gently tapping the whole apparatus on the lab bench several times.
6. Observe the position of the flies in the chamber.
7. Invert the chamber, and observe the position of the flies after 15 seconds and after 30 seconds.
8. Take notes on their general movements in the chamber and interactions with each other. Notice if they seem to keep moving about, move sporadically or settle down in one place. Do not interfere with their behavior in any way.

Question: What was the flies' response when you tapped the chamber on the lab bench?

Investigation III: Chemotactic Response in Fruit Fly

A. OBSERVATION OF FRUIT FLY'S RESPONSE TO WATER

1. 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.

2. Tap 10-20 fruit flies into the chamber using the funnel and quickly cap the other end of the chamber.

3. Place a few (5–10) drops of distilled water on two cotton balls, and place one moist cotton ball at each end of the chamber.

   Note: Do not add too much liquid/water to the cotton as too much liquid will drip down into the chamber, making the flies stick to the bottle.

4. Lay the chamber down on a white surface and observe the flies.

5. Give the flies a few minutes of undisturbed time.

6. Every minute for 10 minutes, count how many flies are on each end (A and B) of the chamber, and then record your data in Table 1. Record even if they all move to one side or stop moving.

B. OBSERVATION OF FRUIT FLY'S RESPONSE TO OTHER SUBSTANCES

1. List all of the substances that you will be testing (lab chemicals, condiments, fruits, etc), and predict what you think the flies will prefer based on your knowledge of fruit flies. Gather data for at least three different substances.

2. Begin to test each substance by placing 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.

3. Place a cotton ball with distilled water on the other end.

4. Lay the chamber down on a white surface and observe the flies.

5. Give the flies a few minutes of undisturbed time.

6. Every minute for 10 minutes, count how many flies are on each end (A and B) of the chamber, and then record your data in Table 1. Record even if they all move to one side or stop moving.

7. Change the caps, andrepeat the above steps to expose the fruit flies to two additional substances. Record your data in Table 1.

Investigation III: Chemotactic Response in Fruit Fly

8. Graph each experimental test on the graph paper provided. Use a different color pen for each condition tested. For each experimental condition tested, determine the following:

   a. The independent variable ____
   Use this to label to horizontal (X) axis.

   b. The dependent variable ____
   Use this to label to vertical (Y) axis.

   c. Graph Title: ____

   d. Hypothesis:____

   e. Which substances do fruit flies prefer? _____________________________________
   Which do they avoid? ____

	Water		Substance 1		Substance 2		Substance 3	
	A	B	A	B	A	B	A	B
Time (min)								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Table 1 - Chemotaxis behavior in fruit flies

Investigation III: Chemotactic Response in Fruit Fly

Investigation IV: Phototactic Response in Fruit Flies

1. 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.
2. Tap 10–20 fruit flies into the chamber using the funnel and quickly cap the other end of the chamber.
3. Wrap one end of the choice chamber in transparent colored film.
4. Lay the chamber down on a white surface and observe the flies.
5. Give the flies a few minutes of undisturbed time.
6. Every minute for 10 minutes, count how many flies are on each end (A and B) of the chamber, and then record your data in Table 2. Record even if they all move to one side or stop moving.

	# of flies at end A	# of flies at end B
Time (min.)		
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Lab Extensions

The following are suggestions for the student-directed lab activities. Students are encouraged to conduct several trials to determine the response of fruit flies to the following environmental conditions.

1. Determine if the ripeness of the fruit makes a difference. For example, ripe bananas could be compared to green bananas.

2. Determine how fruit flies react to carbon dioxide by placing pieces of Alka-Seltzer in moist cotton balls. Are they attracted to or repelled by carbon dioxide.

3. Determine the effect of age or the developmental stage of the fruit fly by using newly emerged flies in the chamber and/or the third instar larva.

4. Work with different mutants of fruit flies to determine if vestigial or white-eyed flies (or other mutants) react similarly or differently to various conditions.

5. Determine if mutant eye colors (white, cinnabar, brown) affect the response of fruit fly to light.

Study Questions

1. Why are fruit flies an ideal organism for genetic study?

2. Give an example of how environmental factors affect the behavior in fruit flies?

3. Can you give a few examples for designing student-directed lab activities besides the activities suggested in this lab?