Advanced Placement Biology

AP Biology
Lab 11

291

EDVO-Kit #

Animal Behavior

Storage:
See page 3 for specific storage requirements.

EXPERIMENT OBJECTIVE

The objective of this experiment is to introduce students to the concept of distribution of organisms in a resource gradient and to learn the difference between kinesis and taxis.

Table of Contents

	Page
Experiment Components	3
Experiment Requirements	3
Background Information	4
Experiment Procedures	
Experiment Overview	6
Part A: General Observation of Behaviors	7
Study Questions, Part A	11
Student Designed Experiment	12
Part B: Reproductive Behavior in Fruit Flies	13
Study Questions, Part B	15
Extension of Experiment	15
Student-Designed Experiment	16
Instructor's Guidelines	
General Information and Pre-Lab Preparations	17
Expected Results	19
Study Questions and Answers	20
Material Safety Data Sheets	21

AP Biology

Lab # 11

Experiment Components

This experiment is designed for 10 lab groups

- Pillbugs or Sowbugs (depending upon availability)*
- *Drosophila* Wild Type*
- Petri Plates
- Transfer pipets
- EDVOTEK® BugOut™ Solution
- Culture Tubes
- Foam Lids
- EDVOTEK® Instant Drosophila Growth Media

Storage: Store entire experiment at room temperature.

* *Drosophila and pillbugs (or sowbugs) must be requested 2 - 3 weeks prior to experiment. Use enclosed card to order either the pillbugs or the Drosophila.*

Requirements

- Spring water
- Scissors
- Filter paper
- Tape
- Vegetable or mineral oil
- Alcohol
- Stereoscopic or dissecting microscope
- Incubation oven (optional)
- Fly Cycle CD, Catalog #1474 (optional)
- Light Source - Incandescent or Fluorescent (optional)
- Soft Paint Brushes

AP Biology

Lab # 11

Background Information

Animal Behavior

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.**

AP Biology

Lab # 11

Background Information

Animal Behavior

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.

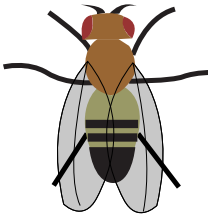


Figure 1:
Drosophila melanogaster

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.

Figure 2:
Pillbug

Pillbugs (**Figure 2**) are crustaceans that belong to the isopod group. Pillbugs derive their name from the fact that they roll up in a ball or "pill" when they are disturbed. Other insects run when disturbed. Although they are of little or no economic significance, they are interesting creatures to use for studying behavioral and environmental responses.

Pillbugs lack a cuticle and breathe through gills so they must live in moist environments. They prefer low light or dark habitats and can be found underneath logs, leaves, rocks, wood, etc. They feed on decaying wood, leaves and other vegetation. Therefore, they play an important role in decomposing dead plant material. The body of a pillbug is flat, 5-15mm in length, and has three body segments; head, thorax, and abdomen. The outer layer is made of shield-like plates. The seven pairs of legs are identical. There is one pair of antenna and one pair of compound eyes. Females hatch their young from a brood pouch that is carried under the thorax. New pillbugs are independent after hatching. Pillbugs molt by shedding their exoskeleton and growing a new one. Pillbugs are safe in that they do not bite, sting, or transmit disease to humans.

AP Biology

Lab # 11

Experiment Procedure

Experiment Overview

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce students to the concept of distribution of organisms in a resource gradient and to understand the difference between kinesis and taxis.

WORKING HYPOTHESIS

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

2. If mating between a male and female fruit fly occurs by following strict behavioral patterns between the two, then one will observe certain behaviors occurring from each sex towards the other.

LABORATORY SAFETY

Gloves and safety goggles should be worn routinely as good laboratory practice.

Part A: General Observation of Behaviors

You will be working with common terrestrial isopods such as pillbugs, sowbugs or roly-polies.

PROCEDURE

1. Place a small amount of bedding material and 10 pillbugs in a petri dish. If they start to climb up the sides, cover the dish with the petri dish cover or plastic wrap.

2. Observe the pillbugs for 10 minutes without disturbing them in any way. Take notes on their general movements about the dish 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.

3. Make a detailed sketch of a pillbug and summarize your observations of their behavior.

KINESIS IN PILLBUGS

1. Prepare a choice chamber consisting of two large plastic petri dishes taped together with an opening cut in between as illustrated in **Figure 3** on page 8. Use a scissors to cut the opening and use tape to hold the dishes together. Place a dry piece of filter paper in each chamber. Moisten the filter paper in one chamber (use springwater).

2. Transfer five pillbugs with a soft brush from the stock chamber into each side of the choice chamber. Cover the chambers.

3. Every 30 seconds for 10 minutes, count how many pillbugs are on each side of the choice chamber, and then record your data in **Table 1** on page 9. Record even if they all move to one side or stop moving.

4. After observing, return your pillbugs to the stock chamber.

5. Graph both the number of pillbugs in the wet chamber and the number in the dry chamber using the **graph** on page 10.

6. Repeat steps #1-5, with 2 or 3 of the following conditions:

   a. Remove the wet filter paper (spring water). Add a dry piece of filter paper. Use a pipet to moisten with the acidic solution.
   b. Remove the wet filter paper (spring water). Add a dry piece of filter paper. Use a pipet to moisten with the basic solution.
   c. Remove the wet filter paper (spring water). Add a dry piece of filter paper. Cover one chamber with aluminum foil.

AP Biology

Lab # 11

Experiment Procedure

Part A: General Observation of Behaviors

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.

Graph Title: _____

Hypothesis:_____

Figure 3:
Setting up the
Choice Chamber

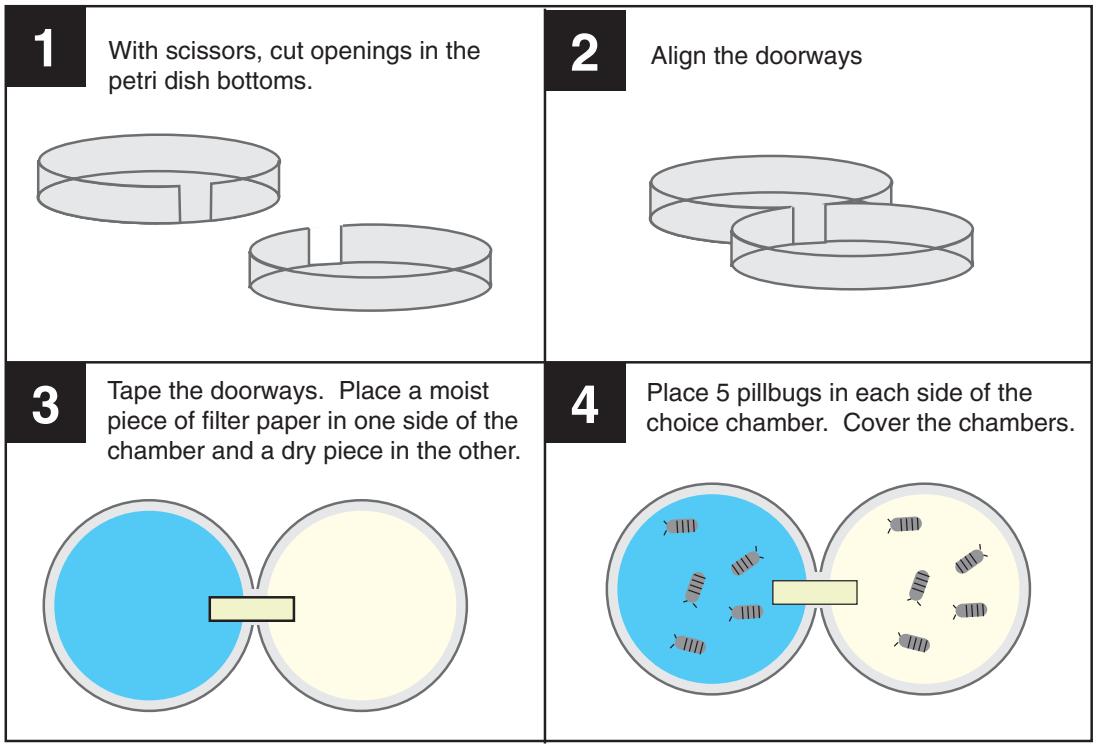


TABLE 1 - OBSERVATION OF PILLBUG BEHAVIOR			
Time (mins)	Wet Chambers	Dry Chambers	Other Observations
0			
0.5			
1.0			
1.5			
2.0			
2.5			
3.0			
3.5			
4.0			
4.5			
5.0			
5.5			
6.0			
6.5			
7.0			
7.5			
8.0			
8.5			
9.0			
9.5			
10.0			

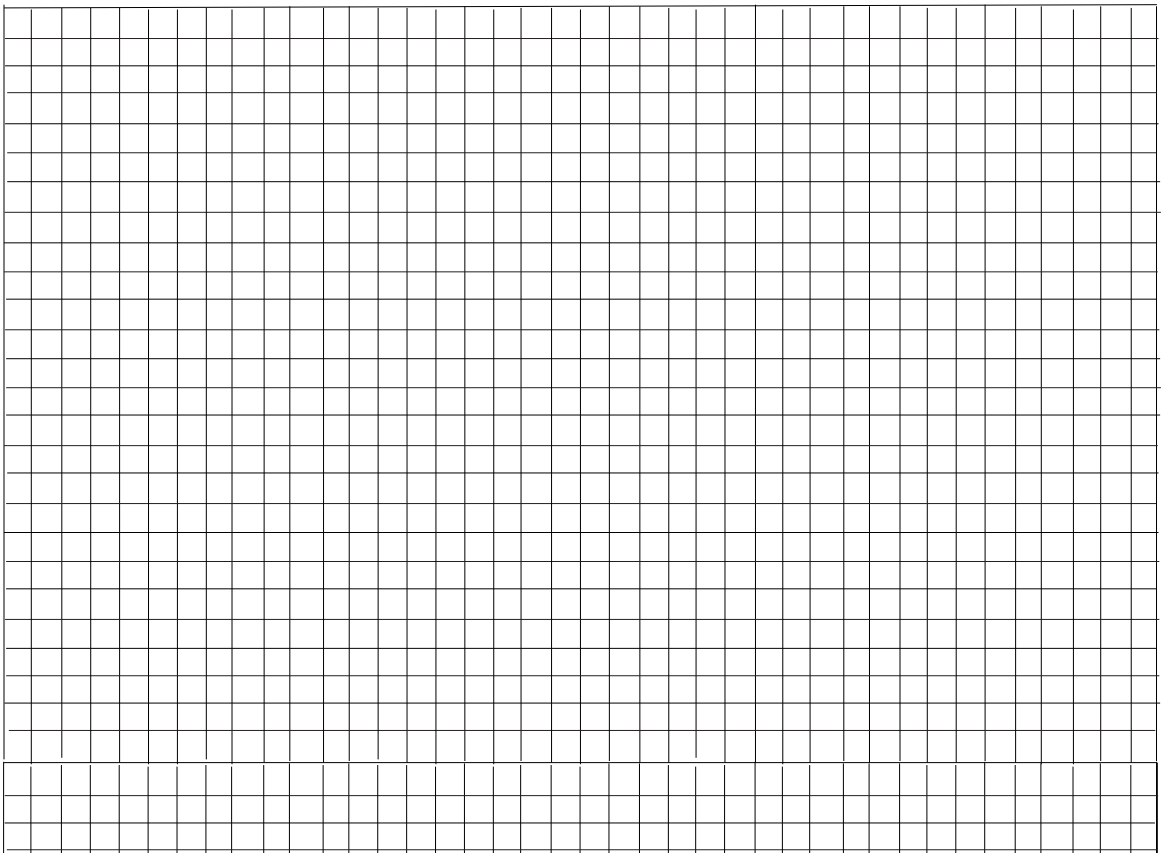
AP Biology

Lab # 11

Experiment Procedure

Part A: General Observation of Behaviors

1. Graph the results from the observations made of pillbug habitat preference.
2. Title the Graph_____
3. Determine the *independent* variable (horizontal (X) axis). Label the graph.
4. Determine the *dependent* variable (vertical (Y) axis). Label the graph.
5. What hypothesis is being tested in this experiment?



AP Biology

Lab # 11

Experiment Procedure

Part A: Study Questions

1. From your data, how do pillbugs seem to sense their environment? Explain physiological reasons for the behavior observed in this activity.

2. Obtain results from all lab groups in your class. With respect to the environmental stimuli tested, humidity, pH, and light which types of environment do isopods prefer? Give specific examples.

3. During a walk in the woods, you turned a rock over and found isopods under it. What would you expect them to be doing? Continue to watch the isopods a few minutes. Would you expect to see their behavior change? Explain.

4. Is the isopod's response to moisture, pH, and light best classified as kinesis or taxis? Explain your response.

AP Biology

Lab # 11

Experiment Procedure

Student-Designed Experiment

Design a simple experiment to investigate pillbugs' response to other environmental conditions such as temperature, background color, vibration or any other variable that you devise.

1. Chose one of the environmental conditions listed above and write a hypothesis concerning the pillbugs' response to the condition.

2. Design an experiment from the materials available in your classroom. (A lamp is a good source of heat).

   a. State the objective of the experiment.

   b. List materials needed.

   c. Outline the procedure in detail.

3. Design the data sheet.

4. Run the experiment. Record data.

5. Return the isopods to the stock culture.

6. Graph the results.

7. Write a conclusion based on your experimental results.

AP Biology

Lab # 11

Experiment Procedure

Part B: Reproductive Behavior in Fruit Flies

Courtship behavior of *Drosophila melanogaster* consists of sequential actions that exchange visual, chemical and auditory signals between males and females. This distinct specific pattern of behavior appears to be genetically programmed. To describe these behaviors, word descriptions are used. In this experiment, you will place virgin female *Drosophila melanogaster* flies in the same vial with male flies and observe the behavior of each sex. Three phases can be distinguished:

I. Courtship Initiation. The male uses visual and olfactory signals.
- A. Orientation. The male aligns himself with the female.
- B. Tapping. The male taps the female's abdomen with his foreleg.
- C. Wing vibration. This produces a male species-specific courtship song.

II. Courtship Intensification. The male uses olfactory signals.
- D. Gently licking of the female genitalia.
- E. Attempt at copulation

III. Culmination of Courtship.
- F. Copulation. The female accepts the male and sperm is transferred.

  or
- G. Rejection. The female rejects the male by moving away and extruding her ovipositor.

Several different mating behaviors have been described. Ten of the most common and easily recognized of these behaviors are listed below. Six of the behaviors are seen in males, four in females. Read the descriptions carefully and refer to **Figure 4** on page 14 so that you will be familiar with the behaviors.

AP Biology

Lab # 11

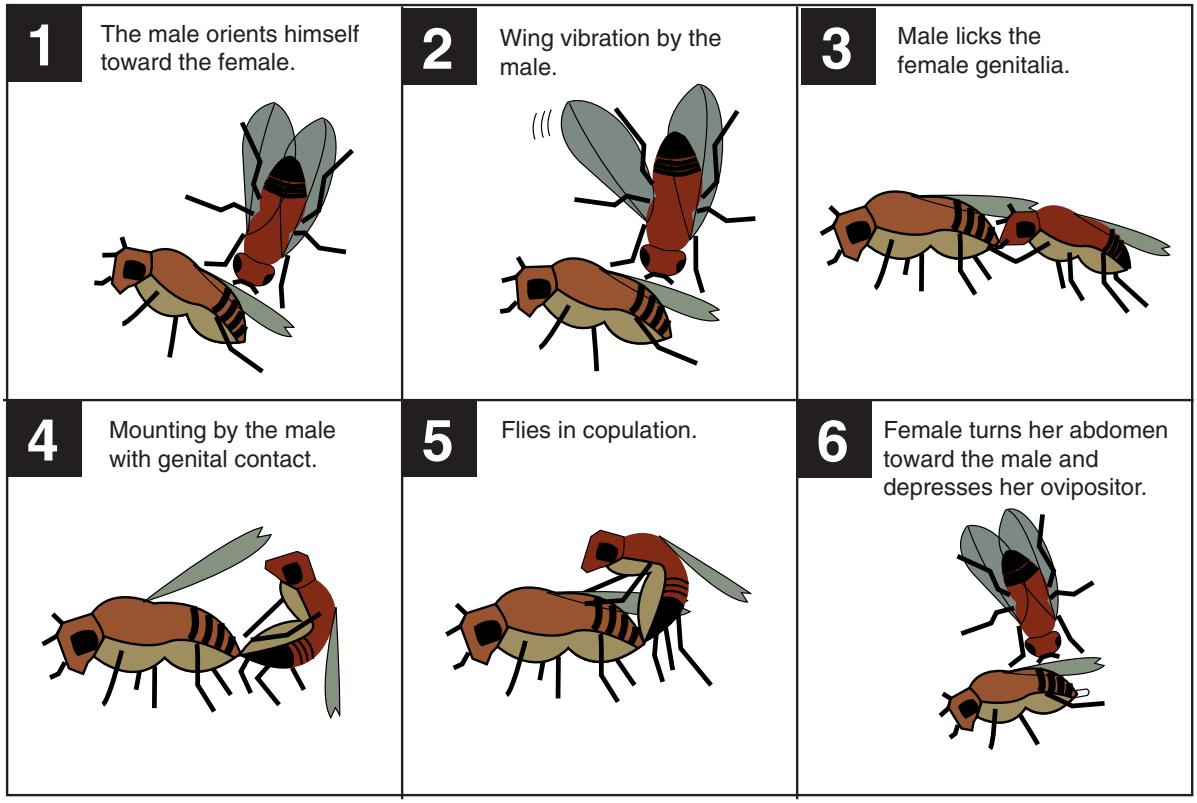
Experiment Procedure

Part B: Reproductive Behavior in Fruit Flies

Male Courtship

1. **Wing vibration.** Extending one or both wings from the resting position, the male moves them rapidly up and down.
2. **Waving.** The male extends his wing and holds it 90° from the body, then relaxes it without vibration.
3. **Tapping.** The female is tapped or struck by the male extending his forelegs.
4. **Licking.** The female's genitalia, located on the rear of her abdomen, are licked by the male.
5. **Circling.** When the female is non-receptive, the male postures and then circles the female.
6. **Stamping.** Without striking the female, the male stamps forefeet as in tapping,

Figure 4 - Reproductive Behavior



Part B: Reproductive Behavior in Fruit Flies

Female Rejection

1. **Extruding.** Extended from the female's genitalia is a temporary, tubelike structure.
2. **Decamping.** A female that is nonreceptive runs, jumps, or flies away from the courting male.
3. **Depressing.** By depressing her wings and curling the tip of her abdomen down, a nonreceptive female prevents access to her genitalia.
4. **Ignoring.** The male is ignored by a nonreceptive female.

PROCEDURE

1. Set up the stereoscopic or dissecting microscope.

2. The behaviors may happen very quickly. Have paper and pencil ready to record observations while your lab partner calls out the behavior.

3. Obtain one vial containing virgin females and one vial containing males, Gently tap the male flies into the female vial.

4. Make observations first with the naked eye. Use the stereoscopic microscope to make observations after the flies have encountered each other,

5. Identify the various behaviors. Record their sequence and duration. Quantify your observations by counting the number of times a behavior takes place and timing the duration of the behavior.

Part B: Study Questions

1. Design possible original experiments investigating courtship behavior in flies.

2. Discuss in detail the behavioral encounters you have observed. Make sketches and perform quantitative analysis as appropriate.

Extension of Experiment

Use a digital video camera to videotape the behavioral interactions between males and females. Quantify results.

AP Biology

Lab # 11

Experiment Procedure

Student-Designed Experiment

Design a simple experiment to investigate one of the following questions or any other that you devise:

a. Will males placed in a vial with only males demonstrate reproductive or aggressive behaviors towards each other?
b. How will males respond to already mated females?
c. Will a recently mated female exhibit an increase or decrease in receptivity to other males?
d. What types of aggressive behaviors does a nonreceptive female exhibit to males?

1. Write a hypothesis concerning the fruit fly behavior.
2. State your objective.
3. List the materials you will use.
4. Outline your procedure in detail.
5. Decide what data you will collect and design your data sheet.
6. Run your experiment. Repeat at least two more times.
7. Make any graphical representation of your data that will help to visualize or interpret the data.
8. Quantify results as appropriate.
9. Write a conclusion based on your experimental results.

General Information and Pre-Lab Preparations

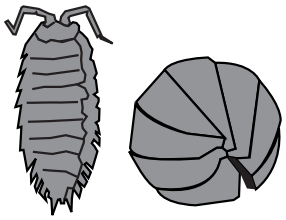


Figure 5 - Pillbugs

REQUEST ORGANISMS

Request organisms a minimum of two weeks prior to setting up for the experiment. You will need an additional two weeks to establish fly cultures.

CARE FOR PILLBUGS OR SOWBUGS

Depending upon availability, you will receive either pillbugs or sowbugs. Upon receipt, store pillbugs or sowbugs in an aerated container (plastic shoebox with a few holes in lid). Add leaf litter (dead leaves) and keep moist but not wet. Each group will need approximately 10 bugs (bugs can be reused after a group has finished making observations).

GENERAL FLY HANDLING TECHNIQUES

It will be necessary to set up and establish flies for the student lab well in advance (two weeks prior) of the experiment. Remember that female fruit flies will use sperm from a single insemination for their entire life span. It is essential you collect virgin female flies for the first Parental (P1) crosses. Females will be capable of mating 10 hours after emerging from the pupae. Therefore, female flies must be less than 10 hours old when collected.

1. Three to five days after arrival of flies, set up and label 6 new vials "Wild type".
    - In each of the vials, add 1 scoop of EDVOTEK Instant Drosophila Growth Media and 1 scoop of water. Use either a small coffee type scoop or a 20ml beaker (15ml of media and 15ml of water). There will be about 1 inch of media in the bottom of the vial. The media will hydrate and absorb the water.
    - Allow the media to set up for 5-10 minutes.
    - Transfer an equal number of adult flies from the vial received from EDVOTEK to the appropriately labeled fresh vials. This is done without the BugOut™ solution.
2. Tap vial with adult flies on lab bench to knock flies off the cap and towards the bottom of vial.
    - Immediately remove the foam lid from the vial with flies and quickly invert and overlap the vial which contains media.
    - Tap flies into the empty vial and cap immediately.
      1-2 Days before the lab, isolate virgin flies.
3. Virgin flies will emerge from the pupae within the vials set up earlier.
    - Collect within 10 hours after emergence.
    - Check vials morning and evening. Males can be collected from an overnight hatching, or at the end of the day from vials, and sexed flies should be placed in separate vials.

Pre-Lab Preparations

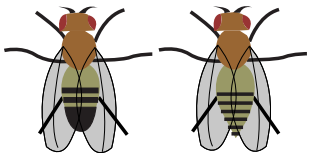


Figure 6 - Drosophila Male (left) & Female (right)

PROCEDURE FOR USING EDVOTEK BUGOUT™ SOLUTION

1. Do not anesthetize fruit flies in a vial containing media because they will drop into the media and die.
2. Transfer newly emerged flies to a vial without media and quickly cap with foam lids. Anesthetize the flies.
   - Place two drops of EDVOTEK **BugOut™** solution (component B) onto a small strip of filter paper with a transfer pipet.
   - Wedge the filter paper between the foam lid and the wall of the vial. The flies will stop moving in a few minutes.
3. Transfer immobilized flies onto the inverted lid of a petri dish.
   - Place the flies under the stereoscopic or dissecting microscope and select the male and female flies as required for your crosses.
   - The flies can be moved by gently pushing with a piece of filter paper. **Figure 6** illustrates differences between male and female flies. Male flies have a blunt posterior with a broad black band. They also have sex combs on their front legs. Females have a narrow pointed posterior and lack sex combs. The females also lack the broad black band on their posterior. This is clearly visible under the microscope.
4. To transfer the anesthetized flies to the vial containing media, remove the foam plug from the vial.
   - Pick up sexed flies by gently brushing them onto a piece of filter paper using another piece of filter paper. Do not allow anesthetized flies to fall onto media or they will die.
   - Hold the new vial slightly horizontally. Slide flies into the new vial so that they land on the side wall of the vial.
   - Replace the foam plug and place the vial horizontally onto the bench until the flies awaken.

THE MORGUE

Set up several fly morgues to collect unwanted flies. Add 50-60ml of mineral or vegetable oil and 50-60ml of alcohol to a 500ml flask. Cap with a cork. Unwanted flies can be tapped into the morgue where they will get stuck in the oil and die.

Expected Results

Table I. Pillbug Behavior Observations, page 9.

Student answers will vary. Refer to Answers to Study Questions, part A, # 2, 3, 4, on page 22.

Graph of Results, page 10.

2. Title: Habitat Preference of Pillbugs

3. Independent Variable: Time (min.)

4. Dependent Variable: Number of Organisms in Chamber

5. HYPOTHESIS: If an organism is placed an unfavorable environment, then the organism (pillbug) will exhibit an orientation behavior that allows the organism to move away from that environment into a more favorable one.

Please refer to the kit insert for the Answers to Study Questions

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200. Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List)

BugOut™

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK	Emergency Telephone Number: 202.370.1500
Address (Number, Street, City, State, and ZIPCode): 1121 5th Street NW	Telephone Number for information: 202.370.1500
Washington DC 20001	Date Prepared: 05/03/12
	Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Triethylamine CAS # 12-44-8				
Ethanol CAS#64-17-5				

Section III - Physical/Chemical Characteristics

N.D. = No data

Boiling Point	N.D.	Specific Gravity (H20 = 1)	N.D.
Vapor Pressure (mm Hg.)	N.D.	Melting Point	N.D.
Vapor Density (AIR =1)	N.D.	Evaporation Rate (Butyl Acetate =1)	N.D.

Solubility in Water

Appearance and Odor: Yellowish solution with strong odor

Section IV - Fire and Explosion Hazard Data

Flash Point (Method Used): Approx. 20° F	Flammable Limits	LEL	UEL

Extinguishing Media: Use dry chemical, CO2 or appropriate foam

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent clothing with skin and eyes

Wear NIOSH approved self contained breathing apparatus

Unusual Fire and Explosion Hazards: Vapors are heavier than air and may travel along the ground to a distant source of ignition and flash back.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: Heat, open flames
	Stable	X	

Incompatiblity (Materials to avoid): Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon dioxide, carbon monoxide

Hazardous Polymerization	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Vapor is a severe irritant to the eyes & mucous membranes. Ingestion may cause gastrointestinal discomfort.

Inhalation may cause irritation to respiratory tract.

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

No data

Signs and Symptoms of Exposure: Irritation to eyes, mucous membranes, respiratory tract, gastrointestinal discomfort.

Medical Conditions GenerallyAggravated by Exposure: Prolonged exposure may cause liver or kidney damage

Emergency and FirstAid Procedures: If inhaled: move to fresh air. If no breating, give respiration. If contact with eyes: flush with water for 15 minutes. Seek immediate medical attention if irration persists. If swallowed: give water and induce vomiting. Call local poison control center.

Section VII - Precautions for Safe Handling and Use

Steps to Be Taken in case Material Is Released or Spilled: Ventilate area of spill. Eliminate all sources of ignition. Remove all non-essential personnel from area. Wear proper protective equipment and clothing. Absorb material with suitable absorbent and containerize for disposal.

Waste Disposal Method: Dispose in accordance with all applicable Federal, State and local regulations.

as this material is highly flammable.

Precautions to beTaken in Handling and Storing: Store tightly closed in a cool, dry, well ventilated area suitable for the storage of flammable liquids.

Other Precautions: Keep all oxidizing materials away.

Section VIII - Control Measures

Respiratory Protection (SpecifyType): NIOSH/MSHA approved respirator

Ventilation	Local Exhaust: Yes		Special
	Mechanical (General): yes		Other
Protective Gloves: Rubber, neoprene, PVC		Eye Protection: Chem. Safety goggles	

Other Protective Clothing or Equipment: Lab coat, eye wash and safety shower

Work/Hygienic Practices