Chapter 7

Animal Behavior Experiments Using Arthropods

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Introduction

The purpose of these laboratory exercises is to acquaint the student with the complex area of ethology, which is the study of animal behavior. The student will observe relatively simple animal behavior in various arthropods. These animals are convenient and easy to handle. Observations include mechanoreception in cockroaches, chemoreception in flies, taxis and kinesis in terrestrial isopods, aggression in crickets, and courtship behavior in crickets and fruitflies. These exercises are designed for use in an introductory biology laboratory. This laboratory can be completed in a single three-hour session with the students working individually or in pairs. These exercises can be run concurrently, and are spaced so that the student will remain busy, but still be able to make the required observations.

Instructors' Materials

Descriptions of Exercises

Exercise I. Simple reflex behavior in the cockroach

This exercise attempts to demonstrate reflex behavior in the cockroach. The procedure is relatively easy and quick. The biggest problem involves familiarizing the students with handling roaches. A roach can easily be picked up by pinning it to the side of the storage container and grasping it firmly between the thumb and index finger. Demonstrate this to the students before starting the exercise.

Sensory hairs on the cerci respond to mechanical and acoustical stimuli. The experiment where brief puffs of air are applied to the cerci is a classical experiment demonstrating an evasive or escape behavior mediated by a giant fiber system. The afferent nerve fibers from the hairs of the cerci synapse in the last (6th) abdominal ganglion and stimulate the giant fibers, which ascend the ventral nerve cord and synapse with motor neurons in the thoracic ganglia controlling the legs (Camhi 1980). Have the students diagram and label such a reflex circuit, including the receptor, the effector and the nerve cells. It may be profitable to point out that insects are capable of doing many things without a head because thoracic and abdominal ganglia have considerable autonomy. The brain is often a site of inhibition for many reflexes controlled in the ventral ganglia. A good example of this is the release of copulatory movements in praying mantis males deprived of their heads by a voracious, courted female praying mantis.

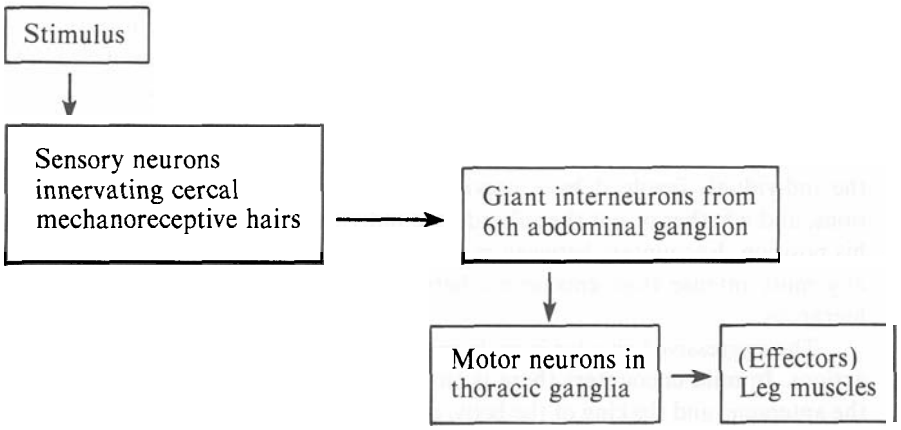


Figure 7.1. Outline of the escape reflex pathway of the cockroach.

Exercise II. Reaction of terrestrial isopods to light and humidity

Isopods (pillbugs or woodlice) demonstrate negative photo-taxis. They have light receptors (ocelli) that are sensitive to general illumination (non-image forming), and they make directed movements away from areas of greater illumination toward dark areas. Isopods are classical examples of animals which orient to humidity gradients via kinesis (more specifically, orthokinesis); i.e. there is a change in the general level of locomotory activity with a change in the stimulus intensity (Gunn 1937). Stress that orientation via kinesis is due to a *non-directed* locomotory activity. Isopods increase their locomotory activity under dry conditions and wander quite randomly, decreasing their activity under preferred humidity. Because of this decrease in activity in areas of high humidity, isopods tend to accumulate or aggregate in damp places. Taxis, on the other hand, is a direct orientation of an organism in response to a stimulus. Kinesis indicates a variation in intensity of locomotor activity that is dependent on the intensity of the stimulation, not its direction (Fraenkel and Gunn 1961). These exercises demonstrate taxis and kinesis separately, and in combinations.

Exercise III. Aggressive and courtship behavior in crickets

In this exercise students will observe the aggressive and courtship behavior of the cricket. Normal male crickets chirp from hiding places, emitting a sound that attracts females. Courtship and mating follow. The male will also defend his territory against the intrusion of other males. Once the female has mated she deposits her eggs in a damp place, usually moist sand. The eggs hatch into nymphs in about two weeks. They develop through a series of molts, acquiring adult characteristics at their final molt. Adults start reproducing in about one week.

Groups of adult male field crickets in small, confined areas form dominance hierarchies, as do many other animals such as chickens and wolves. A male's position in the hierarchy is determined by aggressive contests with other individuals. Most contacts between males involve a display of aggressive behavior. The intensity of aggression upon contact depends upon whether or not the individuals involved have previously established their hierarchial positions, and whether or not the subordinate individual wants to attempt to raise his position. Encounters between males that rank next to each other are usually more intense than encounters between males that are far apart in the hierarchy.

The aggressive behavior in male crickets consists of one or more of several actions. In mild encounters there is either rearing of the forebody, lashing of the antennae, and shaking of the body, or else rearing of the hind body, kicking with the hind legs, and shaking the body. In intense encounters antennal lashing and rearing of the forebody are followed by spreading the mandibles, stridulating (chirping) distinctively, rushing forward, sparring with the forelegs, butting with the head, and grappling, wrestling, or biting with the mandibles. A male is often flipped back or thrown sideways, but mutilation as a result of fighting is rare, and only in the most intense encounters is the winner determined by what seems to be superior strength or fighting ability.

The aggressive stridulation of male field crickets is distinct from the calling and courting songs. Stridulation by one male during combat usually causes the other male to stridulate in most species; both males stridulate during intense aggression. Dominant males stridulate during aggression more frequently than subordinate males, and the dominating male in an intensively aggressive encounter nearly always stridulates after the retreat of the subordinate. The subordinate male rarely chirps after an encounter. If he does it signals an impending rise in his status.

Dominance hierarchies have important consequences in the survival of a species, in that they reduce the total amount of aggression among individuals of a population and ensure that at least some individuals will receive sufficient food and shelter under adverse conditions.

Most encounters between male and female crickets result from the movement of a sexually responsive female toward a stationary, stridulating male as a result of hearing his calling sound. The auditory organs of the female are located on the tibia of the front legs, and are very accurate in discerning the direction from which the calling song is coming.

Upon contact with the female the calling male usually begins courtship immediately. The stridulation of the male in courtship changes from the loud calling song to the softer courtship song. The courtship song is executed with the male's abdomen facing the female, and the male usually sways his body from side to side in time with the motion of the forewings. This activity is continued by the male until the female starts to mount, whereupon he flattens

his forewings against his body, lowers his abdomen to the ground, and moves slightly backward, facilitating the mounting of the female. The female then climbs on top of the male, and the male attaches a spermatophore (packet of sperm) over the female's gonopore. She is inseminated as an osmotic process in the spermatophore causes the sperm to be ejected. The female remains on top of the male for a time sufficient for most of the sperm to be released from the spermatophore. See Alexander (1961), Bentley and Hay (1974), and Cade (1981) for more information on cricket behavior.

Exercise IV. Chemoreception in the adult fly

This experiment attempts to duplicate some classical work on the determination of threshold for the detection of sugars in solution. Stimulation with sugar solution of chemoreceptors located on the tarsi of a fly induces the extension of the fly's proboscis. Since a behavioral assay is being utilized to determine threshold, variations may occur because of motivational and environmental factors and the fly's state of health. This variability is reflected in the way threshold data are presented. Threshold is the concentration of sucrose that elicits proboscis extension in 50% of the flies tested.

Tarsal hairs have been found to be multimodal; that is, they respond to both mechanical deflections and certain chemicals. They are innervated by up to 5 sensory neurons which respond to salts, sugars, water, mechanical stimuli, and possibly anions. The proboscis extension reflex and the resultant feeding is part of a homeostatic mechanism for the regulation of energy flow in the fly. Figure 7.2 is a summary diagram of factors which regulate feeding responses worked out for the blowfly *Phormia*. See Dethier (1963) and Gelperin (1966) for further information.

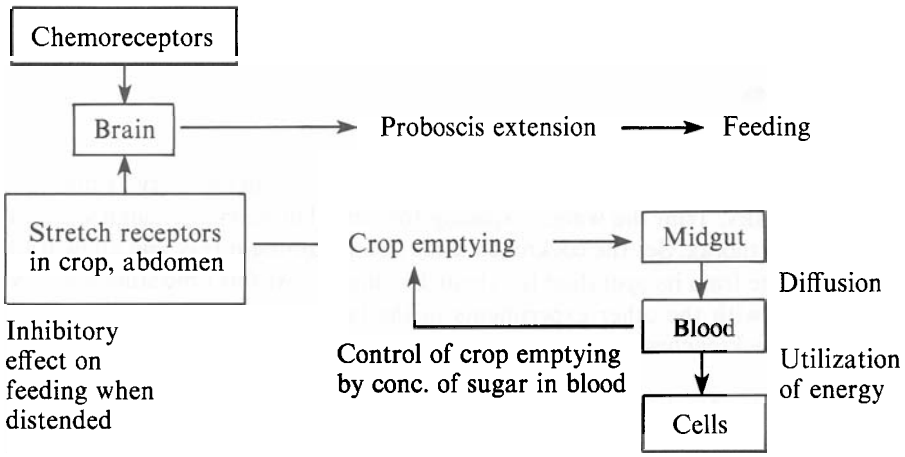


Figure 7.2. Factors that regulate feeding response for the blowfly *Phormia*. (Adapted from Gelperin, 1966).

Two methods of insect preparation for testing chemoreception in adult flies will be explained. Method I allows all the fly's tarsi to be stimulated by the sucrose, which should give a more accurate response. This method requires handling the fly. We have found *Phormia* easy to handle since they are large. However, this method is more time-consuming to set up, and the students often allow flies to escape in the classroom. Method II is easier for the students to work with, requires no set up, but may not be as accurate since only the fly's forelegs are extended.

Exercise V. Courtship behavior in the fruit fly

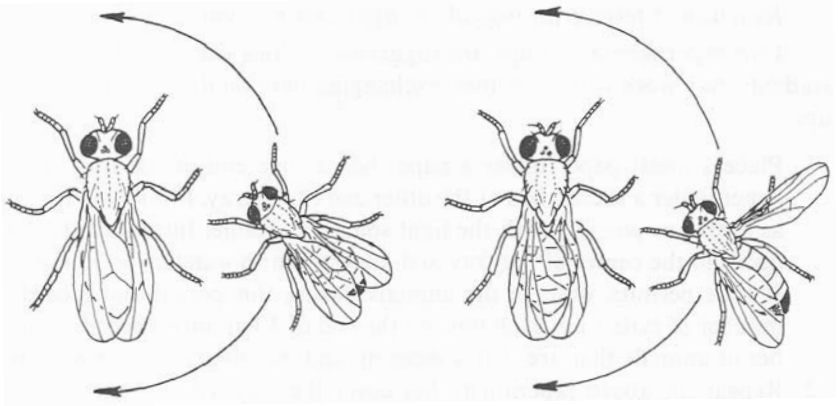
This exercise will deal with the courting sequences of *Drosophila.* Generally, courtship entails highly-specific responses on the part of both animals. If one step is omitted or slightly modified by one of the pair, the entire sequence may be abandoned. *Orientation* is the first component of the actual courtship ritual. The male fly stands close to the female, often behind her. He continually faces her, as he circles around her. In *vibration,* the second courtship step, the male extends horizontally the wing nearest the female's head and vibrates it in a vertical plane. He constantly remains oriented to the female. If the male circles her, he changes the wing elevation as he passes her head or tail to maintain the proper posturing. The third component, *licking,* is performed simultaneously with the other two. The male licks the female genitalia with his proboscis. The male then generally attempts copulation; he mounts the female and reaches for her genital region with the tip of his abdomen. However, she may try to ward him off by twisting, kicking, fluttering her wings, etc. If she is submissive, she spreads her wings and extends her genitalia. All of the above behaviors may occur within several minutes; observe this closely. Figure 7.3 shows the various positions in the courtship behavior movements (Bastock and Manning 1955).

Procedures

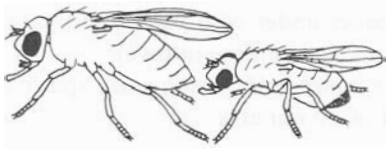
Sample reflex behavior in the cockroach

Obtain a large cockroach and decapitate as close to the body as possible with scissors. Trim the wings, exposing the cerci but leaving enough wing to cover the thorax. Set the cockroach aside in an aluminum tray and allow it to recuperate from its operation for about 15 minutes. At this time students may continue with the other experiments in the laboratory. (Methods for maintaining cockroaches can be found in Chapter 6 of the first volume of this series.)

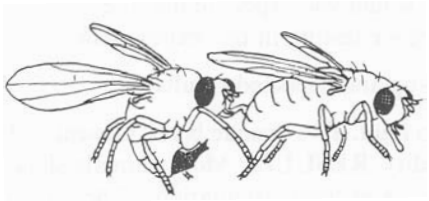
Using a fine brush gently touch various parts of the animal, such as the tarsus of each leg, the sides of the abdomen, the mid-dorsal surface, and note the responses. Usually gentle stimulation rather than too vigorous a stimulation will allow you to observe more specific responses. If possible, allow the



Orientation Vibration



Licking



Attempted Copulation Copulating Pair

Figure 7.3. Courtship behavior movements in *Drosophila*.

response to stop before the next stimulus is applied. Does the animal adapt (cease to respond) to a repeated stimulus? _____

Note the cerci, a pair of prominent dorsal appendages on the tip of the abdomen. Stimulate the cerci with brief puffs of air from a pipette. How does the decapitated cockroach respond to brief puffs of air on the cerci from a pipette? _____

_____

Reaction of terrestrial isopods to light and humidity

Five experimental set-ups are suggested for this exercise. To save time, students may work with a partner, exchanging data on different types of set-ups.

1. Place a moist paper under a paper box at one end of the tray and dry paper under a second box at the other end of the tray. Illuminate the trays as evenly as possible with the light source available. Introduce the 10 isopods to the center of the tray and allow them to wander for 30 minutes. If time permits, observe the animals during this period and record the number of exits from each box. At the end of 30 minutes record the number of animals that are still wandering and the number under each box.
2. Repeat the above experiment, but cover the tray with aluminum foil so that the animals are entirely in the dark. Record your results at the end of 30 minutes.
3. Place a moist paper under the plastic box and a dry paper under the paper box. Record your results at the end of 30 minutes.
4. Place the dry paper under both plastic and paper boxes, and record the results at the appropriate time intervals.
5. Place wet paper under both plastic and paper boxes, and observe the results at the end of 30 minutes.

When students have finished the last exercise they may return the isopods to their container and continue with the other exercises.

Ask your students the following questions: Would you say that isopods show taxis or kinesis with respect to light and humidity? Which is the stronger stimulus, light or humidity? Where would you expect to find these animals in nature? What types of receptors are we testing in this experiment?

Rationale for experimental set-up and expected results:

1. Animals are initially exposed to light. Two opaque boxes present a choice of high humidity vs. low humidity. RESULTS: More animals should be moving at the beginning (because of light stimulation). The amount of movement should decrease under the box with moist paper; hence, isopods will aggregate there. More animals should exit from the dry box than the moist box.
2. Experimental conditions are the same as #1 except everything is done in darkness. RESULTS: The total amount of movement will not be as great as in condition #1, but with time the isopods should aggregate under the moist box.
3. Experimental conditions test whether light or humidity is a stronger stimulus. RESULTS: There will probably be more isopods under the dry,

opaque box than the wet, transparent box because the response to light is a taxis (directed), whereas the response to humidity is a non-directed change in locomotion or kinesis.

4. Experimental conditions test for a response to light alone. RESULTS: The isopods should aggregate under the opaque paper box.
5. The response to light with humidity is examined. RESULTS: There should be a greater aggregation of isopods under the opaque box, but the differences will not be as marked as in condition #4 since the high humidity will tend to suppress locomotion.

Aggressive and courtship behavior in crickets

Crickets should be sexed upon arrival, 5–7 days before use. Separate the males into one aquarium and the females into another. They are easiest to handle when subdued by ether or carbon dioxide. Females can be identified by the long needle-like ovipositor protruding from their abdomens.

We have found the following method of keeping crickets to be successful: Once crickets are housed in their aquarium, place some egg cartons or paper cups inside. This gives the crickets enough space to move about freely. Check for mortality every other day and remove dead crickets. The temperature must be above 20°C before removing dead crickets to avoid confusing them with dormant ones. For drinking water fill a petri dish with aquarium gravel and put water into the dish. Water should not be exposed. Crickets will walk on the gravel to drink. Cages should be kept as dry as possible. Dampness will cause a gas from the droppings that will kill the crickets. Feed the crickets wedges of potatoes using a small plate that can be removed. It is best to change feed and water about every fourth day. Crickets have a life span of about two weeks. Crickets maintained this way will survive that period without any problems.

To remove crickets from the aquarium simply pick up an egg carton or paper cup and shake them into your hand. Males must be isolated two to three days before class. Place a single male into a vial with a piece of potato. Cap and set aside until class. You will need 2 males for each student.

Students can work individually or in groups of 2–4. Give them the following instructions: "The crickets you will use were separated by sex upon arrival. The females can be identified easily from the males by their needle-like ovipositor extending from the abdomen. Be sure your crickets are the correct sex when you obtain them. Crickets prefer low-light situations; therefore the light in the room should be dimmed or you should shade the plastic cage that you are using. Crickets are also extremely sensitive to vibrations. One of their reactions to disturbance is to stop moving for periods of a few seconds to a minute. Do not jar the table the plastic container is sitting on. Do not

move quickly or hover over it, since crickets are also sensitive to changes in light intensity. Assemble a data sheet for each part of this experiment. Note posture, use of antennae, general activity, and directional locomotion."

1. Behavior in a new environment: Release a cricket into the clear, plastic cage supplied for this experiment. Observe the behavior for 5–10 minutes. Take notes as previously specified.
2. Social encounters between males: Place another male in the cage. At first the crickets will appear distracted because of the new environment, but within a few minutes they will test each other to determine their hierarchical rank. Note what happens at the time they start to interact only. How do they appear to sense the presence of each other? What changes in behavior follow mutual recognition? Observe the sound of the aggressive stridulation, the extensive lashing of the antennae, and the positioning of the body during contact.
3. Male-Female Social Encounters: About 10 minutes after the 2 males have calmed down from their aggressive behavior place 4 to 6 females in the cage with the males. In this number there should be at least one sexually responsive female. In this situation the male will often show aggressive behavior to the female upon their initial encounter, but the female will not retreat. The male should then exhibit courtship behavior. Listen for the low courtship song of the male, and observe the posturing of his body. A responsive female should mount a male within 10 to 15 minutes. Copulation usually takes place immediately upon mounting by the female.
4. Mating: Observe what happens during mating. Do you see a white object pass from the male to the female and remain attached to the female? This is a spermatophore. Take the spermatophore from the female immediately and crush it on a slide. Add a drop of saline solution, cover with a coverslip and examine under a microscope. If you do this carefully you should see living sperm. In crickets insemination occurs after mating has terminated. The spermatophore empties in about 15 minutes.

Chemoreception in adult fly

Flies can be maintained in a screen fly cage preferably with a cloth sleeve on one side to permit collection. To feed flies pour water or sugar water into a jar, cover it with several layers of cheesecloth, secure this with a rubberband, invert and place on the top of the fly cage. Flies can also be fed by placing sugar cubes in a container in the bottom of the cage. Do not store flies at a temperature above 29°C. Flies should be starved from sugar (not water) at least 24 hours prior to use.

Method I: Flies will need to be anesthetized to mount them on the applicator stick. They do not respond well immediately after anesthetizing, so

we have students in one class set up enough flies for the students in later classes. This gives the students a chance to become acquainted with the procedure for mounting flies as well as actually testing their response.

Set up a work area for the students to use in mounting flies. Place the Buchner funnel in the ring stand and attach the tubing to the tank outlet. Adjust the flow of CO_2 to maintain a slow, gentle rate. Collect 10 flies by inserting a test tube through the cloth part of the cage and running the tube across the top of the cage. When you have enough flies to work with, cap the tube with your thumb, remove the tube and cap it. Subdue the flies with an initial application of CO_2 by inserting the end of the rubber tubing into the tube of flies. Once the flies have slowed down, pour them into the Buchner funnel, cover it with plexiglass, attach the tubing to the funnel and anesthetize the flies. Flies are ready to handle once they have stopped moving. Remove the plexiglass but let the CO_2 continue to flow.

The next step is to attach a fly to an applicator stick. Lay the applicator stick on the table and gently heat a dissecting needle in a flame. Place a fly dorsal-side-down on the paraffin-coated applicator stick and sear the wings to the paraffin with the needle by laying the hot needle across the wings as shown (Figure 7.4). Insert the stick into the holder and proceed with the next fly. If the flies in the funnel start to awaken while you are mounting them, simply cover the funnel until they are reanesthetized. Turn off the CO_2 flow when the flies have been mounted.

If you have chosen this method demonstrate it to the students at the beginning of the lab. They can mount flies throughout the lab while the other exercises are going on. Flies mounted in this manner can be reused every other day as long as they are fed and restarved.

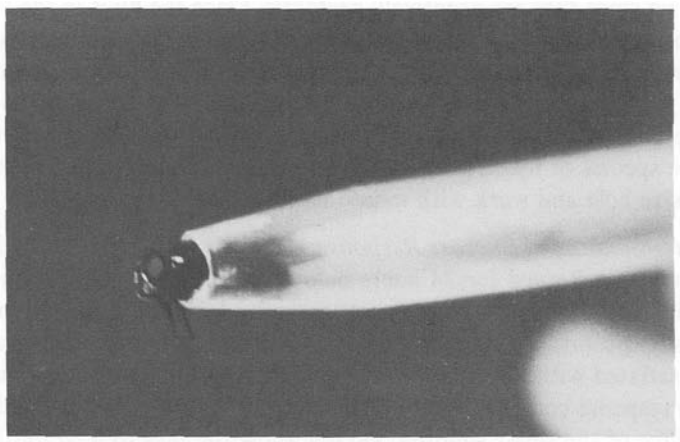


Figure 7.4. Properly mounted *Phormia* using pipette method.

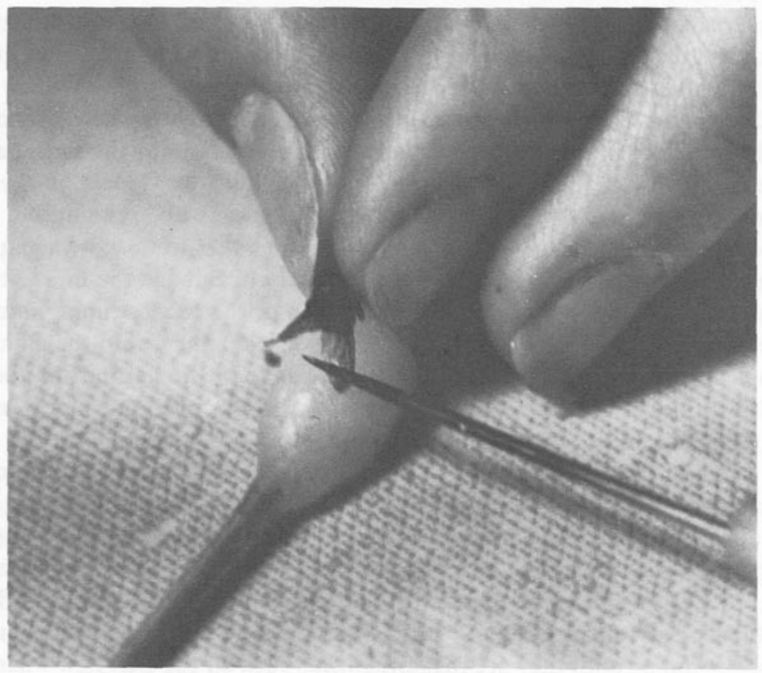


Figure 7.5. Attachment of *Phormia* to applicator stick.

Method II: Students will work with 10 flies. First cut off the ends (2–4 mm) of 10 disposable pipette tips. Next collect a fly in a test tube. Remove the plug from the tube, insert the wide end of a pipette tip into the tube and allow the fly to crawl up the pipette tip towards the end. The pipette tip should be held up since flies are negatively geotactic. Once the fly is in the tip, insert the wooden dowel and gently coax the fly's head and forelimbs through the trimmed end. If necessary, further manipulations of the fly can be made with a small brush. If the hole is too small the fly can be pushed back down and the hole enlarged with a scissors. (Figure 7.5) The size of the hole will vary with the species of fly. With practice students will be able to determine the proper size hole and work with more than one fly in a test tube at a time.

Determination of Sucrose Response: Each student will work with 10 flies that have been starved for 24 hours before the laboratory. Allow the flies to drink their fill of water and then test them by bringing the tarsi in contact with a sugar solution in a watch glass. It is important that the flies are completely satiated with water before starting the sugar response experiments. A positive response consists of an extension of the proboscis and its contact with the surface of the sugar solution. Do not allow the flies to feed until the end of the test. The dilutions of sucrose to be tested are 0.0025, 0.005, 0.01, 0.05, 0.1, 0.5, and 1 M.

Table 7.1. Individual Student Data

	Fly										
Sucrose	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Student*
0.0025 M	−	−	−	−	−	−	−	−	−	−	−
0.005 M	−	−	−	−	+	−	−	−	−	−	−
0.01 M	−	−	−	−		−	−	−	+	−	−
0.5 M	+	−	+	+		−	+	+		+	−
0.1 M		+				+					+
0.5 M											
1 M											

After several negative responses with distilled water, test a fly with each sucrose solution, working from the lowest to the highest concentration. Rinse the tarsi by dipping repeatedly in distilled water between each test solution. Be careful not to drown the fly. Record the threshold concentration of sucrose to which it responds. Test each fly in the same manner. Record your data and determine the point of threshold for the flies, as in Table 7.1. At the end of the experiment feed the flies for 30 seconds on 1 M sucrose and return them to a chamber.

Determine the threshold of taste for sugar for yourself. Who is more sensitive, you or the fly? Compare your data against those of the entire class, as recorded in Table 7.2. It is best to collect all the data from the class and represent threshold as the concentration eliciting proboscis extension in 50% of the flies tested. A graph of the threshold response can be drawn by plotting the negative log of the sucrose concentration versus the total percent of flies detecting sucrose at each concentration. (See Table 7.3 and Figure 7.6.) To determine the sucrose concentration eliciting proboscis extension in 50% of the flies find the point on the graph indicating 50% response and note the corresponding sucrose concentration. This graph denotes 50% proboscis extension at 0.022 M sucrose. A mathematical computation of 50% endpoints has also been devised by Reed and Muench (1938).

Table 7.2. Class data.

	Flies	*Students*
Sucrose	# of positive responses/# of negative responses	
.0025 M		
.005 M		
.01 M		
.05 M		
.1 M		
.5 M		
1 M		

Table 7.3. Individual data.

Sucrose Concentration	*Negative Log_{10}*	*% Flies Responding*
0.0025	2.6	0
0.005	2.3	10
0.01	2.0	20
0.05	1.3	80
0.1	1	100
0.5	.3	
1.0	0	

Courtship behavior in the fruit fly

Flies should be sexed 2–3 days before use. They do not respond well within 24 hours of etherization. Vials can be set up most efficiently during sexing. Set up 2 vials for each student. One should have 10 male *Drosophila* in it and the other 10 females.

Flies can easily be transferred from the vials to the mating chamber. (Fig. 7.7) To transfer flies obtain 2 vials of flies and a mating chamber. Remove the plunger from the mating chamber and set the chamber on your desk with the open side up. Take 1 vial of flies, knock them toward the medium by gently tapping the bottom of the vial on a notebook. Quickly pull the plug from the vial and invert it into the chamber. Tap the chamber and vial on the notebook to remove the flies from the vial. Quickly cover the chamber with your hand (use your hand as a lid). Take the other vial, tap it and the chamber at the same time, invert the vial in the chamber, tap and reinsert the plunger. Invert the chamber and adjust the chamber plunger to about 2.5 cm below the screening.

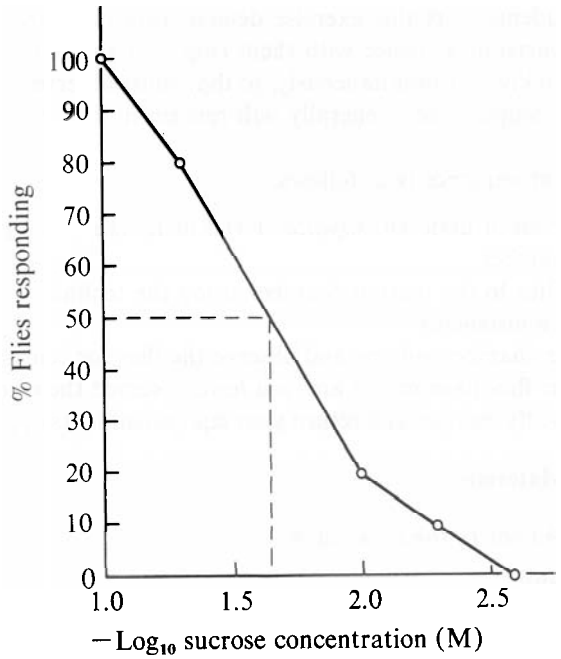


Figure 7.6. Graph of sucrose threshold response in *Phormia*.

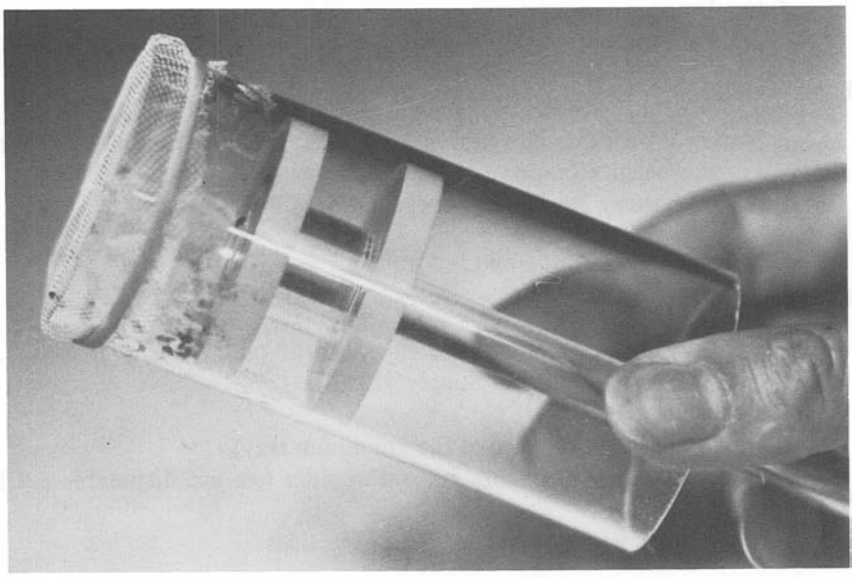


Figure 7.7. *Drosophila* mating chamber.

Before students start this exercise demonstrate the transfer technique. Discuss the courtship sequence with them (Fig 7.3) and reiterate that these steps occur quickly and simultaneously, so they must observe carefully. Once two flies have coupled they generally will remain in that position for 5–15 minutes.

The student sequence is as follows:

1. Obtain 1 vial of male *Drosophila,* 1 vial of female *Drosophila* and one mating chamber.
2. Transfer flies to the mating chamber using the technique demonstrated by your lab instructor.
3. Adjust the chamber volume and observe the flies for courtship behavior.
4. When your flies have mated and you have observed their behavior, place them in the fly morgue and return your equipment to its appropriate spot.

Supplies and Materials

Reflex behavior in the cockroach

For each student:

- 1 cockroach *(Periplaneta americana)* (Carolina Biological Supply #L-730)
- 1 pair of scissors
- 1 aluminum tray (6-½″ × 10-½″ × 1″)
- 1 brush
- 1 pipette

For the class:

- 1 battery jar with vaseline applied to the top 4 cm of the inside rim for cockroach storage
- 1 similar battery jar for used cockroaches

Reactions of terrestrial isopods

For each student:

- 10 terrestrial isopods, woodlouse *Porcellio* or pillbug *Armadillidium.* (Carolina Biological Supply #L-624)
- 1 aluminum tray
- 2 papers (cut to ½ the size of the aluminum tray)
- 2 plastic boxes with notches cut out of sides (we use disposable petri dishes)
- 2 paper boxes with notches cut out of the sides

For the class:

1 container for terrestrial isopods. (We order isopods shortly before use and maintain them in their shipping container.) Isopods can be reused.
1 roll of aluminum foil

Aggression and courtship in crickets

For each student:

1 plastic container with lid (10″ × 7 ½″ × 4″). Tri State Plastic Molding Company, Henderson, Kentucky 42420.
4 female crickets *(Acheta domesticus)*. Flucker's Cricket Farm, Baton Rouge, Louisiana.
2 vials, each containing a male cricket
1 compound microscope

For the class:

2 covered aquaria for male and female crickets
1 container for used crickets
40 vials with cotton wool plug
1 dropping bottle of saline
1 box of microscope slides
1 box of coverslips

Chemoreception in flies

For each student:

Method I

1 applicator stick holder
10 applicator sticks dipped in paraffin
10 flies (preferably *Phormia*)
1 dissecting needle
8 watch glasses

Method II

10 disposable pipette tips (Eppendorf blue 200–1000 VI)
10 pointed wooden dowels to fit into above pipette tips
1 test tube with cotton wool plug
8 watch glasses
10 flies (preferably *Phormia regina*)

For the class:

Method I

1 cage of flies
1 empty cage for used flies
1 4″ diameter Buchner funnel
1 piece of vacuum tubing
1 4″ × 4″ piece of plexiglass
1 Bunsen burner
1 ring stand
1 CO_2 tank with gauge
1 test tube

Method II

1 cage of flies
1 empty cage for used flies

Courtship in fruit flies

For each student:

1 vial of 10 female *Drosophila* (Carolina Biological Supply #L-17-2100)
1 vial of 10 male *Drosophila*
1 mating chamber. (Tube 12cm long with 4.5cm inside diameter containing a mating platform 4.3cm in diameter. See Figure 7.7)

For each class:

20 vials with 10 male *Drosophila* in each
20 vials with 10 female *Drosophila* in each
1 battery jar with alcohol in it
1 funnel

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