Animal Behavior

Perhaps one of the most difficult fields of biology to study is ethology, the study of animal behavior. Observation of a behavior is simple; interpreting what has been observed requires more effort; therefore, more than any other division of research in biology, ethology calls for patience, objectivity, and imagination.

It is important for an ethologist to investigate and interpret the various animal behaviors observed in terms of the animals’ reaction to their own environment. It is too easy to view animal behavior in terms of human habits; if a behavior seems to emulate a behavior that humans perform, it is interpreted as a human interaction. This anthropomorphic view (anthro = human, morph = change)—bestowing human traits and motivation on animals’ behavior—can lead to inaccurate interpretations of the animals’ behavior.

For this reason, ethologists assess various behaviors by placing documented behavior in simple categories. Once the behaviors in these categories are tallied, ethologists can determine the entire lifestyle of the organism, gaining a complete picture of the animal’s existence. Ethology, then, can be considered a unique branch of biology, investigating not only what happens in an animal’s existence, but also why it happens.

In every ecosystem, organisms are influenced by limiting factors—biotic or abiotic factors which regulate the maximum size of a given population—and a relatively narrow range of environmental conditions which are favorable to them and their offspring. Since most organisms cannot change the nature of their environment, they must position themselves in an environment with favorable conditions. This behavior is called habitat selection.

Many organisms exhibit a tactic response to these environmental factors. Tactic responses may be positive, toward a favorable environment, or negative, away from an unfavorable environment. These tactic responses enable an organism to locate prey, avoid predation, seek shelter, or avoid a toxic environment—all vital to habitat selection.

An animal’s orientation behavior, which allows an animal to be placed in a favorable environment, occurs in two ways: taxis and kinesis. Taxis is a deliberate movement toward or away from a stimulus. Kinesis, on the other hand, is a random movement that is not oriented toward or away from a stimulus. A movement classified as taxis defines the physiological needs of the organism, its evolutionary history, its nervous system, etc. For example, an insect found around rotting fruit has most likely responded to a very different environmental stimulus than one found around a decaying corpse.

Agonistic behavior is characterized as a response, either aggressive or submissive, to another organism. These behaviors can range from outright confrontation to a simple display of submission or superiority.

In many ways, mating behaviors are the most complex to observe. Animals have evolved a complex series of behaviors to find a mate, court, and copulate. It is crucial for a female to select a male that is well adapted; this ensures that the genes will be passed on to a suitable offspring, who will in turn pass on his or her genes.
Courtship in Drosophila

Drosophila melanogaster, the common fruit fly, was first used in genetic experiments in 1907 by T.H. Morgan of Columbia University, and has been a staple of genetic research ever since. Drosophila specimens are well suited to investigations into Mendelian patterns of inheritance; they are small, produce large numbers of offspring, have many easily discernible mutations, have only four pairs of chromosomes, and complete their entire life cycle in approximately 12 days. Additionally, Drosophila are relatively easy to maintain, as they are hardy and have simple food requirements.

Since the fruit fly was selected for study nearly a hundred years ago, a great deal has been learned about its genome. In fact, the first chromosome map of any kind was constructed to detail the fruit fly. Chromosomes 1 (the X chromosome), 2, and 3 are very large, and the Y chromosome—number 4—is extremely small. Thousands of genes reside on these four chromosomes, many of which are universal in nature, existing in most eukaryotic forms, including humans. The similarities between the Drosophila genome and those of other species is helpful in determining patterns of evolution and species divergence.

Drosophila embryos develop in the egg membrane. The egg hatches and produces a larva which feeds by burrowing through the medium. The larval period consists of three stages, or instars, the end of each stage marked by a molt. The first instar is the newly hatched larva; the third instar is the final larval stage, where the larva may attain a length of 4.5mm. Near the end of the larval period, the third instar larva will crawl up the sides of the culture vial, attach themselves to a dry surface (the jar, the filter paper, etc.) and form pupae. After a period of time the adults emerge.

It takes one or two days for Drosophila eggs to hatch into larvae, four to five days for the larvae to enter pupae, and four days for the pupa stage. The duration of these stages, however, vary with the temperature; at 20°C the average length of the egg–larval period is eight days, while at 25°C it is reduced to five days. Thus at 25°C the life cycle may be completed in about 10 days; at 20°C, 15 days are required.

Drosophila Life Cycle

Different body features characterize the male and female flies. The females are slightly larger and have a light-colored, pointed abdomen. The abdomen of the males will be dark and blunt. The male flies also have dark bristles on the upper portion of the forelegs, which are known as sex combs.
Mating in Drosophila melanogaster follows a strict behavioral pattern, which generally occurs in six phases:

1. **Orientation**: The male consistently positions himself near the female.

2. **Wing Vibration**: The male produces the equivalent of a “song” by vibrating his wings.

3. **Licking of the Female Genitalia**

4. **Attempted Copulation**

5. **Copulation**

6. **Rejection**: The female signals that she is no longer receptive by extruding her ovipositor (egg-laying structure).
OBJECTIVES

In this experiment, you will

- Observe and note general behavior characteristics of pillbugs.
- Hypothesize as to whether pillbugs have adapted to perceive and react to certain environmental changes.
- Design an experiment to determine how pillbugs respond to environmental changes.
- Examine similarities and differences between male and female Drosophila.
- Observe courtship and mating rituals between male and female Drosophila as an example of a strict behavioral pattern.
- Hypothesize as to whether fruit flies respond to certain environmental changes.

MATERIALS

Part I Materials  Part II Materials
10 pillbugs  vial of collected Drosophila males
1% hydrochloric acid solution  vial of collected Drosophila virgin females
2% potassium hydroxide solution  thermo-anesthetizer
animal behavior tray  petri dish
camel’s hair brush  camel’s hair brush
masking tape  magnifier
magnifier

PROCEDURE

Part I  Behavior of Pillbugs

A. General Observations
1. To become familiar with the organisms, place several pillbugs in the behavior tray and carefully observe them for at least 10 minutes. In Table 1 in the Analysis section, document any behaviors you see. Remember to document even the seemingly unimportant behaviors. Try to document the behaviors observed in chronological order. Note: Do not disturb the pillbugs; shaking or tipping the tray will cause unnatural behavior in the pillbugs.

2. In Table 2 in the Analysis section, sketch a pillbug. Label any structures that you recognize.

B. Taxis
3. Place one piece of masking tape on either side of the behavior tray and label one side A, the other B.

4. Place five pillbugs in each chamber of the tray.

5. Every minute for 10 minutes, count the number of pillbugs in each chamber.

6. Record your observations in Table 3 in the Analysis section.

7. Calculate the average number of pillbugs in each chamber in the 10 minute time period. Enter the results in Table 3.
8. Using the data from every group in the class, calculate the class average for number of pillbugs in each chamber in a 10 minute time period. Enter the results in Table 3.

C. Experiment Formulation

9. Your instructor will assign you one of three environmental factors that affect pillbug behavior. These environmental conditions elicit different types of pillbug taxis: phototaxis, chemotaxis, or hydrotaxis.
   - Phototaxis: The orientation of an organism in relation to the presence of light. Movement toward a light source is positive phototaxis; movement away from light is negative phototaxis.
   - Chemotaxis: The orientation of an organism in relation to the presence of a particular chemical. Chemotaxis is universal in protozoa and most organisms; they avoid most chemicals and exhibit a positive taxis to some weak acids.
   - Hydrotaxis: The orientation of an organism in relation to the presence of water.

10. Formulate a hypothesis regarding environmental preferences and how the pillbugs may react to different conditions. Enter your hypothesis in the Analysis section.

11. Design an experiment to test the environmental factor you were assigned. Explain your experimental setup in the Analysis section.
   - Phototaxis: To test phototaxis, vary the amount of light the pillbugs are exposed to and record their responses.
   - Chemotaxis: To test chemotaxis, simulate an alkaline or acidic environment—place several drops of either a weak acid or weak base on a filter paper on one side of the behavior tray.
   - To test hydrotaxis, simulate a moist environment—place several drops of water on a filter paper on one side of the behavior tray.

12. Check with your instructor before proceeding. Once your instructor has approved of your experiment, collect the data and enter it in Table 4 in the Analysis section. **Note:** Be sure that there is only one possible variable present in your experiment. Multiple variables will invalidate the experiment.

13. Draw a graph to illustrate the data you collected.

14. Using your graph and data from both the preliminary investigation and the experiment you designed, discuss your general findings and draw a conclusion based on your experiment.

15. Discuss your results with the rest of the class.

**Part II Courtship in Drosophila**

A. General Observations

1. Obtain a vial of virgin female flies and a vial of male flies.

2. While in the vial, observe the flies’ traits, particularly body features that distinguish males and females. Use a hand magnifier or stereoscope to assist in your observations. Record the physical features of each in Table 5 in the Analysis section.
3. Anesthetize the flies by placing the vials in a refrigerator or icebath for 10 minutes. After the flies are anesthetized, gently tap several male and female flies into a Petri dish. Cover the dish.

4. Observe the flies’ interaction with each other. Use a hand magnifier or stereoscope for detailed observations. Keep an accurate record of the courtship behavior sequence and duration of each phase. **Note:** It would be easiest to work in pairs, with one team member observing and dictating the behavior and the other team member recording what is observed.

5. Record the courtship behaviors of both the male and female flies in Table 6 in the Analysis section. Remember to record all observed behaviors, even seemingly insignificant ones.

6. Read the information on the Drosophila courtship rituals below. Note which behaviors you were able to observe and which behaviors you may have missed.

   **Male**
   - Vibrating Wings: Extends one or both wings, rapidly moving them up and down.
   - Waving: Holds a wing at a 90° angle from his body, then relaxes it.
   - Tapping: Strikes or taps the female with a foreleg.
   - Licking: Licks the female’s genitals, on the rear of the abdomen.
   - Circling: Postures and circles the female. This is usually done when she is not receptive.
   - Stamping: Stamps his foreleg but does not strike the female.

   **Female**
   - Extruding: Extends her ovipositor, a tubelike structure, from her genitalia. This is often done to prevent copulation.
   - Decamping: Avoids the male.
   - Depressing: Blocks access by pressing her wings down and curving her abdomen down.
   - Ignoring: Ignores the male.

7. Repeat the experiment with new flies. Add to the list any behaviors you may have missed the first time.

8. Pool the class’s data. Develop a series of behaviors that occur in each of the six phases to put together a pattern of courtship and copulation behaviors. Keep in mind that many behaviors are innate (inborn), and some are random.

**B. Experiment Formulation**

The courtship of Drosophila is affected by a number of factors. The female, once fertilized, stays fertilized for life. Because of this, competition for females is very intense. As in all insects, pheromones influence a female’s choice of male to mate with; therefore, the introduction of these pheromones by the male is crucial to his success in convincing a female to mate with him.

9. Choose one of the following questions, or devise your own, and design an experiment to find the answer.
   - Will non-virgin females allow mating?
   - Will non-virgin males attempt to mate?
   - Will males compete for a virgin female?
   - Will one male be successful over another, same male in two different matings?
   - Will a female allow mating if a male cannot perform the courtship behaviors?
10. Formulate a hypothesis regarding behavior under the conditions you are choosing to test. Enter your hypothesis in the Analysis section.

11. Design an experiment to test the conditions selected. Explain your experimental setup in the Analysis section.

12. Check with your instructor before proceeding. Once your instructor has approved of your experiment, collect the data and enter it in Table 4 in the Analysis section. **Note:** Be sure that there is only one possible variable present in your experiment. Multiple variables will invalidate the experiment.

**ANALYSIS**

**Part I  Behavior of Pillbugs**

**A. General Observations**

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
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<tbody>
<tr>
<td>General Observations of Pillbug Behavior</td>
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### B. Pillbug Taxis

<table>
<thead>
<tr>
<th>Time (min)</th>
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<th># Pillbugs in Side B</th>
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<td>Class avg.</td>
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C. Experiment Formulation

Hypothesis:

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Design:

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Table 4

Pillbug Taxis with Altered Environmental Conditions

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<tr>
<th>Condition Tested:</th>
<th># Pillbugs in Side A</th>
<th># Pillbugs in Side B</th>
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Conclusion:

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Graph your results as directed by your instructor.
II Courtship in Drosophila

A. General Observations

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Characteristics of male and female Drosophila</th>
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<tr>
<td>Drosophila</td>
<td>Characteristics</td>
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<tr>
<td>Male</td>
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<td>Female</td>
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<tr>
<th>Table 6</th>
<th>Mating behaviors of male and female Drosophila</th>
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<td>Drosophila</td>
<td>Characteristics</td>
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<td>Male</td>
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B. Experiment Formulation

Hypothesis:

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Design:

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Drosophila Observations

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<tbody>
<tr>
<td>Male</td>
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<td>Female</td>
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Conclusion:
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QUESTIONS
1. Below are some structural features of pillbugs. Discuss as a class what advantages you think they might offer the organism.
   - Multi-segmented body
   - Antennae
   - Color

2. In Part I, B of the experiment, you placed pillbugs in a chamber and acquired data. What do you think was the purpose of this experiment? What numbers did you expect? Why?

3. In Part I, C, you tested your hypothesis with regards to pillbug behavior and environmental preference. Did your conclusions agree with your hypothesis? Are you confident with your conclusions or would you need to test more factors?

4. Your teacher wants you maintain some pillbugs in the classroom and has asked you to set up a terrarium to keep them in. What would you include in the terrarium to insure they have the best chance of survival?

5. Would you describe the pillbugs’ response to moisture as kinesis or taxis? Why?
6. In Part II, B of the lab, how did your initial observations compare to the list of Drosophila courtship behavior? Was there anything you observed the second time that you missed the first time?

7. Why do you think you were provided with virgin Drosophila females?

8. Why is it necessary for a scientist to have good observational skills and maintain a sense of objectivity when observing animal behavior?

9. While you were observing the various behaviors throughout the lab, do you think you remained objective? What did you learn about yourself as a scientist?

10. In this lab you investigated behavior in two different terrestrial organisms. Pick an aquatic organism and design an experiment to test a behavior of the organism. State what you would like to test and explain or draw your experimental setup below.

11. As you learned in Part II, B of the experiment, Drosophila courtship and mating follows a strict set of behaviors. Name something in which you engage that also follows a strict set of behaviors.
Vernier Lab Safety Instructions Disclaimer

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- Essential instructor background information
- Directions for preparing solutions
- Important tips for successfully doing these labs

The complete Advanced Biology with Vernier lab manual includes 27 labs and essential teacher information. The full lab book is available for purchase at:
http://www.vernier.com/cmat/bioa.html