HOW-TO-DO-IT

Forensic ENTOMOLOGY for the Laboratory-Based Biology Classroom

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Insect studies offer a wide range of teaching opportunities as well as providing a positive learning experience for students in the laboratory-based biology classroom. A staggering array of potential resources features insects or other invertebrates as teaching tools. When one considers the sheer numbers, diversity, adaptability, and evolutionary success, it makes sense to consider insects as a prerequisite to a broader understanding of life (Creager 1976; Fischang 1976). Moreover, one might take into account their impact, both beneficial and detrimental, on human affairs, as well as the aesthetic value of insects, as manifested in the beauty of their many forms. Because of elaborate protocols required to handle and keep mammals and other vertebrate organisms, it is becoming more difficult and expensive to conduct laboratory exercises that illustrate fundamental biological principles. As a result, alternative models are needed in today's laboratory-based biology classrooms. Insects can serve as models to illustrate many of the physiological processes that occur in all living organisms, as well as basic principles such as ecology and behavior. Furthermore, insects can be observed first-hand with few restrictions such as those that have been placed on vertebrate animals. Still further, insects are inexpensive, easy to rear, take up little space, and readily available during warm weather. In response to the need for alternative models for teaching biology, an abundance of new and innovative instructional materials using insects and other invertebrates has been and is continuing to be developed and marketed (see National Science Resources Center). Our goal in presenting these activities is to integrate an understanding of insect biology with fundamental principles of ecology to form the foundation for an appreciation of forensic entomology.

Forensic entomology is the study of insects and other arthropods associated with suspected criminal events for the purpose of uncovering information useful to an investigation (Keh 1985). The use of insects, especially blow flies (Figure 1), as medicolegal and medicocriminal indicators has been recognized for many years (Megnin 1894; Smith 1986; Catts & Haskell 1991). Over the past several years, a resurgence of interest in forensic investigation by entomologists has resulted in a number of reviews summarizing this important field of criminal investigation (Meek et al. 1983; Keh 1985; Erzinclioglu 1983, 1985; Johnson 1990; Catts & Goff 1992). Moreover, the use of insects in homicide investigations was highlighted in the motion picture *Silence of the Lambs*.

Using insects in the classroom can be easy and fun, especially in the context of forensic biology. Because the timing of events in the insect lifecycle is predictable, insects are extremely useful in determining time of death (Haskell et al. 1997). A decomposing body is a miniature ecosystem that shows succession as insects visit in a predictable order. Forensic entomologists collect the various life stages of insects on or near the body, return

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them to the laboratory, and observe their development. The length of time since death is determined by counting backward from the number of days it takes to rear the insect to the adult stage.

In this paper, we describe activities that use insects commonly found outdoors (Figure 1) to illustrate basic ecological principles as they relate to forensic science. The activities presented are best done between May and October or when the outdoor temperature is consistently 10˚C (50˚F) or higher (Bass 1997). It is helpful to have some field guides available for identifying various insects. Students should recognize that this activity can generate odors some may find offensive. In addition to the maggots, bacteria will be decomposing the flesh, thus producing an objectionable smell. Students should be instructed to not open the cups, and always wash their hands with soap and water after handling them. Odor can be minimized by storing the paper cups in a well-ventilated area.

Figure 1.
The Blow Fly (Diptera: Calliphoridae).

The use of insects in murder investigations is based on the occurrence of a sequence of "colonizations" by various insects on a human corpse (i.e., faunal succession). The first wave of insects is made up mainly of flies (i.e., blow flies). Analysis of the larvae collected around the corpse, compared with both laboratory-rearing data and results of decomposition studies, can be of value in establishing the time of death. (Photograph by J.S. Miller)

Materials

- Chicken gizzards and/or hearts
- String or dental floss
- Paper cups (8 oz or larger)
- Plastic wrap
- Rubber bands
- Sticky traps
- Wire cage (or equivalent)
- Hand lens or dissecting microscope
- Data sheets, metric ruler, and pencil or pen
- Field guides for the identification of insects (See Field Guides and Books of Interest, p. 142)
- A well-ventilated area suitable for cool, room temperature, and warm temperature environments

Activity 1

Basic Procedure

1. Provide students with a chicken gizzard or heart and tie string or dental floss around it.
2. Take the "gizzard on a string" outside onto school grounds. Students should tie the gizzard to a tree branch, bush, or on a stick that is planted into the ground. Tying reduces the risk of predators taking the meat. Leave the gizzard outside for up to 24 hours. An intuitive variation of this procedure would be to extend the time of exposure outdoors (see Optional Variations of Activity 1: Comparing Different Times).
3. Retrieve the gizzard and place it into a paper cup.
4. Sprinkle a small amount of water on the specimen, as it usually dries out overnight.
5. Cover the cup with plastic wrap and secure with a rubber band. Punch small holes in the wrap to allow air to circulate.
6. Label the paper cup with students' names, place it in a warm spot, and observe for the next two weeks.
7. Have students set up a journal (Figure 2) to describe the environmental conditions in which the samples were exposed and begin recording their observations on a regular basis.

How To Use the Data Table

Each student should complete a data sheet (Figure 2) for each observation. Recording observations accurately, completely, and throughout the experiment is essential to drawing meaningful conclusions. To avoid confusion, showing the experiment title on each data sheet, the date and time of day are necessary. Insect species diversity, presence and activity differ through the day as well as by season. Recording local weather conditions (temperature, relative humidity, and most recent precipitation) allows students to interpret anomalous results that might derive from unusual weather conditions. Flying insect distribution is subject to vagaries of wind strength and direction; these factors might affect the colonization of the experimental specimen. Identification of insect families at a given time and distance from the specimen indicate the rate of progress of colonization. The section titled "Notes" allows students to record the unique details of each observation, and "Conclusions" to list immediate conclusions for later evaluation with all data from the experiment.

Optional Variations of Activity 1

Comparing Different Times

Vary the time the meat is left outside and determine how quickly the adults lay eggs on the sample. Bring the meat in after various time intervals (30 min, 1 hr, 24 hrs, 48 hrs), place in the cups, as previously described, and watch larva/pupae development.

Comparing Different Temperatures During Development

Temperature plays an important role in determining the time of death. Have students vary the temperature of the developing insects and observe the time it takes for the insects to develop into adults. Place a group of the samples in a cool place (approximately 21˚C), another group at room temperature (approximately 25˚C), and a third group in a warm place (28˚C).

Development slows as the temperature decreases and speeds up as temperature increases. Have students pool data from the various conditions and draw conclusions about the effect of temperature on insect development. Relate this to different weather or seasonal conditions and have students speculate how this would influence the evidence presented by a forensic entomologist in a legal investigation.

Results

Allow students to make daily observations. This should take about 10 to 15 minutes to complete. Typically, flying insects will be the first to discover the meat samples. Blow flies (Figure 1, Diptera: Calliphoridae) are usually the first to arrive and reach a meat sample within minutes. Blow fly larvae emerge in about two to three days under suitable conditions (25 to 26˚C). Because the meat samples are exposed for relatively short periods of time (i.e., 24 hrs), blow flies will most likely be the only insects observed. However, other flies such as flesh flies and Diptherians (cheese skippers) may be seen. The longer the meat sample is left outdoors, however, the greater the number of flies can be recovered.

It takes from one to two weeks for blow flies to go from egg to pupae, depending on temperature. The eggs are approximately 2 mm in length. Depending on the temperature, the egg stage lasts around one to two days. One can expect to see five to six larvae on a piece of meat that has been left outside for 24 hours. The blow fly has three instars (molts) during the larval stage. The first instar is approximately 5 mm long after about two days, the second instar is approximately 10 mm long after about three days, the third instar is approximately 17 mm long after four to five days. The simplest way to identify the instar is by size. The time it takes to reach the different instars depends on microclimate, i.e., temperature and humidity immediately surrounding the larva. At the end of the third instar the larvae will move away from the meat sample. This stage is referred to as prepupae. The prepupae is about 12 mm long, and is seen 8 to12 days after oviposition. The prepupae gradually become the pupae, which darken with age. The pupae are about 9 mm long and are typically seen 18 to 24 days after oviposition.

How Forensic Entomologists Determine the Time of Death

Based on previous descriptions, finding empty puparia on a corpse should indicate that the body has been dead for more than approximately 20 days. A more precise way to determine age of larvae and eggs is to rear them. For example, when a human body is found with unhatched egg masses on it, the forensic entomologist will note the time of discovery and the time when the first, 1st-instar larvae occur. Subtract the discovery time from the occurrence time and call this time "A." Next, rear the blow flies to adults, let them mate, lay eggs on a piece of raw meat under conditions similar to where the corpse was found, and note the time it takes for the first 1st-instar larvae to occur. This time can be called "B." By subtracting time B – A, one gets the estimated time between oviposition and discovery. Similar calculation can be done for other instars. By creating base-line data as just described under different temperatures and for different species, one only needs to rear the flies to a stage where they can be identified. Careful consideration must be made with respect to the biology of various insects. For example, some fly species lay eggs only in

Figure 2.
Example of journal setup for making observations and recording data.

Data Table

Experiment title: ______________________ Date: __________ Time: __________

Observers' names: ______________________

Experiment start date: __________ Expected duration: __________ Time: __________

Weather conditions: ______________________

Temperature: __________ Wind strength and direction: ______________________

Relative humidity: __________ Most recent precipitation: ______________________

Description of experimental setup: ______________________

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Insects present (keyed to family level) and distance from sample: ______________________

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Notes: ______________________

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Conclusions: ______________________

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In a given region, characteristic insect species take part in "colonizations" or waves in a consistent fashion, which is influenced to some extent by variations in environmental factors, especially temperature.

shaded or sunny areas. Likewise, some species only lay eggs during cool or hot temperatures.

Activity 2

Spoiled Meat Sample vs. Fresh Meat Sample

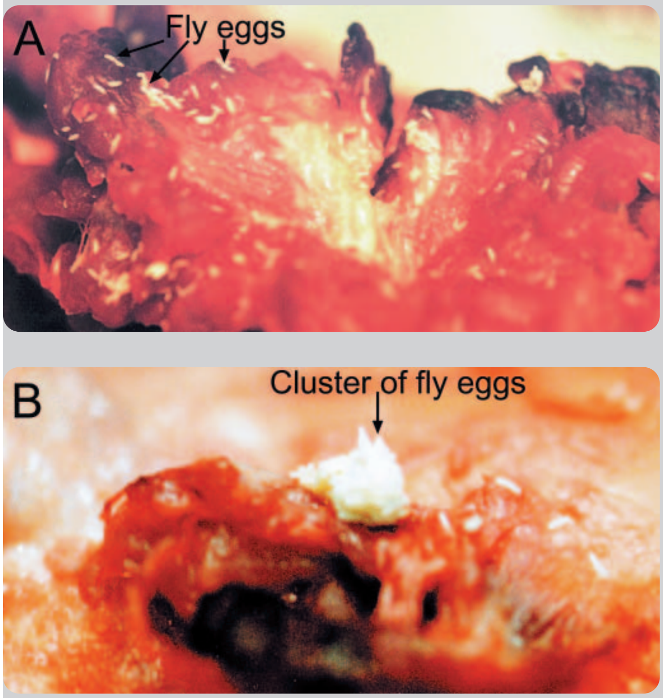
Rot the meat first in a glass jar, then take the sample outside to attract insects. Keep a portion on top of the soil, and bury the rest. When collecting insects from around the meat sample, collect underneath the sample as well as on top, and from the soil within a six-foot radius. Note the different insects or the timing of their arrival and compare these results with the results from the basic procedure (described above). Have students speculate how this might relate to an investigation of a corpse that has been covered with dirt or submerged in water, then exposed to the open air.

Results

For the most part, with the exception of ground meats, the type of meat used will not produce a noticeable (significant) difference in the number or variety of insects recovered. However, this may vary according to geographic location such as urban vs. rural or northern states vs. southern states. Ground meats are to be avoided because they tend to dry out quickly, fall apart, and thus become unsuitable for oviposition. The results will be disappointing. If an instructor wants to try using ground meat with a class, he/she should discuss the reproductive biology of insects and the significance of a suitable oviposition site before attempting the activity.

Intuitively, spoiled meat produces a stronger odor and therefore will attract more flies and possibly other insects as well. Predator insects and/or spiders may be observed because they are attracted to the carrion to prey upon the larvae or adult flies. Students should be able to distinguish predator from prey.

Figure 3.
Blow fly eggs on a piece of meat.



Eggs may be scattered across the surface of the meat (A), or laid in a clump (B). (Photographs by V.L. Naples)

Activity 3

Dead Animal or Road-Kill

Instead of grocery-store meats, leave a mouse (small rodent) or bird body outside, enclosed in a cage to allow insect access, but to prevent scavengers from carrying off the specimen. Watch the decomposition and succession. Have students make detailed observations of the process. Students could possibly set up a photo system to record progressive changes. Put out "Sticky Traps" near and around the cage to capture insects that may have walked to or from the animal corpse. Note the time (day) of their capture. Try to identify the insects stuck to the trap. "Sticky Traps" can be ordered from biological supply catalogs, your local exterminator, and some hardware and home improvement stores.

Results

We do not endorse the idea of deliberately killing a small vertebrate for the purpose of this activity. If one is looking for a "road kill" specimen along a busy roadway, care should be taken to avoid being hit by a motor vehicle or causing an accident. Under all circumstances, instructor and student safety is a major priority.

This experiment takes a considerable amount of time. It is difficult to predict the results of this activity because of the environmental diversity that exists across the United States or in foreign countries (refer to Suggested Readings, p. 142). Generally, the length of time after death of the road kill will determine which insects are present. Flies generally will be the first insects observed on fresh carrion. Carrion that has been exposed for some time will attract beetles as well as other insects. Beetles are good decomposers and are major contributors to the recycling of dead animals such as road-kill. Beetles will colonize a corpse later than flies, and different beetles will arrive at different times after a death, thus defining specificity with a particular stage of decomposition (Cragg 1956; Rodriguez & Bass 1985). Histerid and Staphylinid beetles are the first to arrive, followed by Dermestid beetles that appear when bone and hair only remain (Day 25 – indefinite). Other types of beetles such as Rhizhophagidae, Ptinidae and Tenebrionidae may be observed as well.

Conclusions

Traditionally, forensic work was restricted to a subfield of anthropology, and these techniques are still interesting and useful to learn. However, recent advances in research from many fields of the biological sciences have provided new tools that can be employed in a forensic setting. Most relevant here is the understanding of the lifecycles of a variety of insects and their roles in recycling the nutrients provided by a piece of meat or a dead animal (Figure 3). Many biological disciplines contributed to this understanding, including entomology, ecology, physiology, taxonomy and anatomy. Forensic studies of biological materials also utilize the *Scientific Method* to deduce previous events by analysis of present information, or in the case of forensics, evidence. These methods allow questions to be framed in the form of hypotheses and permit the investigation of physical phenomena to allow the investigator to draw conclusions.

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Suggested Readings

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Field Guides & Books of Interest

National Audubon Society Field Guide to North American Insects and Spiders. (2000). By Lorus & Margery Milne; visual key by Susan Rayfield. A.A.Koft, New York, distributed by Random House. (ISBN 0-394-50763-0)

Peterson Field Guide to Insects. (1998). By D.J. Borror & R.E. White. Houghton Mifflin, Boston. (ISBN 0-395-91171-0)

Peterson Field Guide to Insects. (1987). By C. Leahy. Houghton Mifflin, Boston. (ISBN 0-395-90664-4)

How to Know the Insects, 3rd ed. (1978). By R. Bland & H. Jaques. W.C. Brown and Co., Dubuque, IA. (ISBN 0-697-04752-0)

Simon and Schuster's Field Guide to Insects. (1981). By R.H. Arnett Jr. & R.L. Jacques Jr. Simon and Schuster Inc., New York. (ISBN 0-671-25014-0)

Immature Stages of Some Flies of Forensic Importance. (1989). By D. Liu & B. Greenberg. *Annals of the Entomological Society of America, 89, pp.* 80-93. (Contains a key and diagnostic description.)

A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes. (2000). By M.L. Goff. Harvard University Press, Cambridge, MA. (ISBN 0-674-00220-2)

Introduction to Insect Biology and Diversity. (1998). By H.V. Daly, J.T. Doyen & A.H. Percell III. Oxford University Press, New York. (ISBN 0-19-510033-6)

An Introduction to the Study of Insects, 6th ed. (1989). By D.J. Borror, C.A. Triplehorn & F.J. Norman. Saunders College Publishing, Harcourt Brace College Publishers, New York. (ISBN 0-03-025397-7)

Useful Web Sites

American Board of Forensic Entomologists:

http://web.missouri.edu/cafnr/entomology/index.html

Forensic-Entomology.Com:

http://www.forensic-entomology.com

National Science Resources Center:

http://www.si.edu/nsrc