

Chapter 13

Forensic Entomology

Objectives

After reading this chapter, you will understand:

- The stages of death.
- The role insects play in the decomposition of carrion.
- Postmortem interval.
- How insects can be used to estimate postmortem interval.
- The life cycle of insects.
- How variables affect results of scientific experiments.

You will be able to:

- Distinguish among major insect types associated with carrion.
- Identify the relationship between insect type and the stages of death.
- Perform the same experiments that forensic entomologists do.
- Estimate time of death from case description.
- Rear flies from pupae and larvae to adult.
- Explore variables affecting the determination of time of death.



**“When I was young I used to wait
On master and hand him his plate
And pass the bottle when he got dry
And brush away the bluetail fly
One day he ride around the farm
The flies so numerous they did swarm
One chanced to bite him on the thigh
The devil take the bluetail fly
The pony run, he jump, he pitch
He threw my master in a ditch
He died and the jury wondered why
The verdict was the bluetail fly
They lay him under a ’simmon tree
His epitaph is there to see
‘Beneath this stone I’m forced to lie
A victim of the bluetail fly!’”**

*—from early American folk song,
“Jimmy Crack Corn and I Don’t Care”*



Advance Preparation

Make copies of Blackline Master 13.1 from the Teacher Resource CD to hand out to students.

Teacher Note

The TRCD for this chapter includes a PowerPoint presentation, which is an overview of the chapter. It can be used as introductory material or at the end as a review.

The TRCD also contains a crossword puzzle that can be used after students have learned the vocabulary from the end of this chapter.

More than likely, the “bluetail fly” was a horsefly.

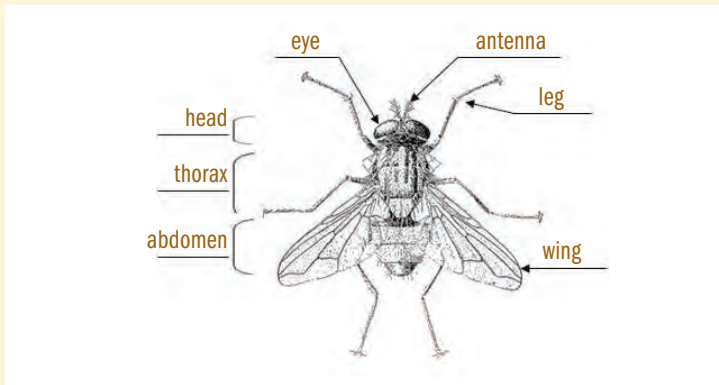
**Teacher Note**

The first two activities of this chapter can be skipped depending upon time and prior knowledge. Some of the activities as written are dependent upon climate. Using purchased pupae and larvae can circumvent natural conditions, but exploration is not as exciting. The activities can also take weeks to months, depending on temperature, so plan accordingly. The different lab activities can also be performed simultaneously to save time. For these reasons, estimating a time frame for completing this chapter, or even each lab activity, is problematic; however, we have tried (see the Estimated Time Charts in the TRCD).

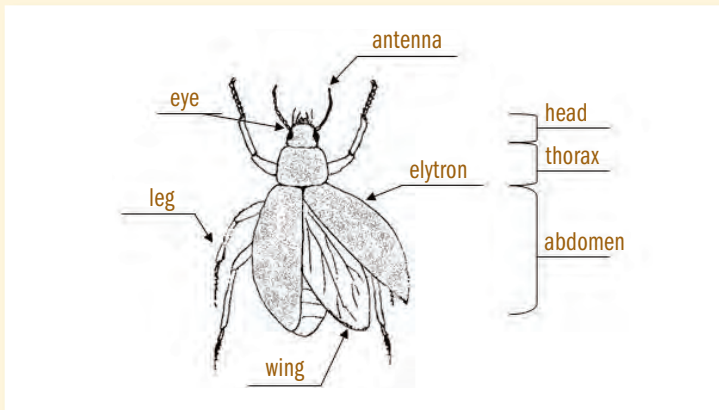
Use the handout provided by your teacher to record your answers.

1. What is a bug?
 - a) an insect
 - b) a pest
 - c) a hidden microphone
 - d) any or all of the above, depending on who you are talking to
2. A person who studies insects is an:
 - a) etymologist.
 - b) insectologist.
 - c) entomologist.
 - d) erythologist.
3. Insects are the most numerous living things on earth: true or false?
4. Taxonomy is:
 - a) the science of taxicabs.
 - b) the classification of things.
 - c) a book in the Bible.
 - d) income tax evasion.
5. What is the proper name for insects with a hard, outer-body casing and jointed legs?
 - a) arthropods
 - b) snails
 - c) mammals
 - d) lobsters
6. Most insects live on land: true or false?
7. The three basic body parts of an insect are:
 - a) head, eyes, tail.
 - b) head, wings, legs.
 - c) head, abdomen, wings.
 - d) head, thorax, abdomen.
8. How many legs does an insect have?
 - a) four
 - b) six
 - c) eight
 - d) any of the above
9. All adult insects have four wings: true or false?
10. Which is not an insect?
 - a) spider
 - b) ant
 - c) bee
 - d) beetle
11. Metamorphosis is:
 - a) a change in the body of insects.
 - b) Sting's new CD.
 - c) the middle earth.
 - d) a process of extraterrestrial travel.
12. Larva is:
 - a) a Hindu god.
 - b) a volcanic rock.
 - c) the immature stage of insect development.
 - d) a skin disease.

13. Exoskeleton is:
 a) the outer structure of a spacecraft.
 b) the tough outer covering of insects.
 c) X-man's skeleton.
 d) bones found in the woods.
14. Hypothetically, if a pair of houseflies bred in April, and all the offspring lived, how many flies would there be by August?
 a) 10^5
 b) 10^{10}
 c) 10^{15}
 d) 10^{20}
15. Label the parts of this housefly as indicated in the diagram:



16. Label the parts of this beetle as indicated in the diagram:



1. any or all of the above;
 2. entomologist; 3. true;
 4. the classification of things;
 5. arthropods; 6. true; 7. head, thorax, abdomen; 8. six; 9. false, they have two wings; 10. spider;
 11. a change in the body of insects; 12. immature stage of insect development; 13. tough outer covering of insects; 14. 10^{20} ;
 15. Insects' compound eyes are made up of many hexagonal lenses; insects have two segmented antennae which are used for smell and balance; all adult insects have six legs; legs and wings attach to the thorax; abdomen is the segmented tail area; 16. The elytron (plural: elytra) is a hardened forewing that protects the longer hind wings in beetles; beetles have two hind wings used for flying; they can be folded under the elytra when not in use. Although the students may not be able to answer some of the questions, this exercise can provoke a discussion

The common housefly is a major transmitter of diseases such as typhoid fever, conjunctivitis, poliomyelitis, tuberculosis, anthrax, leprosy, cholera, diarrhea, and dysentery.

It is estimated that there are 300 million insects for each person on earth!

about insects and their role in our lives (e.g., spreading diseases; being on the low end of the food chain; and, most germane to this chapter, as nature's trash recyclers).

macrofauna:

animals visible to the naked eye, generally 1 mm and longer

Teacher Note

The purpose of this activity is to acquaint the students with the vast numbers of organisms that live in the soil, as well as to familiarize them with methods of collection that are used by forensic entomologists at the site of a corpse.

The heat from the light and drying of the soil drives the animals down into the funnel. Test this by turning off the light, or keeping it on but also keeping the soil moist. The sampling environment could be changed by adding wet leaves or placing a board on the ground for several days before sampling. Both should increase populations. Different organisms may inhabit deeper layers, and in lesser numbers.

There are 1,462 recorded species of edible insects. A few treats:

- Thailand – bee grubs in coconut cream
- Laos – dragonfly nymphs
- United States – locust stew
- Philippines – parched locusts
- Japan – boiled wasp larvae
- South Africa – deep-fried mopani worms
- And a favorite – mealworm chocolate chip cookies

A Berlese funnel is used to separate **macrofauna** from collected soil samples. Different types of organisms can be examined with a stereomicroscope, and perhaps some can be identified.

Materials

- Berlese funnel, either purchased or constructed from a large, 6- to 8-inch-wide funnel; galvanized screen with 1/8- to 1/4-inch mesh; and cheesecloth. Cut the screen with tin snips into a round disc that will fit snugly about two-thirds of the way down into the throat of the funnel. Line the mouth of the funnel with a single layer of cheesecloth and press it down so that it lies on top of the screen. The cheesecloth should drape over the funnel's top rim (see Figure 13.1).

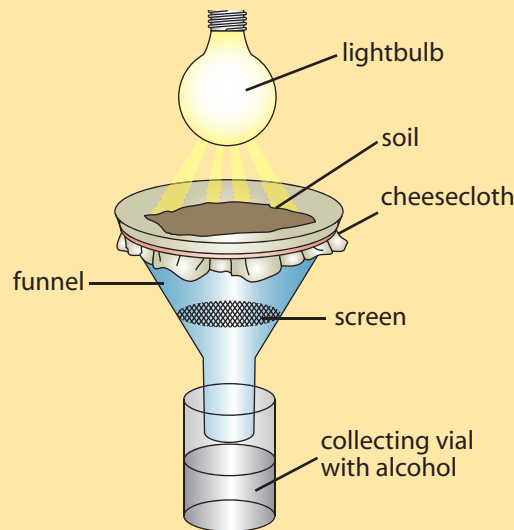


Figure 13.1 Berlese funnel

- ring stand to hold funnel and lamp
- vial wider than funnel stem filled one-half full with rubbing alcohol (70 percent isopropyl alcohol) and a few drops of glycerin to retard evaporation
- stereomicroscope
- forceps or tweezers
- trowel or spoon to collect soil samples
- plastic ziplock bags, 1 gallon
- marking pen
- 15-cm ruler
- lamp with a 40- or 60-watt bulb

Procedure

1. Collect soil samples; start with one about 6 inches square, 1 inch deep. Different lab groups should collect from different areas.
2. Place the sample in a plastic bag. Label it with date; group or student identification; description of the collecting site, including whether it was in shade or partial/direct sun; whether it is moist or dry; and type of soil (sandy, clay, organic, etc.).
3. If the sample is very wet, let it dry overnight in a cardboard box lined with newspaper. It may still be damp the next day.
4. Place the soil sample on top of the cheesecloth in the funnel. (No need to remove twigs, leaves, grass, etc.)
5. Place the collecting vial with alcohol beneath the funnel stem.
6. Turn on the light and watch what happens. Make daily records of the number of specimens that fall into the collecting vial.
7. After a day or two, pour off the alcohol, use forceps or a toothpick to remove specimens, and observe them with the stereomicroscope. Measure each organism and sketch it in your notebook. The chart below (Table 13.1) can serve as a model. Do not write in the textbook!

Table 13.1: Soil Specimens

	No. Found	No. of Body Segments	No. of Pairs of Legs	No. of Pairs of Wings	Common Name	Class & Order
1						
2						
3						
4						
5						
6						

The types of organisms will vary according to the habitat. You may find ants (Hymenoptera), pillbugs (Isoptera), beetles (Coeloptera), cockroaches (Blattodea), earwigs (Dermaptera), springtails (Collembola), and immature insects that are difficult to identify, as well as earthworms, mites (Arachnida), spiders (Arachnida), and even centipedes

Teacher Note

Table 13.1 is reproduced as Blackline Master 13.2 on the TRCD.

Laboratory Activity 13.1, *continued*

(Chilopoda) and millipedes (Diplopoda). Figure 13.2 depicts a few of these insects.

Teacher Note

Blackline Master 13.3 from the Teacher Resource CD contains a Dichotomous Key.

dichotomous:

“divided into two parts”; therefore, dichotomous keys always give two choices in each step in identifying an organism

“On my return to the laboratory [from the crime scene] . . . I put the soil samples into Berlese funnels to extract the arthropods.”

—From M. Lee Goff’s *A Fly for the Prosecution*, p. 82

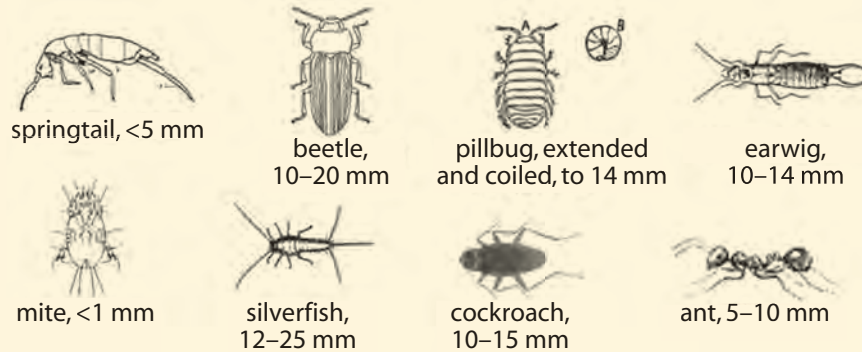


Figure 13.2 Common soil macrofauna

- If you want to go further in identification, use a **dichotomous** key for insect orders, available from your teacher or online at <http://earthlife.net/insects/orders-key.html> or www.amnh.org/learn/biodiversity_counts/ident_help/Text_Keys/text_keys_index.htm.
- Compare your data with those of other groups. Is there a relationship among collection site, type of soil, and other factors? What do you think caused the animals to leave the soil in the funnel? How could you test your hypothesis?

Taxonomy

taxonomy: the classification of things in an orderly way that indicates natural relationships

Taxonomy of modern humans:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Primates

Family: Hominidae

Genus: *Homo*

Species: *sapiens*

Carl Linnaeus (1707–1778) is credited with devising an orderly system of classification which has been especially useful in the organization of biological systems. This method of classification is called **taxonomy** and is applicable to many different systems. Linnaeus established conventions to impart a unique name for every living species. How does this work?

A *kingdom* is the broadest category; it is subdivided into a smaller unit, *phylum*, and so forth until we come to *species*, which is assigned a unique name.

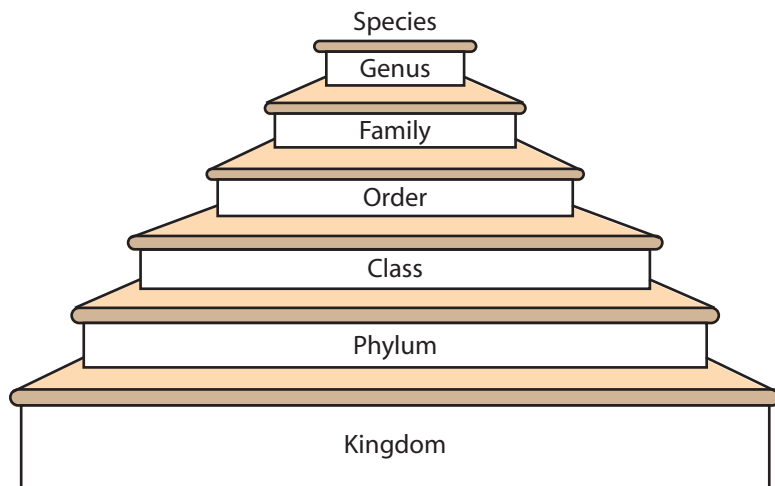


Figure 13.3 The steps of taxonomy

	GO TO	www.scilinks.org
	TOPIC	classification systems
	CODE	forensics2E375

We are especially interested in **arthropods**, especially beetles (Coleoptera) and flies (Diptera), as you will learn shortly. Let's take a look at the classification of a particular beetle that eats **carrion**:



Kingdom: Animalia
 Phylum: Arthropoda (arthropods)
 Class: Insecta (insects)
 Order: Coleoptera (beetles)
 Family: Silphidae (carrion beetles)
 Genus: *Nicrophorus*
 Species: *humator*

What does all this have to do with forensic science? Forensic **entomology** is the use of insects and other arthropods to aid in legal investigations. There are three general areas of application: urban entomology, affecting man and his environment (e.g., insect damage to structures); stored products entomology, such as insects infesting foodstuffs; and medicolegal entomology (or forensic medical entomology), which deals with **necrophagous** (carrion-feeding) insects that typically inhabit human remains. This is the subject that will occupy the rest of this chapter, particularly as it relates to the determination of the **postmortem interval (PMI)** associated with the time of death.

arthropods: animals characterized by jointed legs, a segmented body, and a hard, nonliving exo- (outer) skeleton. The exoskeleton does not keep up with the growth of the insect, and so must be shed periodically. This process is called molting. The stages between molts are termed instars.

carrion: the carcass of a dead and decaying animal

entomology: the scientific study of insects

necrophagous: feeding on carrion; from the Greek word *phagos*, to eat, and *necro*, meaning dead

postmortem interval (PMI): the time elapsed since a person has died

Most animals and plants are known only by their genus and species names. Genus name is spelled with a capital letter, whereas the species name is normally spelled with a small letter; in printed text the pair are normally written in *italics*.

To solve a crime, investigators must answer the five “W’s”: *Who* was the victim, and/or the perpetrator; *what* happened; *when* did it happen; *where* did it happen; and *why* did it happen?

The Process of Death

Let’s look at death in order to relate it to various methods of estimating when it occurred. Realize that death is a process, not an event. Different

tissues and organisms in a living body die at different rates. For example, brain cells deprived of oxygen die within 3–7 minutes, whereas skin cells can live up to 24 hours. Even the definition of “death” varies within the legal and medical fields, especially now when a person can be on life-support systems.

The *cause of death* usually is quite obvious, especially to a medical examiner or forensic pathologist. The *manner of death* usually requires detective work as to what happened. For example, the corpse’s lungs are full of water: The cause of death is drowning; the manner of death could be accidental, suicide, homicide, natural (pulmonary edema), or undetermined.

Physical Methods of Determining Time of Death

A pathologist usually determines the time of death. It is most accurate if the body is found within the first 24 hours after death, using the indicators of algor mortis, livor mortis, and rigor mortis. After that time period, other methods must be employed; estimations are made by studying the environmental conditions and other information regarding the scene.

algor mortis: the cooling of body temperature after death

Temperature conversions:
 $^{\circ}\text{C} = (5/9) \times (^{\circ}\text{F} - 32)$
 $^{\circ}\text{F} = (9/5) ^{\circ}\text{C} + 32$

Algor mortis refers to the cooling rate of the body after death. Immediately upon death, the body can no longer metabolically maintain its temperature of 98.6°F (37°C) and begins to equalize its temperature to that of its environment. Measuring the internal body temperature can give some indication of the time of death, theoretically following Newton’s law of cooling, which states that the rate of change of the temperature of an object is proportional to the difference between its own temperature and the ambient temperature (i.e., the temperature of its surroundings). This can be expressed mathematically by a simple first-order differential equation:

$$\frac{dy}{dt} = ry \quad \text{or} \quad \frac{dT}{dt} = -K(T - T_s)$$

This method of measurement was used for some time, but it applies only to small, inorganic substances, so it works with hot coffee or a pizza

Teacher Note

For a nice little applet showing the exponential decay and effect of K, see <http://orion.math.iastate.edu/algebra/sp/xcurrent/applets/cooling.html>.

coming out of the oven, but not for corpses. In some instances, the temperature of the corpse remains the same for a while or even rises due to reactions in cells as they shut down, as well as by bacterial generation of heat. Simpler to apply than Newton's exponential temperature decay, and more accurate, is the **Glaister equation**:

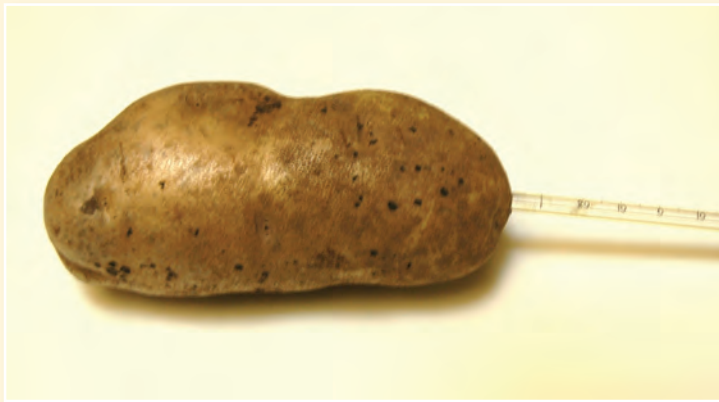
Glaister equation: a formula used for determining the approximate time period since death based on body temperature

$$\text{Hours since death} = \frac{98.4^{\circ}\text{F} - \text{internal body temperature}}{1.5}$$

This equation can be used from one to 36 hours after death, but is most accurate within the first 12 hours. Generally, a body cools about 1–1½ degrees Fahrenheit per hour until it reaches ambient temperature. Consideration must be given to the temperature of the environment, type of clothing on the body, wetness of the clothing, air movement, how many layers of clothing, and other conditions. Also, the greater the ratio of surface area to mass, as with children or smaller adults, the faster the body cools.

The Potato Corpse

Laboratory Activity 13.2



Materials

- several large baking potatoes of different sizes
- several large sweet potatoes of different sizes
- a large beet
- a large baking apple
- microwave oven
- thermometer

Procedure

1. Weigh each item and record the dimensions.
2. Heat each food product in a microwave. Prick them prior to heating to let steam escape. The beet and apple should be placed in a tray to catch any juices.

Teacher Note

Graph paper can be found on the TRCD as Blackline Master 13.4 for use in this activity. See if any student thinks of wrapping the hot potato corpse in “clothing” such as a cloth napkin, or cooling the corpse with a blower, or laying it on a conducting versus insulating surface, etc.

You may also wish to give the students a prepared hot potato and have them estimate time of death, with error limits.

For advanced students in chemistry, this experiment can be used to illustrate, or lead into, the concept of heat capacity. See www.ausetute.com.au/heatcapa.html for a simple tutorial, and www.chm.davidson.edu/ChemistryApplets/calorimetry/SpecificHeatCapacityOfCopper.html for an experiment to determine heat capacity (in this case copper, but it can be applied to a potato).

Laboratory Activity 13.2, *continued*

3. Remove the product and insert a thermometer into the center. It will take a little practice to get the internal temperature to around 100°F.
4. Record room temperature and the temperature of the “body” as a function of time.
5. Graph your results in your science notebook.

Analysis Questions

1. Describe the data mathematically.
2. Compare results from the different materials with your classmates.
3. Explain any differences.
4. How reproducible are the results?
5. What other variables could be explored?

Livor mortis refers to the pooling of blood in the body due to gravity after the heart stops. It appears on the skin as a purplish-red discoloration and can give an indication of the position of the body at the time of death. It does not occur in the areas of the body that are in contact with the ground or constricted by other objects, as the capillaries are compressed in those places. Livor mortis begins within a half hour after death and is most evident within the first 12 hours. After this time, the discoloration of livor mortis will not move regardless of how the body is disturbed. This fact can be useful in determining whether a body has been moved after death.

livor mortis: a purple or red discoloration of the skin caused by pooling of blood after death

rigor mortis: a stiffness in the muscles that occurs shortly after death

Rigor mortis refers to the rigidity of the skeletal muscles after death. Immediately upon death muscles begin to relax; ATP (adenosine triphosphate, an enzyme in the muscles) begins to break down, fluid concentrations change, and the muscles become

rigid. Rigor mortis begins in the smaller muscles so is first observed in the face, neck, and jaw. The noticeable stiffness of rigor mortis can occur

NAME: RAMSEY, JONBENET

AUTOPSY NO: 96A-155

Page 5

PAIRS OF THE LEFT HAND. THE FINGERS OF BOTH HANDS ARE OF SUFFICIENT length for clipping. Examination of the back is unremarkable. There is dorsal 3+ to 4+ livor mortis which is nonblanching. Livor mortis is also present on the right side of the face. At the time of the initiation of the autopsy there is mild 1 to 2+ rigor mortis of the elbows and shoulders with more advanced 2 to 3+ rigor mortis of the joints of the lower extremities.

Extract from JonBenet Ramsey's autopsy report

within a few hours of death and is gone within approximately 30 hours, leaving the body limp. The effects of rigor mortis begin to disappear in the same order in which they began, the small muscles becoming limp first, and then the larger muscles of the trunk, arms, and legs. Rigor mortis is affected by environmental conditions such as temperature, dehydration, condition of muscles, and their use prior to death; hence there are uncertainties in determining the time of death from rigor mortis also.

The observations in Table 13.2 may be used in estimating time of death, but must be used with caution:

Temperature of Body	Stiffness of Body	Time Since Death
warm	not stiff	not dead more than 3 hours
warm	stiff	dead between 3 and 8 hours
cold	stiff	dead between 8 and 36 hours
cold	not stiff	dead for more than 36 hours

Estimating Time of Death

A body, quite stiff, has been found lying on the floor in the basement of a tenement building. Body temperature is measured to be 86°F; lividity (discoloration) is not complete. The police want you, as medical examiner, to estimate the time of death. You have available to you a study done on 114 natural deaths in 1872 (see Figure 13.4). Justify your conclusion. How accurate is it? What factors could affect your opinion?

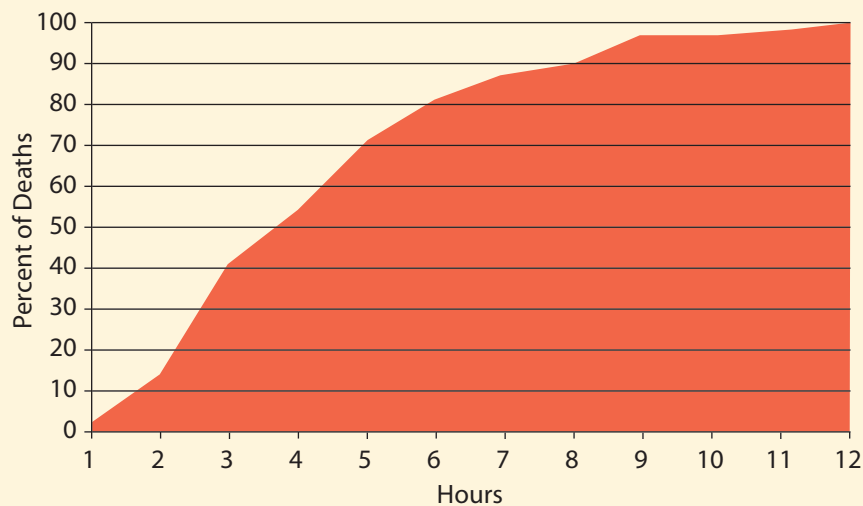


Figure 13.4 Time for the onset of rigor mortis

Activity 13.2

Teacher Note

Livor mortis not set, <12 hours; temperature change 12°F/1.5°F = 8 hours (Glaister equation); rigor mortis complete, so consistent with data from Figure 13.4. All methods are approximate and depend on such variables as ambient temperature, air movement, body size, age of the victim, etc. The three indicators are consistent with a PMI of 8, ±1 hour. See Table 13.2.

Within four minutes of death, decomposition begins. Cells are deprived of oxygen, carbon dioxide in the blood increases, pH decreases, and wastes accumulate, poisoning the cells. Enzymes dissolve cells from the inside out, causing them to rupture, releasing fluids. This entire process is termed **autolysis**.

autolysis: a process by which a biological cell self-destructs

putrefaction: the decomposition of animal proteins, especially by anaerobic microorganisms

Autolysis is first observed after a few days by the appearance of fluid-filled blisters on the skin and skin slippage where sheets of skin slough off areas of the body.

Putrefaction is the next step of decomposition. It is the destruction of the soft tissues of the body, primarily by bacteria. Usually the first visible sign of putrefaction is a greenish cast to the skin. Gases such as methane and ammonia, released by continued decomposition of tissues, cause bloating. The odor of volatile butyric and propionic acids is unpleasant. Further decay of proteins and fats yields such interesting and odoriferous compounds as skatole, methyl disulfide, and the aptly named cadaverine and putrescine. By this time, the body is a mass of fluids and smells just awful. Some call this stage “black putrefaction.” (See Table 13.3.) The stages are continuous; the times are for general reference and are dependent upon many variables.

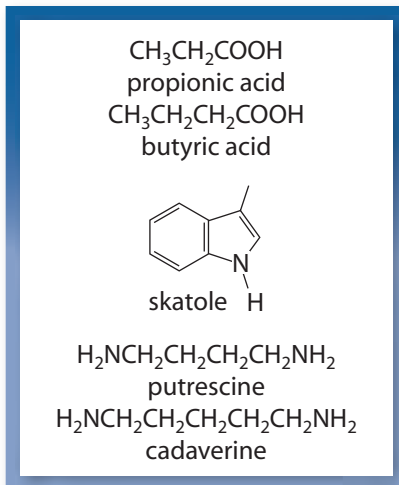


Table 13.3: The Stages of Decomposition

Stage	Description
Initial or fresh decay (autolysis)	The cadaver appears fresh externally but is decomposing internally due to the activities of bacteria present before death (0–4 days).
Putrefaction or bloating	The cadaver is swollen by gas produced internally, accompanied by the odor of decaying flesh (4–10 days).
Black putrefaction	Flesh of creamy consistency, with exposed body parts black. Body collapses as gases escape. Fluids drain from body. Odor of decay very strong (10–20 days).
Butyric fermentation	Cadaver drying out. Some flesh remains at first; cheesy odor from butyric acid (20–50 days).
Dry decay (diagenesis)	Cadaver almost dry; slow rate of decay. May mummify (50–365 days).

Adipocere formation sometimes occurs months to years after death.

Adipocere is usually a yellowish-white, greasy, waxlike substance that forms as a result of the **saponification** of fatty acids, most often catalyzed by a particular anaerobic bacterium. Conditions most favorable to its formation are burial in a moist, alkaline soil (which retards putrefaction and scavengers), high body fat of the corpse, and burial in a casket within a burial vault. This process helps to preserve the body, which may aid in identification and recognition of injuries. (For a fascinating mystery involving this subject, read *Carved in Bone* by Jefferson Bass.)

Mummification is the result of dehydrated tissue, usually skin, that has survived decay. It commonly develops under hot, dry conditions with persons of low body fat and can occur as early as one month after death.

In the final stage, bone is chemically altered, especially by moisture and the pH of soil. This process is called **diagenesis**.

The rate of decomposition depends on many variables; most important are the environment, temperature, and the presence of scavengers. For example, a body decays twice as fast underwater and half as fast underground. The following equation gives a very rough estimate for time of decomposition of soft tissue for a person lying on the ground:

$$\text{Number of days to become skeletonized} = \frac{1,285}{\text{average temperature, } ^\circ\text{C}}$$

Thus, if the average temperature around the corpse is 20°C (68°F), it will take about 64 days to leave only a skeleton. In the tropics, at an average temperature of 30°C, it would take only 30 days or less because of the higher humidity. This gives investigators a ballpark time frame with which to begin their investigation.

adipocere: also called “grave wax,” insoluble fatty acids left as residue from preexisting fats from decomposing cadavers. It is formed by the slow hydrolysis of fats in wet ground and can occur in both embalmed and untreated bodies.

saponification: the alkaline hydrolysis of fatty acid esters. In chemistry, it refers to the reaction of a base with a fat to form soap.

diagenesis: the process of chemical and physical change in deposited sediment during its conversion to rock

13.1: Colonel William Shy

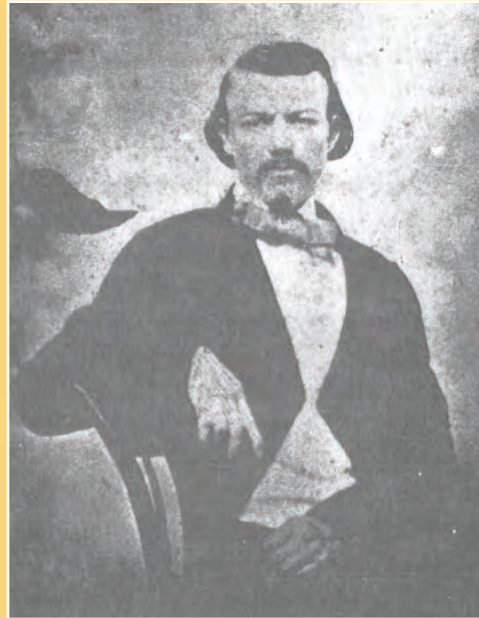
An interesting anomaly occurred when Dr. Bill Bass, of Body Farm fame, was called to investigate an apparent defiling of the grave of a Confederate colonel, William Shy, who was killed in 1864. The disturbed grave was thought to contain a recent burial. The hole had presumably been dug by those who dumped the body; the cast-iron coffin had also been broken open. On top of the coffin, Dr. Bass found the body

CASE STUDY

of a large man, dressed in a tuxedo, decayed, but with flesh of a pink, healthy color. Judging from the state of the decayed flesh and the odor, Dr. Bass estimated the time since death to be six to 12 months.

**CASE STUDY
CONTINUED**

However, there was no other body in the grave. After comparing the body's physical dimensions to those of Colonel Shy, and considering the method of embalming bodies in the mid 1800s, the teeth, and the type of clothes on the corpse, Dr. Bass came to the conclusion that the corpse was indeed Colonel Shy, right where he was supposed to be. The body was 113 years old, not 12 months! Apparently, the lead-lined coffin and embalming method (using arsenic compounds) had prevented normal decomposition beyond autolysis which had occurred prior to burial. The disturbance at the grave site was caused by grave robbers looking for Confederate souvenirs to sell.



Lt. Col. W. M. Shy, 20th Tenn. Infantry, C.S.A. Born May 24, 1838. Killed at Battle of Nashville, Dec. 16, 1864.

Life Cycle of Insects

Many insects are carrion eaters. Indeed, if it weren't for them, our forests and meadows would be full of animal parts. The life cycle of insects is well known. For example, the four principal stages of **metamorphosis** of a fly are: (1) egg; (2) larva (maggot); (3) pupa (plural, pupae); (4) winged adult.

Figure 13.5 shows the detailed life cycle of the common housefly (*Musca domestica*).

The female fly deposits 100–150 eggs at a time. They hatch as larvae, which eat a lot and grow fast. With a somewhat rigid exoskeleton, they must **molt**. The first larval stage is called the first **instar**; the second, the second instar. The third instar is the longest. By this time, the larva is full and no longer interested in eating. It becomes restless and moves away from the food source. It is now in its prepupal stage, during

metamorphosis: biological process in the development of animals, usually involving conspicuous changes in the animal's form or structure. About 88 percent of all insects go through complete metamorphosis in four major stages.

molt: the shedding of an insect's outer skeleton during a growth stage

instar: a developmental stage of arthropods, generally referring to changes in the size of the larvae

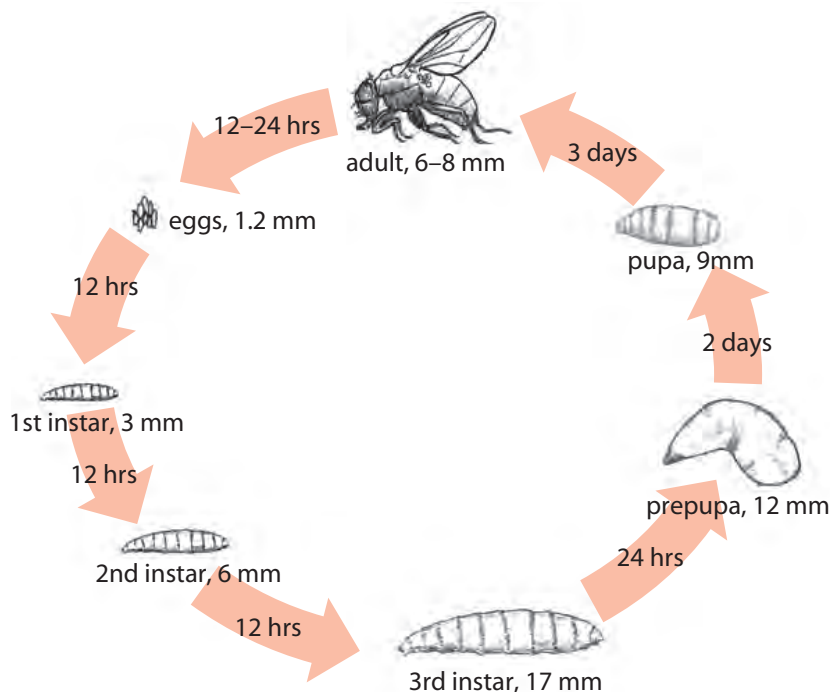


Figure 13.5 The life cycle of *Musca domestica*

which it gradually darkens and hardens to form a pupa, the brittle casing from which the adult fly emerges.

Forensic Entomology

Knowing the stage of insect inhabitation of a corpse and the duration of stages in the insect's life cycle can lead to an estimate of the time since colonization. Estimating the time it takes for the insect to find the corpse and start laying eggs allows the investigator to calculate the PMI.

For example, a corpse found with empty pupae of the housefly tells us only that the time since colonization is more than seven days (Figure 13.5). Suppose, however, that unhatched pupae of another species of fly are also found, and it is known from laboratory studies that it takes nine days from egg-laying to pupation for this species of fly under similar conditions. Assuming **oviposition** within one day of death for both flies, the PMI can be estimated to be about ten days.

As a body undergoes successive stages of decomposition, it attracts a succession of different insects; some feed on the products of decomposition, and some feed on the feeders themselves. Like the life cycle of individual insects, the succession of insect predators on a

oviposition: the depositing or laying of eggs. To oviposit means to lay eggs.

A vulture boards an airplane, carrying two dead raccoons. The stewardess looks at him and says,
"I'm sorry, sir, only one carrion allowed per passenger."

corpse follows a predictable pattern, although it is somewhat influenced by geography as well as by local conditions. Insect species also vary from region to region, from habitat to habitat, and from season to season.

The Insects of Death

Flies (Diptera) and beetles (Coleoptera) are the two most common orders of insects found on corpses.

Over 1 million species of flies are estimated to inhabit our planet.

Adult flies have one pair of wings (see question 15 in Activity 13.1). The larvae appear wormlike. Diptera undergo complete metamorphosis.

Table 13.4: Major Families of Diptera Found On or Near Carrion

Family	Examples
Calliphoridae	Blowflies such as the blue- and greenbottle flies. In the early stages of decomposition, often the most abundant larvae on a corpse. Different species have specific preferences for oviposition (in shade or light) and habitat (urban or rural).
Sarcophagidae	Flesh flies. These are large flies that lay live larvae instead of eggs, and may be present shortly after death. Their larvae eat blowfly maggots.
Muscidae	A large family that includes the ubiquitous housefly. Sometimes found during the later stages of decomposition.
Piophilidae	Dark, shiny flies such as the cheese skipper. The larvae are scavengers and are associated with the late stage of decomposition.

Beetle adults have two pairs of wings, but the top pair is hard and protects the flight wings folded underneath (see question 16 in Activity 13.1). Like Diptera, they have three pairs of legs. The larvae have different shapes and

The heaviest beetle in the world is the African Goliath beetle, which can weigh 3 ounces and measure 5 inches in length. The longest beetles in the world are the long-horned beetles from South America and Fiji, which attain a length of about 8 inches.

sizes—some wormlike, some with legs, many soft and C-shaped (grubs); pupae are like pale, mummified versions of the adult. Coleoptera undergo complete metamorphosis. Most beetles are nocturnal and may be found under a body or in the soil surrounding the remains. See Table 13.5.

Blowflies and flesh flies arrive at a body first, sometimes within minutes of death, attracted by the molecules of decay. Later to come are the houseflies. The female fly lays eggs within natural body openings and wounds. Inhabitation is a complex

Table 13.5: Major Families of Coleoptera Found On or Near Carrion

Family	Examples
Staphylinidae	Rove beetles. Can be present within hours of death as well as months later. Adults and larvae feed on eggs and larvae of other species.
Silphidae	Carrion beetles, burying beetles. Example is the sexton beetle, found during the early stages of decomposition. Adults and larvae feed on maggots as well as carrion.
Histeridae	Clown and hister beetles. Present from early on to the start of the dry stage of decomposition. Adults and larvae feed on maggots and pupae, and on the larvae of <i>Dermestes</i> beetles.
Dermestidae	Skin beetles, hide beetles. Feed on dried skin and tissues during the later stages of decomposition.
Scarabidae	Hide beetles. Some of the last arrivals at a corpse (dry decay stage).
Cleridae	Examples are ham beetles and checkered beetles. These are predators of flies and other beetles.
Carabidae	Ground beetles. Larvae and adults are predatory. Found during all stages of decomposition.
Tenebrionidae	Darkling beetles. Larvae and adults are predatory.

interaction of different insects and decaying body chemistry.

Fly eggs hatch as maggots that feed only on soft, mushy body parts. Maggots can form large, moving masses that, along with bacterial decomposition, can raise the temperature around them to well above **ambient** temperature. Maggots account for the loss of most of the body's mass. Predators, such as ants, wasps, and beetles (hister, rove, and burying beetles), continue to arrive at the scene of death. Beetles feed on the corpse and other arthropods present, and they lay their eggs on or under the corpse. The first fly families to arrive only like the semifluid environment, so as the corpse dries, cheese skippers and coffin flies take over. Checkered beetles now arrive to feed on flies and other beetles. This feeding frenzy has now reduced the body to 20 percent or less of its original weight, with primarily skin and bones remaining. The flies and maggots are gone. The rove beetles remain, and hide beetles arrive. In the soil under the corpse, the number of **mites** increases. By the end of the decay stage of decomposition, the insects have left, and the corpse has been reduced to about 10 percent of its original weight.

ambient: concerning the surrounding area or environment

mites: tiny eight-legged creatures belonging to the order Acarina, related to spiders and ticks. Some mites live freely, others as parasites.

There are 15 board-certified members of the American Board of Forensic Entomology (ABFE) in the United States and Canada. There are only 62 scientists involved in this field of study worldwide.



13.2: Body in the Basement

From FBI files



In the southeastern United States during mid-November, police were called to investigate a foul odor that was coming from a small, single-family home in an impoverished section of town. Investigating officers soon discovered the badly decomposed body of a young, black female in a shallow grave in the dirt basement of the dwelling.

The victim had died of a single bullet wound to the head inflicted with a small-caliber rifle. A careful examination of the corpse and excavation of the soil in and around the grave site revealed the presence of numerous larvae of *Calliphora vicina* (a blowfly) and larvae and pupae of a relative of the housefly, *Synthesiomyia nudesita*.

Specimens collected from the scene were reared in the laboratory. Supplemental information including climatic data and soil temperatures was reviewed in an effort to determine the intervening climatic conditions. Using information on the developmental biology of both of these species of flies, an estimate was made that the victim had died and was colonized by flies approximately 28 days prior to the time of discovery.

Investigators were able to target their investigation in and around the estimated time of death. Shortly thereafter, a suspect was identified. This individual eventually confessed to having killed the victim 28 days prior to the time the body was found. She had buried the victim in the basement of the house shortly after committing the homicide.

In this case, the larvae of two species of flies provided investigators with the only scientifically reliable method of estimating the time of the victim's death.

Why were specimens collected at the scene reared in the laboratory? It is difficult to characterize fly species from their larvae, so they are raised to the adult fly, which is easier to examine and classify. The life cycle for the particular species can then be used to estimate PMI.

Why were data on climatic conditions gathered? Insects are cold-blooded; therefore, their development is temperature-sensitive. Data obtained in lab rearing at 72°F will not reflect accurate life cycle times at other temperatures; for example, at 62°F, metamorphosis will take longer. There are methods to estimate the effect of temperature on metamorphosis, which we will explore next.

The Effects of Temperature on Rearing of Maggots

Laboratory Activity 13.3

This activity demonstrates the effect of temperature on the rate of metamorphosis. The data can then be used to explore the concept of thermal units and their application for estimating PMI.

Materials

- pupae
- rearing medium such as raw liver or ground beef or canned dog food containing beef
- a pint-size clear plastic container with a tight lid or screen
- vermiculite or sterile sand (heated above 100°C)
- thermometer, or data logger if available
- paper towel
- stereomicroscope
- ruler
- forceps or tweezers
- labels and marking pen
- nail or awl or a screen
- vial with isopropyl alcohol

Procedure

Construct a rearing chamber as follows:

1. Add $\frac{1}{2}$ inch of vermiculite or sand to the bottom of the plastic container.
2. Place the rearing medium on a moist, crumpled paper towel occupying about $\frac{1}{3}$ – $\frac{1}{2}$ of the surface of the vermiculite. It is important to keep the medium moist by sprinkling the protruding paper towel with water. (Liver dries out more readily than ground beef or dog food).
3. Punch a number of holes in the lid with a small nail, small enough so no flies can escape, or stretch vinyl screening over the top of the container using a rubber band.
4. The flies will like a bottle cap of water, with a sponge cut to fit in the cap so they don't drown, and a bottle cap with moist sugar.
5. Place the rearing chamber in areas of different but constant temperature (from 55° to 85°F), where observations can be made on a daily basis.



A rearing chamber

Forensic entomologists use domestic pigs for research because they closely follow human decomposition and are inexpensive and readily available.

Teacher Note

Ward's Natural Science sells pupae of *Calliphora* and *Sarcophaga*. Carolina Biological Supply sells *Sarcophaga bullata* as well as housefly pupae. Obtaining two species allows the students to compare larvae, pupae, and flies as well as their respective periods of development—a good illustration of succession. Allow about 40 days for a full metamorphic cycle at 80°F.

Extremely valuable in identifying flies and beetles of forensic importance is a deck of 52 cards, each with a color photo, information, and a life-size silhouette of the insect. A few scientific supply houses sell these for about \$35–\$40; they are well worth it.

An example of a time log that student groups might use for this activity can be found on the TRCD (Blackline Master 13.5). Ask the students to present their data as a graph, plotting larvae length (y) against time (x). Then all the temperature results can be graphed, time (y) versus temperature (x). The representative graphs on page 388 show what the results should look like. These can also be found on the Teacher Resource CD as Blackline Masters 13.6 and 13.7.

Teacher Note, continued

A simple environmental chamber can be constructed with a lightbulb in an open cardboard box. The temperature can be regulated by moving the top opening.

Laboratory Activity 13.3, continued

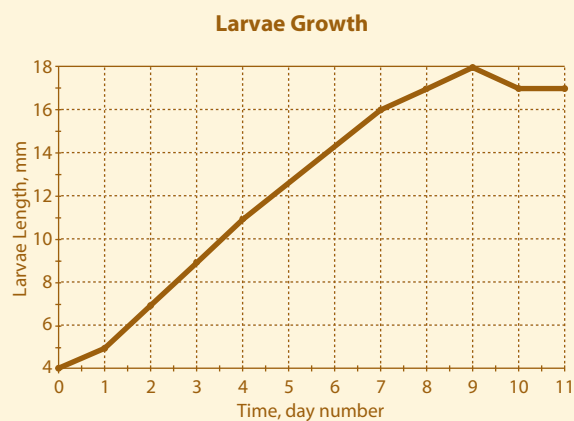
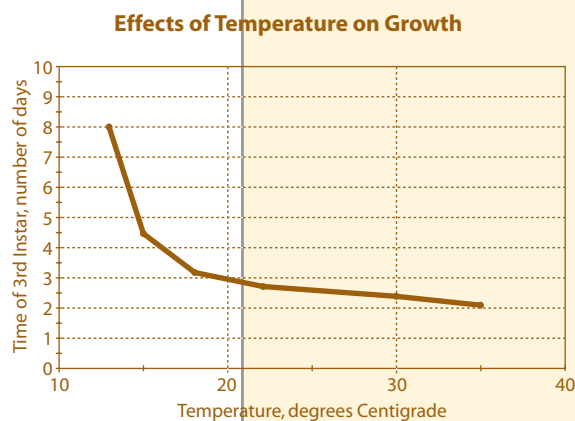


Figure 13.6 Common flies

6. Keep a time log and note when the pupae were received, when they were put into the chamber, when the first and last flies appeared, and so forth. Watch closely for oviposition.
7. Describe (draw or take a close-up photograph) and measure each stage of the metamorphosis.
8. To obtain an accurate length, you must kill the larva by immersing it in alcohol.
9. Note the time of the third instar (when growth levels off and then decreases). Soon thereafter, the maggots will stop feeding, leave their medium, and initiate pupation.
10. Continue recording data until flies emerge (**eclosion**).
11. Cool the chamber to 32°F or below to immobilize the flies, then pick out several and kill them in 70 percent isopropanol.
12. Observe the flies under a stereomicroscope and try to identify them.
13. Present the larvae growth data as a graph. Your teacher will collect growth data for the different temperatures from your classmates so that you can graph the effect of temperature on larvae growth.
14. What conclusions can be made?

eclosion: emergence of an adult fly from its pupal case

GO TO www.scilinks.org
TOPIC graphing
CODE forensics2E388



Temperature Dependence: Degree-Days

Insect development is dependent upon temperature. As you noted in Activity 13.3, the higher the temperature, the faster the growth. However, while an insect will not develop below a threshold temperature, above a certain temperature its development also stops. Within the development temperature range, however, insect growth is regulated by the amount of thermal energy absorbed, or, simply, per unit of heat. Growth rate is expressed in temperature-time units such as a **degree-day** or degree-hour. Accumulated units represent the energy needed to effect a change from, say, eggs to the third instar.

degree-day: a unit of measure of the energy absorbed by a biological system, causing growth

Entomologists borrowed the concept of degree-day from agriculturists, who used it to predict certain stages in the growth cycle of plants for timely application of fertilizer or when to spray their fields, for example.

Refer back to Figure 13.5. Assuming a constant temperature of 68°F, it takes 36 hours to grow from eggs to the third instar, or $36 \times 20^\circ\text{C} (68^\circ\text{F}) = 720$ accumulated degree-hours (ADH). This is the total amount of energy required to effect this change for this particular species of fly. At 10°C, it will take twice as long to get to the third instar: $720 \text{ degree-hours} / 10^\circ = 72$ hours. Theoretically, any combination of time and temperature that adds up to 720 is valid.

How do forensic entomologists use this concept to find a PMI? We will use an example to illustrate the process.

On September 16, two squirrel hunters found a body in the woods under a large oak tree. The forensic entomologist called to the scene found lots of maggots on the body, the largest being 18 mm long. There were also some new prepupae wiggling around the corpse. The forensic entomologist decided that the development stage was the very end of the third instar.

At 3 PM, he recorded an ambient temperature of 74°F. He collected many live specimens and got them back to his lab quickly in order to raise them to flies. Several weeks later, he was able to identify them as blue blowflies with the disgusting name of *Calliphora vomitoria*. It took 536 hours at 68°F (20°C) for the collected maggots to



Calliphora vomitoria

become adult flies. A literature search revealed that this particular species of fly has a life cycle (from egg to eclosion) of 555 hours at a constant temperature of 27°C, and that it prefers shady places and has a low threshold temperature (temperature below which this fly is not active).

The forensic entomologist now needed to know the temperatures that the corpse experienced during its stay under the oak tree. A remote weather station was located about 3 miles from the site of the corpse. As with most such stations, it recorded maximum and minimum temperatures each day. These temperatures are shown in Table 13.6.

Table 13.6: Weather Station Log

Silver Falls Weather Station [NWS-467R] Temperature Log for September 2007			
Temperature, °F			
Date	Maximum	Minimum	Median
Sept 1	76	62	69
2	78	66	72
3	79	69	74
4	80	72	76
5	78	70	74
6	77	71	74
7	76	70	73
8	74	68	71
9	74	66	70
10	76	68	72
11	75	69	73
12	76	68	72
13	74	64	69
14	76	68	72
15	78	68	73
16	76	66	71
17	74	62	68
18	72	60	66
19	70	60	65
20	70	58	64

Now, how does our forensic entomologist estimate PMI? The first step is to convert the available data to accumulated degree-hours (ADH).

1. published life cycle: $555 \text{ hours} \times 27^\circ\text{C} = 14,985 \text{ ADH}$
2. lab study: third instar to eclosion: $536 \text{ hrs} \times 20^\circ\text{C} = 10,720 \text{ ADH}$
3. thermal energy needed from eggs to third instar: $14,985 - 10,720 = 4,265 \text{ ADH}$

The average median temperature for the first 20 days of September = 70.9°F (21.6°C); therefore:

4. $4,265 \text{ ADH} / 21.6^\circ\text{C} = 197 \text{ hours}$ (8 days, 5 hours), the amount of time since the bluebottle flies deposited their eggs on the body. This would be September 8 at 10 AM.

But the body was in the woods for about eight days before it was found; thus, the average of the median temperatures from September 8 to September 16 should be used. Will this make a significant difference?

Some entomologists subtract the base or threshold temperature before calculating ADD or ADH, but the species of fly must be known, and even then the reading is an approximation. When temperatures are close to the lower limit, this calculation can become important.

All these calculations do not account for the time it takes for flies to find a body. Sometimes they arrive within minutes, depending upon the location, amount of blood, and temperature. So calculations such as these give only the minimum PMI. What other variables might skew the results?

- The weather station was 3 miles from the corpse. Would the temperature have been the same at both places? Perhaps the station was in a sunnier location. To check, our forensic entomologist would have left a temperature recorder at the site for 4–5 days and then compared its readings to those from the weather station. Suppose the site temperature readings were as follows:



A remote weather station

Teacher Note

The answer is no. Using the more accurate median temperatures makes the approximate time of death two hours later, September 8 at 12:00 noon.

Date, September	Max Temp, °F	Min Temp, °F
17	72	60
18	70	58
19	68	58
20	68	56

Teacher Note

It would make it earlier in the day by 9 hours, or 3:00 in the morning. Most flies are not active at night. Had oviposition occurred before twilight on the 7th, the life cycle would have begun then; more than likely, the flies found the corpse at first light on the 8th, so best estimate of TOD is between 8 PM, 9/7, and 6 AM, 9/8. There is an exercise for determining PMI using ADH at www.nlm.nih.gov/visibleproofs/education/entomology/index.html. Ward's Natural Science has a nice lab activity on the same subject: "Critters on Cadavers."

A mass of maggots feeding on a corpse sounds like a bowl of Rice Krispies after pouring on the milk.

How would these readings affect the estimated time of death?

- The time for the first fly to find the body was an estimate. The more blood, the faster the flies' arrival.
- The part of a body exposed is a factor; for example, the liver, heart, and lungs are choice sites for flies. Burn victims also attract flies more quickly.
- Sometimes clothes can retard colonization. How about insect repellent? Burial, water, and plastic coverings may also delay oviposition.
- Maggots feed in masses that generate their own heat. It has been recorded that the temperature of a maggot mass can be well above ambient temperature. This would tend to accelerate decay.
- Toxins and drugs consumed by the person can sometimes accelerate, sometimes retard decomposition. Indeed, traces of drugs have been found in maggots feeding on bodies. We will explore this topic later.

Habitat

Fly species can vary geographically according to climate, season, and habitat. For example, in the summer, green blowflies of the genus *Phaenicia* are common, whereas in the fall, bluebottle flies, *Calliphora*, are prevalent. Insects found in urban settings, such as *Calliphora vicina*, may differ from those in the countryside, like *Calliphora vomitoria*. Some fly species prefer warm weather, such as the hairy maggot blowfly *Chrysomya rufifacies*; others are more active at cooler temperatures. Some like sunlit areas, like the greenbottle fly *Lucilia illustris*; some prefer shade, such as the black blowfly *Phormia regina*. (See Figure 13.7.) Some flies are not active at night. An experienced forensic entomologist can use this knowledge, for example, to corroborate field evidence, infer postmortem movement of a corpse, or even infer prior burial, freezing, or wrapping of a body. Each case is unique due to the high number of variables involved.



a) *Phaenicia sericata*



b) *Chrysomya ruffacies*



c) *Lucilia illustris*



d) *Phormia regina*

Figure 13.7 Flies

Fly Infestation as a Function of Habitat

Laboratory Activity 13.4

Do different flies have different preferred habitats? Are there different fly species in your testing area? Can you identify them?

Materials

- raw beef liver, 30–50 g
- plastic container, 1 qt or larger, with tight-fitting lid
- sand or vermiculite (the latter is better)
- thermometer
- masking or duct tape
- killing vial with isopropanol
- ruler
- stereomicroscope
- labels and marking pen
- forceps or tweezers
- flypaper trap

Laboratory Activity 13.4, *continued*

Teacher Note

Larvae to the inexperienced observer without a good microscope all look about the same. However, the posterior (big) end of a maggot has distinctive features that differentiate species as well as identify the stage of development.

Fly traps should be rigid, and can usually be found in a hardware or super store. Cut a 2-inch square and mount it vertically near the container.



Trap and rearing container (flypaper stuck to outside of container)

Procedure

1. Punch holes in the sides of the plastic container to allow access to flies while keeping rainwater out. Paper-punch-size is good.
2. Place the meat on a crumpled, wet paper towel, then place both on the sand or vermiculite.
3. Insert the thermometer through a hole in the side of the container into the sand.
4. Place containers outside, one on or near the ground in a consistently shady area, another in a sunny area.
5. Anchor them by using long nails punched through the bottom of the container into the soil or by some other means that won't inhibit sampling, like with a rock on top.
6. Another container should be placed outside on a windowsill. Try to anchor this one also so that birds won't disturb it.
7. Another container can be placed inside, away from any windows; under an exhaust hood is a good place, as the container will smell.

8. Place a fly trap near each habitat; collect any flies, examine them, and compare them with those that have hatched.
9. Record temperature and insect activity each day as often as you can, starting when each container is first put out. A data logger can record temperature and humidity hourly.
10. Look for eggs, maggots, and pupae, taking a sample at each stage of the life cycle and immersing it in alcohol so that it can be measured and described. Remember that the prepupal and pupal stages will be in the vermiculite.
11. Calculate degree-days/hours for each size or stage.
12. Any time after maggots become apparent, cover the holes in the container with tape so the hatched flies cannot escape.

Analysis Questions

1. Identify the flies from identification cards or photographs.
2. Compare the data with those from the other habitats. Explain any differences.
3. Why can't identification be made from larvae alone?

Beetles

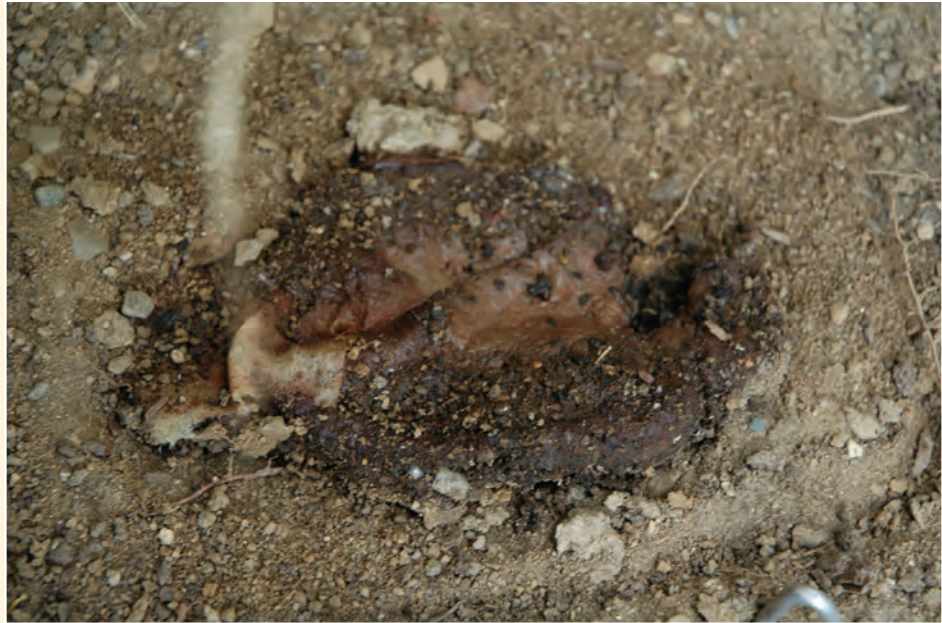
As mentioned earlier in the chapter, flies arrive first at a corpse; then the maggots take over and stay until most of the soft, mushy parts of the body are consumed. Some beetles (Table 13.5) may appear soon after death to feed on the maggots. As the body dries, different families of beetles arrive. They are generally nocturnal and may be found under the remains.

Beetle Infestation of Carrion

Laboratory Activity 13.5

Materials

- slab of raw liver, 20–40 g
- plastic container, 1 qt with a lip
- nails
- stereomicroscope
- forceps or tweezers
- killing vial with alcohol
- ruler



Liver after several days

Procedure

1. Place the liver on the ground and cover it with the plastic container turned upside down. Punch holes in the sides to allow access to flies. Insert nails through the lip into the ground to anchor it and protect the sample from predators.
2. Allow the container to sit for a few days before removing it.
3. Note any activity on top of the meat before gently lifting it up to observe what may be going on underneath it. Be sure to check in the soil also.
4. Continue your observations for several weeks. We are more interested in the activity of beetles than of flies and their larvae.

Some beetles feed on fly larvae (like the Staphylinidae), some on carrion (Dermestidae), some on both larvae and carrion (Histeridae and Silphidae), and some on other beetles (Cleridae and Carabidae). Capture different arthropods and kill them in alcohol for examination and identification.



a) Rove beetle, Staphylinidae



b) Dermestid beetle, *Dermestes maculates*



c) Clown beetle, *Hister nomas*



d) Sexton beetle, *Nicrophorus hybridus*



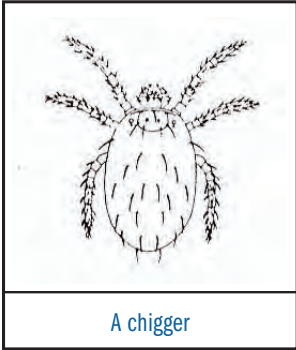
e) Red-legged ham beetle,
Necrobia rufipes



f) Hide beetle, *Trox* sp.

Figure 13.8 Beetles

Other Uses of Insects in Forensic Science



- The presence of wounds can often be observed by maggot activity away from the usual body orifices. For example, activity on the forearms and palms of the hands would imply defensive wounds, probably from a knife attack.
- Insects can place a suspect at the scene of a crime. For example, a murder suspect had chigger bites like the ones investigators got at the crime scene. The chiggers were found to be localized to that one area, yet the suspect denied he had ever been there.
- Contraband trafficking can sometimes be traced by identifying trapped insects. For example, marijuana shipments seized in New Zealand contained nine species of Coeloptera (beetles) and Hymenoptera (bees, wasps, ants). Only one of the species was native to New Zealand. The others were found to live in other specific areas and habitats, allowing for location of a probable source of the marijuana.
- The species of insects plastered on an automobile radiator allowed investigators to refute a suspect's alibi.
- The presence of drugs in a body can sometimes be detected by harvesting and testing the feeding maggots.
- In civil cases, insects found in stored food products or clothing can cause considerable discomfort as well as monetary losses. Structural entomology involves damage to buildings such as from termites and carpenter ants.



A carpenter ant

Flesh-eating insects have been found to have drug residues in their flesh as well as in their **puparia**. This allows examiners to sample for drug use in a corpse long after stomach contents, urine, and blood have disappeared. This activity simulates such a drug analysis and explores the effect of certain chemicals on metamorphosis.

puparia: cases formed by the hardening of the last larval skin in which the pupa is formed. Color starts out white and turns brown with time. Singular: puparium.

Materials

- Ground beef, 40–60 g
- Fly pupae
- Benadryl (diphenylhydramine hydrochloride), ferric chloride [$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$], potassium ferrocyanide [$\text{K}_4\text{Fe}(\text{CN})_6$], potassium thiocyanate [KSCN], 2 percent cobalt thiocyanate solution [$\text{Co}(\text{SCN})_2$]
- clear plastic container, 1 qt, with a few small holes punched in the top, or cover the top with a screen
- vermiculite or sterile sand (heated above 100°C)
- spot plate
- Beral pipette
- a small killing vial with alcohol

Teacher Note

See pages 191–193 in Chapter 7 where many of these chemicals were used.

Procedure

The teacher may assign one of the four “drugs” to each investigative group and the control (no “drugs”) to another group.

1. Make up a small amount of a saturated solution of one of the chemicals listed above. Mix about 1–2 cc into the sample of ground beef. Mix it well to evenly distribute it.
2. Place the meat on a moist, crumpled paper towel in the plastic container on top of about $\frac{1}{2}$ inch of vermiculite or sand. Add water and sugar as described in Activity 13.3.
3. Label each container.
4. Add ten or so pupae.
5. Place each container in a warm spot.
6. Observe what is happening. When you are sure that the flies have laid their eggs or if maggots can be seen, let the flies out and remove the sugar and water. If the meat dries out, sprinkle a little water on the towel.



A pupa

Laboratory Activity 13.6, *continued*

Teacher Note

Flinn Scientific has a nice lab activity simulating insects feeding on drugs in a body.

7. When the larvae reach the post-feeding stage (third instar) and are found in the vermiculite or sand, drop half of them into the killing vial. Allow the remaining ones to reach eclosion. Later, harvest the empty pupae and test for drugs in the same way that you did for the larvae.
 8. Crush the larvae in the vial, and allow the alcohol to evaporate.
 9. Add a small amount of hot distilled water and mix.
 10. Take up a few drops of the maggot soup and place it in a well of the spot plate. Each investigative group should also add its sample to the spot plate. Color spot tests will be used to confirm the presence of the “drug.”
- Benadryl reacts with cobalt thiocyanate solution to form a deep blue precipitate; ferric chloride reacts with potassium thiocyanate solution to make a bright red color; the thiocyanate reacts with ferric chloride solution to make a red color; potassium ferrocyanide reacts with ferric chloride solution to make a deep blue color.
- The control should be placed in four separate wells of the spot plate and tested with each of the four reagents. No color should show.

Analysis Questions

1. Record the results of the study in your notebook, and assess any problems that occurred.
2. Which test gave the best results? Why?

Collection of Evidence

A description is needed of the habitat, and of whether conditions are sunny, shady, or cloudy.

Climatological data at the site would include temperature, humidity, evidence of rain, and weather data for a period spanning from 1–2 weeks before the victim’s disappearance to 3–5 days after the body is discovered. Temperature should be recorded of the ground by the body and beneath the body, and of the maggot mass.

All the different types of insects on the body, in the body, beneath the body, and flying over the body should be collected and labeled. Collection of the largest larvae is most important. Why? Live maggots should be packed carefully so that they can be reared in order to identify them and determine degree-days.

Figure 13.9 summarizes collection requirements at the location of the corpse.

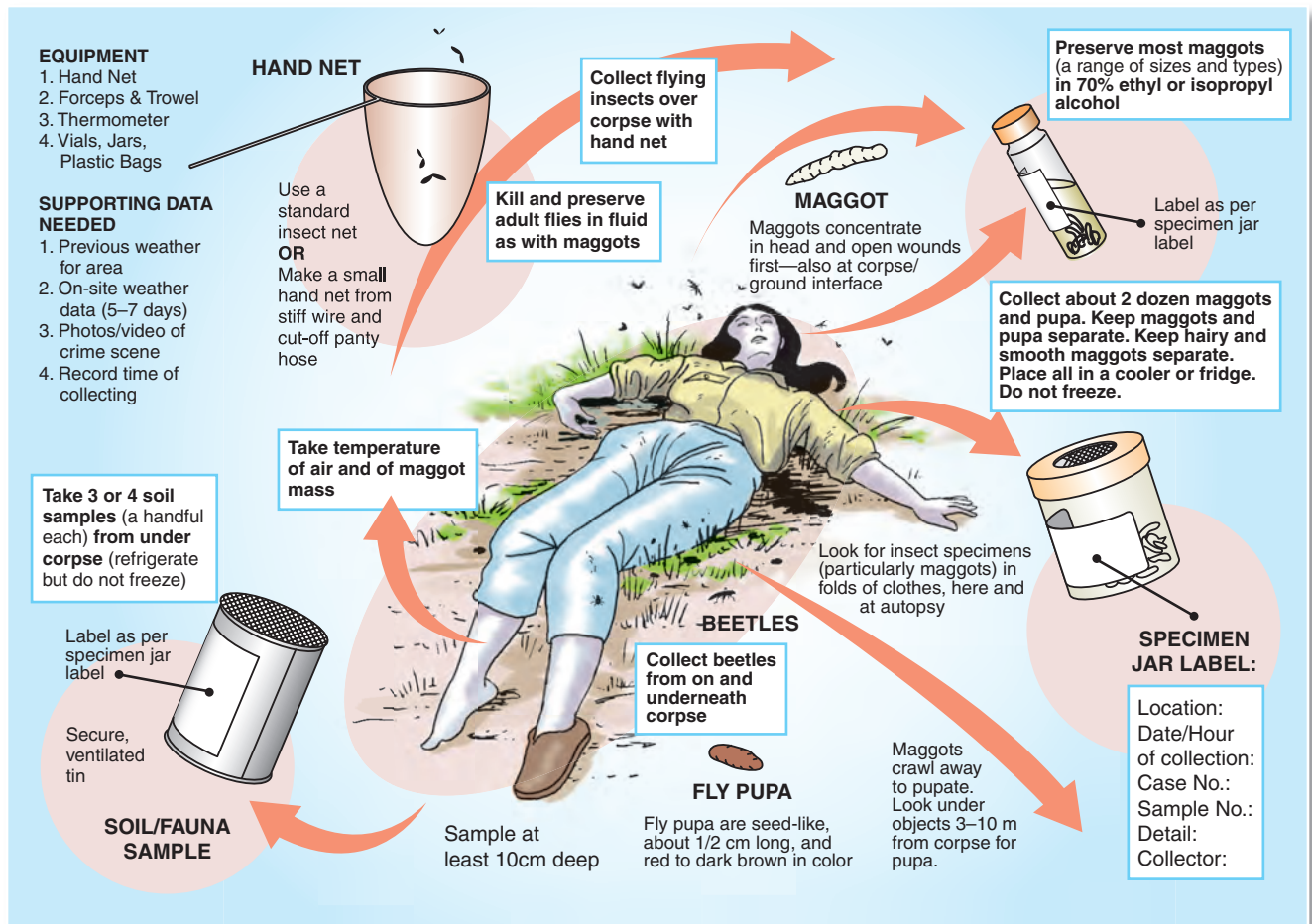


Figure 13.9 Collecting insects for forensic investigations

New Developments in Forensic Entomology

It must be remembered that the PMI is an estimate based upon many variables; not all experts agree. See, for example, www.courtvtv.com/trials/westerfield/timeline/time_of_death.html and www.timeshighereducation.co.uk/story.asp?storyCode=151843§ioncode=26, for two murder trials where very famous forensic entomologists disagreed on the PMI. Clearly, more research needs to be performed.

DNA is an important tool in many areas of forensic science, forensic entomology notwithstanding. DNA fingerprinting of adult fly species can be used to identify eggs and early-instar maggots. Computer modeling of the life cycle of flies may decrease the effect of the number of variables and unknowns in estimating PMI. A forensic entomologist named Arpad Vass has been analyzing the chemicals released into the soil and air from aging corpses in an effort to refine the PMI. A trend is also apparent in

forensic entomologists joining forces with botanists and anthropologists to evaluate evidence at the scene of death.

On a shorter time scale, research is aimed at improving the measurement of relevant body temperatures in algor mortis. Also, there may be a relationship between the electrical conductance of tissue and the time since death. At one time it was thought that the potassium level in the vitreous humor of the eye was related to postmortem interval; now, mathematical modeling may be used to refine this method of measurement.

As with most new developments in forensic science, the *Daubert* ruling must be satisfied. This is especially true in forensic entomology.

13.3: PMI Determined from Photographs

In the summer of 1976, Chicago police raided the apartment of three suspected drug dealers. These men figured that two men living below them had turned them in, so they killed them. Their bodies were dumped in the basement of an abandoned building, one inside a trunk and the other wrapped in a blanket and carpeting. The suspected drug dealers were charged with murder.

Three years later, when the case was finally ready for trial, the state's attorneys needed to corroborate the time of death. A professor from the University of Illinois, Bernard Greenberg, was called on to help. But all he had to work with were a few black-and-white photographs that showed the victims covered with post-feeding larvae and a few pupae. The pupae were light-colored, implying that they were fresh, not old. Dr. Greenberg surmised that the insects were *Phaenicia sericata* because it was a common carrion fly in urban Chicago. He performed research to obtain ADH data for the development of the life cycle of several fly species at four different temperatures. He then used temperature data from the nearest weather station to the crime scene at the time of the crime in order to adjust his ADH data. He calculated the time of death to within $2\frac{1}{2}$ days of the actual shooting—from three-year-old photographs and his research. His testimony at the trial confirmed that of one of the state's key witnesses (one of the three drug dealers—not the most reliable of witnesses). The men were convicted of murder and sent to prison.

A similar case occurred in 2006. In 1959, Stephen Truscott, then 14 years old, was convicted of murdering his schoolmate and friend,

CASE STUDY



Abandoned building

Lynne Harper. Evidence consisted of an erroneous eyewitness account of where they were last seen together and the pathologist report that death occurred precisely between 7:15 and 7:45 PM, based on stomach contents. Truscott was convicted of murder. His death sentence was later commuted, and he was released on parole after serving ten years.

Forty-seven years later, a forensic entomologist from Michigan State University, Prof. Richard Merritt, examined photographs of the body and data on the maggots' measurements that were recorded at the time. From the size of the maggots, he was able to conclude that Harper's time of death was *not* in the early evening before dark when Truscott was last seen with her, but after dark or the next morning when Truscott was home or in school. As a result, his conviction was set aside, but the court was not able to declare his innocence because of legal technicalities.



Dr. Richard Merritt

Answers

1. amino acids
2. Refer to Vass's work on p. 401.
3. No, there is an interval between death and the first oviposition that can naturally range from minutes to even days under certain circumstances. Freezing, burial, and drowning are types of conditions that can also delay infestation.
4. Diptera and Coleoptera
5. Run the experiment keeping two of the variables constant and look at the outcome. Repeat, always keeping two of the parameters constant.
6. One would expect to separate, say, screws by type (wood, sheet metal), then description (flat head, round head), then material (steel, brass, chrome), then size.
7. The answer depends on experimental results.
8. Egyptian burials, "bog bodies," Inca mummies of Peru, Oetzi, Col. Shy
9. Egg, larva, pupa, adult. The time required for each stage can be used to estimate PMI from the insects found on the corpse.

Checkpoint Questions

Answer the following questions. Keep the answers in your notebook, to be turned in to your teacher at the end of the unit.

1. On page 380, some of the chemicals that contribute to the odor of a decomposing corpse are listed. A living person does not contain such compounds. Where do they come from, i.e., what is the starting material?
2. Why is it important to know what chemicals are produced in the body as decomposition proceeds?
3. The time of colonization is used to describe insect activity in a body. Is this the same as the postmortem interval? Explain.
4. What are the two insect orders most commonly found on a decaying corpse?
5. In an experiment, you know that you have to deal with three variables. How do you proceed?
6. Prepare a dichotomous key for sorting and identifying a set of common objects, such as screws or paper clips.
7. Was the "potato corpse" activity (page 377) representative of algor mortis? Explain.
8. Sometimes remains are preserved on purpose, sometimes by accident. Name at least two examples of each.
9. What are the four principal stages of metamorphosis of Diptera? Why are they important to forensic entomology?

10. A body is found still warm, but with rigor mortis. What is the estimated time of death? How could you be more exact?
11. Why is it important to know what kinds of fly larvae inhabit a corpse?
12. What are the variables that affect the period of development of a particular species of fly?
13. What do beetles have to do with estimating the PMI?
14. The ADD for the complete life cycle of *Sarcophaga bullata* has been observed to be 37 days at 27°C. What would it be at 18°C?
15. A fully clothed male was found, facedown, in the basement of a house at 8 AM on July 17. There was a maggot mass in his back, and infestations were apparent in his mouth and nose. The temperature in the basement was 70°F. The house was closed up,



Photograph of a fly reared from the corpse

Answers, continued

10. Dead for 3–8 hours. Take the temperature; use the Glaister equation.
11. Different species of flies require different times to develop, which is related to determining PMI.
12. temperature, drugs, predators
13. As with flies, there is a somewhat predictable succession of beetles devouring a corpse or its inhabitants, depending on the stage of death; thus, a time sequence can be established.
14. 55½ days: $(37 \times 27)/18$
15. It took 36 hours at 75°F (23.9°C) to raise eggs to 12 mm; $36 \times 23.9 = 860$ ADH. Dividing by the temperature in the basement of 70°F (21.1°C) gives 41 hours for the oldest larvae to grow from oviposition to 12 mm. Counting 41 hours back from 8 AM on July 17 gives a TOC of 3 PM, July 15. The PMI was estimated to be about 15 minutes earlier because it was noted that there were flies in the house, so it would not have taken long for them to smell the blood. The insects are *Musca domestica*, the common housefly with its characteristic four stripes on the thorax (see p. 371, question 15, and Figure 13.6, p. 388), and a sexton beetle, which looks very similar to the one in Figure 13.8. Normally, a blowfly would find the corpse first if it were outside, but the houseflies had the advantage in the closed house. The Silphidae beetle is an early visitor to a corpse, feeding on both larvae and carrion; the dirt floor would allow it to live indoors. The development time for the flies was consistent with that of *Musca domestica* (see Figure 13.5). The maggot mass in the back of the victim implies a wound in the back, which would be evident at autopsy.

Answers, continued

- 16.** The arsenic embalming and the lead-lined coffin retarded decomposition—interesting in that both elements hasten death. Egyptian mummies were eviscerated and dried out with natron. Some corpses were said to have been “pickled” in alcohol to preserve them. In modern times, bodies are kept frozen with liquid nitrogen in the hopes that they may be “revived” in the future.
- 17.** the species of fly; weather conditions, especially temperature; time of day the body was found



Photograph of a beetle under the corpse

yet there were a lot of houseflies buzzing here and there. The forensic entomologist collected about 40 of the largest maggots, from 10–12 mm long. She did not see any puparia; however, there were two beetles under the body. The temperature in the basement was monitored for the next five days and found to be very stable at 70°F.

Back at the lab, half the maggots were placed in an incubator at 75°F at 9 AM. Flies started emerging from their pupae about 7½ days (180 hours, to be exact) after incubation of the larvae. The forensic entomologist identified the fly and let it run through its life cycle. She found that it took 36 hours to grow larvae 12 mm long.

What did she estimate the time of colonization (TOC) to be? The PMI? Identify the two insects shown. Is their presence consistent with the estimated PMI? Explain all your reasoning as if you were in court.

- 16.** Why was the body of Col. Shy so well preserved? What other methods of preservation have been used on bodies?
- 17.** Dr. Merritt used data on the size of the maggots on Harper’s body to estimate PMI. What else would he have needed in making his estimate?

Additional Project

It is known that maggots, even puparia, will contain drug metabolites as a result of feeding on the drug user's body. Does this apply to beetles that devour body parts during the later stages of decomposition? Follow an appropriate procedure, but substitute for the rearing medium a portion of dried roadkill, pigskin, a dried-out pork chop, or a piece of leather. Pierce it while it is being soaked overnight in a solution of the "drug" that worked the best in Laboratory Activity 13.4. Oven-dry it and place it in a container with dermestid beetles that your teacher has supplied. Add some wood chips or pieces of bark and a small piece of cotton or filter paper that has been dampened. After the dried meat has been consumed, harvest some of the beetles and test for a color reaction.



Dermestid beetle

References

Books and Articles

- Bass, J. *Carved in Bone: A Body Farm Novel*. New York, NY: HarperCollins, 2006.
- Catts, E. P., and N. H. Haskell. *Entomology and Death, a Procedural Guide*. Clemson, SC: Joyce's Print Shop, 2000.
- Goff, M. L. *A Fly for the Prosecution*. Cambridge, MA: Harvard University Press, 2000.
- Sachs, J. S. *Corpse*. New York: Basic Books, 2001.
- Sachs, J. S. "A Maggot for the Prosecution," *Discover*, November 1998, p. 103.
- Gannon, R. "The Body Farm," *Popular Science*, September 1997, p. 77.
- Schoenly, K. G., N. H. Haskell, D. K. Mills, C. Bieme-Ndi, K. Larsen, and Y. Lee. "Using Pig Carcasses as Model Corpses," *The American Biology Teacher*, Vol. 68, 2006.
- Carloye, L. "Of Maggots and Murder: Forensic Entomology in the Classroom," *The American Biology Teacher*, Vol. 65, 2003.

Websites

- www.aronline.net.au/insects/insects/resources.htm#forensic; many good links
- www.forensicentomology.com/introduction.htm; a very complete exposure to forensic entomology
- <http://research.missouri.edu/entomology/>; more links
- www.nhm.ac.uk/nature-online/life/insects-spiders/webcast-forensicentomology/forensic-entomology.html; video explaining forensic entomology