10.2. Forensic Entomology: Mute "Witnesses"

Learning Goals and Objectives

Forensic entomology has been used in a variety of ways in forensic investigations. In order to understand the potential uses and limitations of the insect life in legal proceedings, you will need to demonstrate an understanding of:

- the general features, classification and morphologies of insects,
- the three areas of study of forensic entomology,
- how insects form a part of the complex ecosystem of decaying remains,
- the features of the different stages of decay after death,
- what factors affect insect colonization and development in remains,
- what are the features of insect succession in the decomposition of a body,
- what are the stages of insect growth and development,
- how to estimate the post mortem interval (PMI) from insect life.

Background and History

Since all organisms on the Earth are part of a highly interactive biosphere, it's not surprising that forensic science has begun to look carefully at the relationships both between organisms with their environments in the hopes of finding useful criminalistic information. Nowhere has the development of this idea been clearer than in the field of entomology, to the point that it is now a powerful investigative tool.

Our living world is divided most broadly into five general groupings or kingdoms into which all organisms fit (taxonomy): animals, plants, fungi, protists, and monerans (types of bacteria and their relatives, Figure 10.1.12). This system has arisen from an attempt by scientists to organize and understand both the similarities and differences among all living things. The largest and most complex of these broadest groupings is the animal kingdom. Within the animal kingdom, organisms are known that range in size from just a few cells linked together to humans to the massive blue whale. The most diverse form of animal life, in fact the most diverse and numerous of all life forms, is found in a subgroup (phylum) referred to as the arthropods. This diverse phylum contains the crustaceans, spiders, insects, and their relatives. It is within this grouping that forensic entomology is focused.

So what is forensic entomology? First, entomology is the field of science that deals specifically with the study of insect life. Insects are truly and undisputedly the most successful branch of organisms in the animal world, with well over 900 thousand varieties known, representing over 80 percent of the Earth's known species of living organisms. But this seems to be just the tip of the iceberg. Scientists estimate that less than one-half of all insect...
species have been so far identified, with estimates ranging from 2 to 30 million for the true number of insect species on the planet. Insects probably also represent the largest biomass on the planet, with an estimate of $10^{19}$ individual insects alive at any given time or about 200 million insects per person.\footnote{This has been calculated to equate to about 300 pounds of insects per person.} Two recent studies have estimated that one acre of soil in North Carolina would contain about 124 million insects and a study acre in Pennsylvania could contain as many as 425 million individual insects - a truly astonishing number.

Historically, insects have been dominant on Earth, beginning with their first appearance over 400 million years ago. Some insect species, such as the cockroach, have remained relatively unchanged for hundreds of millions of years.

Insects are defined as having three pairs of legs and segmented bodies that are divided into a head, thorax, and abdomen (Figure 10.2.2). They are relatively small, air-breathing arthropods that often have true wings (not modified limbs as are found in birds and bats). Adult insects have their skeletons on the outside in the form of an exoskeleton, mostly made of a hard material called chitin (a polymeric sugar molecule). Most insects hatch from eggs and go through a series of stages (molt) before finally reaching their final adult form. The insect phylum ranges from the ants and earwigs to butterflies, dragonflies, and beetles but it does not include many species commonly called insects, such as spiders, scorpions, and centipedes, among others. While these latter organisms do have some similarities with the insects, such as segmented bodies and exoskeletons, they do not have the six legs required to be classified as an insect.

Insects occupy almost every climate, region, and environment in the world. The fly, burrow, walk, crawl, and swim. Every aspect of human life is intimately intertwined with insect life, from the moment of birth to well past the point of death. Insects are responsible for much of the food we eat, the products we consume, and ultimately for the process of recycling dead organisms to provide the basic resources needed for the next generations of life. All of these processes, however, can also provide valuable forensic information to a variety of investigations.

Forensic entomology uses what is known about insects to answer questions of legal importance such as time of death, product storage integrity, drug and toxin identification, and the connection of crime scenes with suspects and victims. The use of insect information in legal settings...
has a very long history. As far back as at least the mid-thirteenth century in China, the value of the insect record in law was appreciated. A Chinese judge named Sung T'zu wrote a book in 1247 called "The Washing Away of Wrongs" that is the oldest known handbook of forensic science for judges

Case History: Dr. Ruxton and the Flies

On September 29, 1895, two young women were gazing over the side of a small bridge near Edinburgh, Scotland, when they noticed what appeared to be a human leg. The police were called and after carefully examined the vicinity, they found the remains of two people "surgically" cut into many small fragments, some wrapped in newspaper, and most badly decomposed.

The bodies were later identified as those of Mrs. Isabella Ruxton and her housekeeper, Mary Rogerson. The identity of Mrs. Ruxton was aided by overlaying a scale photo of Mrs. Ruxton onto a similar scale photo of one of the recovered skulls (left).

The prime suspect in the murder of Mrs. Ruxton was her husband, Dr. Buck Ruxton. Dr. Ruxton, formerly Gabriel Hakim from India, first arrived in Scotland in 1927 to attend medical school. He soon married Isabella Kerr and, by 1935, they lived with their children and housekeeper in Lancashire, England, 100 miles south of where the bodies were found (a place now locally known as "Ruxton's Dump"). Dr. Ruxton, who was known to have a particularly violent temper and a deeply suspicious nature, frequently suspected his wife of infidelity. Police were often called to the Ruxton household to settle family disputes and do we really calm threats??? Although on a personal level I like the feel of the phrase. I'm just not sure calm threats made by Dr. Ruxton against his wife. On one occasion he even told the police officer "Sergeant, I feel like murdering two persons …. my wife is going out to meet a man."

The prosecution built it's case upon the plausible scenario that Dr. Ruxton had confronted his wife with charges of adultery after a trip that she had made alone to Edinburgh. The confrontation turned violent and he killed his wife. Unfortunately, their housekeeper, Mary Rogerson, must have witnessed the murder and was also killed by Dr. Ruxton to silence her. Scientific evidence had carefully identified the bodies and determined that they were surgically dismembered to try to remove all traces of their identities, including the removal of fingerprints. In investigating Dr. Ruxton, bloodstains were found throughout the Ruxton home - he had even asked a patient to help clean up the mess. But the prosecution's case needed to accurately determine the time of death in order to convict Dr. Ruxton. For this, they turned to forensic entomology. Dr. A.G. Mearns identified a species of blowfly (Calliphora vicina) on the remains and determined their stage of development. He was able to say that Mrs. Ruxton had died between 12 to 14 days before her body was recovered. This evidence agreed with other scientific testimony and led directly to the conviction of Dr. Ruxton for murder. After an unsuccessful appeal, he was hanged in 1936 in Strangeways Prison in Manchester, England.

(Photograph at left of superimposed photographs of Mrs. Isabella Ruxton and skull no. 2, 1935. Investigators laid a photo-transparency of this skull over Mrs. Ruxton's portrait to establish that the skull belonged to Mrs. Ruxton. from www.nlm.nih.gov/news/press_releases/visibleproofphotos.html. Credit, University of Glasgow. Photo at right is of Dr. Buck Ruxton from heritage.scotsman.com/myths.cfm?id=2418212005).
attempt to identify the assailant, he had all the suspected field workers line up with their scythes and stand in the hot noonday sun ... and he waited. Before long, the scythe of only one man had attracted a large number of flies, presumably from invisible remains of human blood and tissue on the scythe. When confronted with the evidence, the man confessed to the crime and "knocked his head on the floor". This is the first recorded use of forensic entomology to quite literally "sniff out" a murderer.

Since the time of Sung T'zu, the use of insects in legal settings has made slow but steady progress. One important step came in 1626 when Francesco Redi (Figure 10.2.3) showed that maggots (larvae of flies) did not "spontaneously arise" from the organic matter in rotten meat but only came from eggs deposited by adult flies on the meat.

A landmark case came in 1850 when the body of a small infant was found behind a fireplace mantle-piece in an apartment building near Paris. Since the apartment had been occupied continuously by four different sets of tenants in the preceding few years, it became important to determine how long ago the infant died in order to suggest a possible suspect. A local physician with forensic interests, Dr. Bergeret d'Arbois, was called in. By carefully determining the insect activities and the state of the remains, he was able to conclude that the body had been there since 1848, clearing one set of tenants and throwing suspicion on others. Later, in 1894, J. P. Mégnin published the first textbook on how entomology could be used in forensic investigations ("La Faune des cadavres: Application de l’entomologie à la médecine légale"). In the first half of the twentieth-century, a number of important scientific studies of the lifecycles of insects, especially flies, provided a necessary foundation for understanding what a forensic insect record could tell (Figure 10.2.4). Interest really took off, however, in the 1970s with a series of cases in which insect evidence was used to great effect in several murder investigations. Since then, forensic entomology has developed into a well-respected and often critically important forensic tool.

**Forensic Entomology**

Forensic entomology involves the use of insects in law and has been broken down into three distinct areas of study; urban, medicolegal, and stored product issues. Urban entomology is focused upon the relationships between man and insects, most often centered upon insect pests and their eradication. This area includes considerations of the damage, disease, and elimination of insect species such as termites, mosquitoes, wasps, fleas, ticks, and many other "pest" insect species. Medicolegal uses of entomology tend to focus upon insects that feed directly upon human remains, often called necrophagous insects. This type of insect data can provide critical information about the time of death, the cause of death (including the presence of drugs and toxins), whether a body has been moved, and who might have been involved in the crime. Often, insects are the sole remaining "witnesses" of a violent crime, able to shed light on the sequence and timing of events (see "A Fly for
the Prosecution" by M.L. Goff). Interpreting this record correctly can provide insights that may help to either confirm or cast doubt on the testimony of suspects and victims. Analyzing insect life on a cadaver can also provide a much more accurate estimate of time of death than a medical examiner's estimate in the several days to several weeks range. This is often a crucial time period in understanding criminal events where medical pathology cannot provide definitive answers. The final area, stored product forensic entomology, deals primarily with insect pests that can contaminate and destroy commercial products, such as food, water, medicine, and building supplies. In this section, however, we will deal mostly with medicolegal and stored product uses of forensic entomology.

**Medicolegal Forensic Entomology**

The human body after death is an amazingly nutrient-rich but fleetingly ephemeral object. Its nutritional resources are vigorously contested by a huge variety of living organisms, each having carved out specific niches that gives them a competitive advantage for a particular aspect of the remains. Some thrive due to their early arrival on the remains, some flourish by focusing on certain tissue types for nourishment, and some succeed by scavenging the residue at the end. Other species prey on organisms that feed directly on the remains while yet other species use the body as a convenient environment to life.

Arthropods can, therefore, generally be placed into one of four groups of organisms that are part of the ecosystem of the remains:

1. **Necrophagous species** (species that feed directly on the remains) - e.g., blowflies, fleshflies, skin beetles, carrion beetles, etc.
2. **Predators and parasites** (feed on the necrophagous species, not the remains) - e.g., rove beetles, some fly species, etc.
3. **Omnivorous species** (feed both on the remains and the resident species) - e.g., wasps, ants, and some beetles.
4. **Adventive species** (use the remains as part of their habitat) - springtails, spiders (incidental predators), etc.

Some of species fall in different groups as they progress through their life cycles. For example, some fly larvae begin by feeding solely upon the remains (necrophagous) but become predatory at later stages of their development. Also, soil dwelling species that are not involved with the ecosystem of the remains are quickly leave the site, not to return for a significant time period.

Within a short time after death, a complex ecosystem is rapidly established on and around the body, with its own unique set of inter-species relationships and a recognizable progression of species that are successively active over the time of decay (faunal succession). In the end, the chemical resources contained within the body are efficiently returned to the biosphere to support new generations life - in this sense, insects perform an absolutely necessary biochemical recycling.
function for our planet. The critical part that insects play in this ecosystem and the profound effects of their action on animal remains are illustrated in Figure 10.2.5 in which the decomposition of an animal is shown both with and without exposure to insects.

A mammal, such as a human, undergoes a series of identifiable stages of decay after death. This predictable series of stages, shown for a pig in Figure 10.2.5, are often referred to as the fresh stage, bloated stage, decay stage, post-decay stage (or advanced decay stage), and dry stage. The time of onset and duration of each of these stages may be highly variable and depends upon a variety of chemical and environmental conditions, such as weather conditions (temperature, rainfall/snowfall, wind speed, humidity, sunlight, etc.), location (e.g., temperate, semi-tropical, arid locations, forest, apartment, urban, arid, etc.), position of the body (lying on the surface, underwater, partially buried, etc.), body composition (e.g., size, age, presence of drugs and toxins), and other factors. For example, the dramatic effect of the seasonal temperature and weather conditions on the duration of these stages is shown in Figure 10.2.7 - the entire first three stages takes less time to complete in the summer than just the first stage in winter. Also, the decomposition of bodies in freshwater may occur at less than half the rate.
of remains found on land. Some species of flies cannot penetrate to buried remains while others have been found in deeply buried coffins. Bodies located indoors or tightly wrapped with cloth or plastic may also be inaccessible for insect colonization. An understanding of the stages of decay is important to the accurate interpretation of insect evidence and is briefly summarized here.

The **fresh stage** of the decay starts at the point of death and may last up to several days. There may be few outward signs of change and the person may appear to be simply "sleeping". There are, however, very significant biochemical changes occurring during this stage at the cellular and organ levels within the body. The body first slowly cools to match the temperature of its surroundings (*algor mortis*). During this time, the cells begin to go through a carefully programmed death cycle that produces enzymes that catalyze the breakdown of proteins, carbohydrates, and fats in the body. The muscles stiffen from the chemical decomposition of glycogen and the formation of lactic acid (*rigor mortis*). The stiffening process is usually noticeable within 6 to 8 hours of death and may last two to three days, depending upon conditions. Bacteria proliferate and begin to further breakdown internal tissues and organs while producing gases as byproducts of the decomposition process (e.g., ammonia, carbon dioxide, hydrogen sulfide, and others). Ectoparasites, such as fleas, lice, and ticks, leave the body relatively soon after the death of their host organism. If environmental conditions are right and the body is accessible, blowflies are able to very quickly locate the body from extremely faint decay odors and deposit their eggs (*oviposition*) in body orifices. This can occur under optimal conditions within just a few minutes after death. The eggs are laid around the nose, ears, mouth, eyes, anus, genital openings, and any wound areas on the body. Location of this latter type of site, wound areas, can become very important since the locations of

**BRIEF ON INSECTS AND DECAY**

(1) Four types of species found on dead remains: those that feed directly on the remains (*Necrophagous*), those that feed on the necrophagous species but not the remains, (*Predators and Parasites*), those that feed both on the remains and the resident species (*Omnivorous species*), and those that use the remains as part of their habitat (*Adventive species*).

(2) Five stages of decay: fresh, bloated, decay, post-decay, and dry.

(3) Duration of stages depends on environmental and other factors.

(4) Insects often critical in the decomposition of remains.

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**Case History: The Fly Nose**

After a murder in southern Indiana, an attempt was made by the assailant to hide the body by putting it down a long-abandoned well shaft. He then filled the shaft with rocks, dirt, and tires, burying the body under the mountain of debris. He also hid the entire opening to the well by mounding up tires and junk. While investigators were searching another farm site for the missing person, they immediately noticed thousands and thousands of blowflies swarming over and around a mound of tires. This was clearly unusual. Upon excavation, they found the well and, ultimately, the body. While the blowflies could not directly reach the hidden body to lay eggs and continue the decay cycle, they were clearly attracted to the odor of the decay. (Story from en.wikipedia.org/wiki/File:Australian_sheep_blowfly.jpg).
"hidden" trauma on badly decayed bodies (not readily observable with normal physical examinations) can sometimes be found by noting where the insects have penetrated into the body, such as through gunshot or knife wounds. Eggs are also deposited in other dark and moist places such as in folds of clothing or skin or underneath the edges of the body. In order for the flies to be active and find the body, however, the air temperature must be within a relatively narrow range (too hot or too cold prevents their activity) and conditions must permit insect flight (e.g., it must not be too windy or raining too heavily to preclude flight). Certain species of flies are attracted preferentially to the remains of different animals - some insects prefer small mammals while others focus on human-sized remains. Some species also have additional requirements, for example, bluebottle flies strongly prefer shady settings while the closely related greenbottle flies prefer sunny locations. This difference is dramatically illustrated by the following quote from a World War I veteran "In the shade afforded by the deep portion of the trench, round the traverses, any moist patch of chalk wall would be hidden by a dense, indigo-coloured cluster of Calliphora [Bluebottle fly], large as a soup plate, whereas, where the trench was shallow or blown in [sunny locations], the green shimmer of Lucilia [Greenbottle fly] was everywhere" (See inset box “World War I Trench Entomology”). The species distribution may, therefore, indicate whether a body had been lying in direct sunlight or in more shady conditions. Blowflies also typically do not lay eggs at night so remains must wait until morning to be colonized.

Insects are also not immediately and equally attracted to all tissues and organs after death. For example, exposed liver (such as found at a slaughterhouse) typically remains untouched for about the first three hours while kidneys and entrails are usually attractive to flies immediately. This may be due to the fact that some tissues are too acidic for the flies immediately after death but become less acidic with time. The fresh stage ends when the buildup of gases becomes visible, leading to the bloated stage.

In the **bloat stage**, putrefaction begins and the large amount of gas produced from anaerobic bacterial action inflates the body cavities, especially the intestines and abdomen. This stage typically lasts from two to six days, depending upon environmental conditions. External insect activity also becomes very noticeable and active during this stage. The body begins to change dramatically in its external appearance, with pronounced discoloration caused from blood pooling at the bottom of the body due to gravity (livor mortis). During this stage, blowflies continue to lay eggs but other species, including flesh flies and cheese skippers, make their first appearance and begin egg laying and feeding. Feeding maggots are clearly visible on the outside of the body during this period. Maggots representing several stages of development, from freshly hatched to larger, later stages of growth, are found mixed together.

As mentioned before, the insects that inhabit dead remains have developed unique traits that allow them to compete effectively for the rich nutritional resources contained within the body. While blowflies are usually the first to arrive on site and lay eggs, the later-arriving flesh flies make up for...
the apparent disadvantage of their late arrival by depositing live larvae rather than laying eggs on the remains. Some species compensate by becoming predators on other larvae at later stages of their development. These competitive factors play an important role in the type (species), number and sizes of the larvae found on the remains.

The **decay stage** begins when the abdominal wall ruptures, allowing the built-up gases to escape and the carcass to collapse and flatten. This is an exceptionally busy time for insect life on the remains. While adult blowflies no longer visit, very large numbers of maggots can be found throughout the body, feeding both internally and externally. The number of maggots can become so large that the internal temperature of the body can be raised dramatically, often by tens of degrees C, from the combined heat generated by the **maggot mass**. The mass, in reality, creates its own microenvironment, causing changes in the developmental rates of the larvae themselves. Maggots from the inner portions of the mass may have to occasionally move to the edges of the mass to cool because the temperature in the center of the mass has risen too high. The large number of feeding larvae also attracts other insect species that parasitize or prey directly upon the larvae, such as wasps and fly parasites. Some wasp species, for example, lay their eggs directly within the fly pupae. When the wasp eggs hatch, they consume the fly larvae as food from the "inside". The decay stage may last about a week or more and continues until the moist tissues of the remains have been largely depleted, leaving mostly skin, cartilage, bones, hair, and teeth.

During next stage, referred to as the **post-decay** stage, the remains continue to dry out more fully. The larvae have consumed most of the soft tissues and have reached the developmental stage where they leave the body to pupate. New species arrive, including varieties of beetles, that continue the decomposition process. This stage can last several weeks or even months.

The final stage, or **dry stage**, can last for months or even years, depending upon body placement and environmental conditions. It is often difficult to use this stage to accurately determine the age of the remains, except within very broad ranges. Insects that have evolved mechanisms for digesting keratin (a tough, insoluble protein found in hair, fingernails, skin, and other resistant tissues) are the primary inhabitants of the body during this last stage. These include the dermestid beetles, clothes moths, and sap beetles. At the end of the process, very little besides bones and teeth remains of the body.

The variation of insect species through the stages of decomposition (faunal succession) provides valuable information about how long the body has been decomposing. Figure 10.2.8 and Table 10.2.1 lists some specific species found during the different decay stages. Representative

### Table 10.2.1. Selected Insect Arrival Times During Decay.

<table>
<thead>
<tr>
<th>Decomposition Stage</th>
<th>Example Insect Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>Blow Flies (<em>Calliphoridae</em>), Flesh Flies (<em>Sarcophagidae</em>), Horse and House Flies (<em>Muscidae</em>), Small Dung Flies (<em>Sphaeroceridae</em>)</td>
</tr>
<tr>
<td>Decay</td>
<td>Blow Flies (<em>Calliphoridae</em>), Flesh Flies (<em>Sarcophagidae</em>), Horse and House Flies (<em>Muscidae</em>), Rove Beetles (<em>Staphylinidae</em>), Cheese Flies (<em>Piophilidae</em>), Carrion Beetles (<em>Silphidae</em>)</td>
</tr>
<tr>
<td>Post-Decay</td>
<td>Rove Beetles (<em>Staphylinidae</em>), Cheese Flies (<em>Piophilidae</em>), Checkered Beetles (<em>Cleridae</em>)</td>
</tr>
<tr>
<td>Dry Stage</td>
<td>Sap Feeding Beetles (<em>Nitidulidae</em>), Skin Beetles (<em>Dermestidae</em>)</td>
</tr>
</tbody>
</table>

pictures showing each of these insect types are found in Figure 10.2.9. For example, finding dermestid beetles on the remains suggests that the body is in the final stage of decomposition while finding silphidae insects (Carrion Beetles) suggest an age placing the remains within the earlier decay stage. A careful understanding of the climate and the presence of any drugs is necessary, however, in order to interpret the timing. For example, the presence of cocaine, a stimulant, in the body can accelerate the entire larval development process dramatically. Also, bodies deposited outside during winter are typically not accessible to blow

*Introduction to Forensic Science*

Figure 10.2.10. Effect of burial (summer) on the decomposition of an animal (adapted from Nabaglo, L. *Ekologia Polska* 1973, 21, 251).
flies. In this case, beetles are often the primary colonizers of the remains without the usual pattern of insect faunal succession.

As mentioned previously, burial or submersion in water changes the decomposition characteristics of a body, often quite significantly. Burial virtually eliminates aerobic bacteria and many commonly found insect species associated with surface decay. Some species of bacteria and insects, however, are still able to reach and flourish on buried remains, depending primarily upon the depth of burial. The effects of burial, even from a shallow burial under an inch of soil, is to significantly lengthen the time necessary for decomposition relative to that for remains found on the surface, as illustrated in Figure 10.2.10. Finding blowfly larvae on buried remains can often indicate that the burial was not immediately after death but was delayed long enough for the blowflies to colonize the remains.

Submersion in water usually also results in different organisms (fauna) involved in the decomposition and may include crustaceans, caddis-flies, and other aquatic species, depending upon the local environment (salt or freshwater, water temperature, currents, etc.). If a body floats, the exposed parts can be colonized by typical surface species such as blowflies and beetles. When the remains sink, however, the fly larvae and beetles either migrate away from the body or are drowned. After sinking, the decomposition process is usually completed by bacteria, fungi, and carnivorous aquatic larvae.

The activity of vertebrate scavengers can also dramatically affect the sequence and timing of the decay process (Figure 10.2.11). By opening alternative pathways into a body or by scattering the remains, they may provide earlier than typical access of insects to the interior of the body. This process also usually causes the remains to dry out faster, leading to an earlier start of later decomposition stages than would normally be expected. Larger scavengers can also slow the process by partially or even completely burying or transporting the remains. Their action, however, can often be discovered by a careful physical examination of the remains (e.g., tooth marks, bone abrasions, unusual tissue shearing/tearing, etc.).

Different regions of the world show a diversity of species involved in the decay steps. It is important to know the insect fauna at the locale of any remains to accurately interpret the insect record. One of the most important pieces of information this record can provide is the length of time that the body has been dead.

**Estimating Post Mortem Interval (PMI)**

After a human body has been dead for more than just a few days, many of the tools that the medical examiner has available for estimating the time of death (rigor mortis, algor mortis, etc.) are
no longer useful. The most important time period that needs to be defined in a typical forensic investigation is referred to as the post mortem interval (PMI), or the length of time from the death of the organism to the time that the remains are found. By knowing the PMI, it is possible to calculate backwards from the time of the discovery of the body to determine the approximate time of death.

A determination of a PMI estimate starts with several reasonable assumptions. First, it is assumed that the flies have ready access to the body beginning at the time of death and that conditions are right for them to both find the remains and to deposit eggs quickly. To evaluate the validity of this assumption requires an understanding of the weather patterns at the time of death (temperature, wind speed, rainfall, etc.) and an estimate of the first point in time at which the flies had free access to the body. If access has been in some way limited, then the PMI needs to be adjusted accordingly. Access can be limited not only by weather patterns but also by underground burial, submersion in water, wrapping the body in cloth or plastic, or depositing the body in an enclosed space. For example, an investigation of a murder crime scene found evidence of a burglary, with the body found near the open window entry point. Pathology suggested a PMI of at least 24 hours but the insect life found on the body, however, suggested that it had not been exposed to the outdoors for very long at all, no more than just a few hours. It was later determined that the person reporting the crime had actually committed the murder on the previous day in a closed apartment (no insect access) but had subsequently returned to the crime scene the next day, opened the window, and set the scene to make it appear as though a burglary had occurred. The murderer then called the police to report the crime after resetting the scene. Once the proper sequence of events was established, all the physical and entomological evidence then fit clearly into the same timeline.

Second, PMI estimates assume that the succession of insect colonization on the body follows a predictable course of faunal succession. Noted exceptions to the usual succession, once understood, can provide additional evidence about whether the body had been moved and where the original crime scene might be located.

A third assumption in making a PMI estimate presumes that the course of insect development follows a predictable pattern and timeline (e.g., egg → larvae → pupae → adult). This requires a sufficiently detailed understanding of the stages of development for each species present along with an understanding of

![Image of blowfly life cycle](www.cmnh.org/site/ResearchandCollections_InvertebrateZoology_Research_ForensicEnt_ForensicBlowfly.aspx)
how climate conditions affect insect growth and development. Luckily, much is known about the lifecycles and growth patterns for many of the insects particularly important to PMI investigations.

Finally, an accurate estimate of PMI times requires that environmental conditions at the body site can be reasonably estimated beginning at the time of death and running continuously through the time when the remains were found. This can often be estimated by obtaining climatological data from the nearest weather station and then estimating the local conditions at the body site (microclimate). This information is critical since the rate of insect development, including time for each developmental stage, is very strongly affected by environment.

Probably the most useful insect in determining a PMI, especially during the first few weeks following death, is the fly. The life cycle of a fly, such as the blowfly or housefly, proceeds through a series of well defined stages, as illustrated in Figure 10.2.12. It begins with the deposition of eggs by an adult female fly (oviposition) in a dark and sheltered place. These eggs hatch to form a tiny maggot, or fly larva, that is about 2 mm long (about the size of two grains of salt). This freshly hatched larval form is referred to as the first instar stage of development. A maggot is really little more than an efficient eating machine, equipped with a simple intestinal digestive tract, salivary glands, a hook on the "front" end to allow it to stuff food into its mouth, and structures on the posterior end (called spiracles) that allows it to breathe when "head-down" in tissue feeding, shown in Figure 10.2.13. The presence of this "rear-end" breathing tube allows the maggot to continuously immerse its head in the semi-fluid mixture it feeds upon. When the maggot reaches about 5 mm in length, it can no longer continue to grow within the confines of its original skin. In order to continue its growth, the maggot molts or sheds its first skin to reveal a larger skin underneath. This begins the second instar stage of its development. During this stage, it grows to about 10 mm (the size of a small marble) before again molting for the third instar stage. This final instar stage continues until the maggot grows to about 20 mm (about the length of a penny) before it migrates away from the body as a pre-pupae. Once well away from the body in a dry and well-protected place, the larvae forms a puparia in which the outer skin of the pre-pupae larvae hardens and darkens to form the outer pupal case. Inside this protective casing, the final transformation of the larvae into an adult fly occurs. This is biochemically a very complex time as the tissues of the larvae are completely rearranged to form the structures, organs, and tissues of the adult fly. The pupae are very durable and the insect may remain in this stage until environmental conditions are right for it to emerge as an adult, such as over the winter or through droughts, although under optimal conditions they may
emerge as adults within about a week. Only the adult stage insect has wings and after emerging from the purpurium they fly off to repeat the cycle.

An adult fly typically lives between two to four weeks but during this time an adult female fly can lay thousands of eggs, usually in clumps of 50 to 100. It has been estimated that if a single pair of flies began egg-laying in April, they would have about $2 \times 10^{20}$ descendants by August if all the offspring lived. This, of course, does not occur but it gives an indication of the prolific nature of fly egg deposition.

While this is the general pattern for a fly lifecycle, there are notable variations. For example, the flesh fly gives birth to live larvae on dead remains instead of laying eggs. Other insect species important to PMI estimates, such as the beetles, also have varying lifecycles and stages.

There are two primary tools for helping entomologists determine the PMI; species succession and developmental stage. The range and types of the insects present is particularly important for determining the age of remains that are more than a month old while individual insect developmental information is typically most useful in narrowing down the timeline within the first month.

The first tool, insect succession, involves the identity of the various insect species found on the remains. As discussed before, insect species arrive and depart at different stages of the decay process in a typically well-ordered and overlapping procession (or more accurately, succession) of species. Observing which species are present, therefore, provides an opportunity to estimate how long it would take for that stage of insect infestation to occur. The succession times are correlated with the observed condition of the remains (bloated, decay, post-decay, etc.). For example, fly egg-laying typically stops when the maggot population becomes sufficiently large, usually within a few days. Maggot growth also slows as the food supply runs short and the remains begin to dry significantly. Once the moist food supply runs short, the maggots usually move away from the body while, at the same time, the beetle population increases. While these are overlapping events, clearly understandable patterns can be observed as illustrated in Figure 10.2.14. It is useful to note that the shape of each population curve in the Figure becomes more elongated as time progresses. The first few stages may completely occur within a few days while later stages may extend over months or years. This broadening of ranges at later stages also leads to more uncertainty and less accuracy in PMI estimates as the PMI time increases. The stages of insect succession can also be interrupted or stages even eliminated when the body has been moved between contrasting environments. For example, a body deposited in an arid climate may become dried out so quickly as to virtually eliminate the possibility of colonization of the remains by flies, leaving the beetles as the primary insect decomposers. Other possibilities already mentioned, such as burial, submersion in water, and depositing the remains outside in winter, can strongly influence the pattern of insect succession.
The second tool for establishing a PMI estimate relates to the age and developmental stage of the individual insects found on the remains, especially the largest and, presumably (but not always) the oldest larvae. The particular stage of development for a maggot can usually be determined by measuring its size and, in some instances, measuring the spiracles (breathing tubes). Each stage of the fly's development lasts for a specific amount of time that depends principally upon the local temperature and available food resources as illustrated in Figure 10.2.15 that shows the growth rates at different temperatures. Insects, as cold-blooded organisms, typically grow faster in warmer environments than in colder settings, within limits. Therefore, as the temperature around the larvae goes up, the time necessary for each stage decreases and, conversely, as the temperature decreases the development slows. If it is too cold or too warm, larval growth is greatly limited or even stopped entirely. The temperatures for these upper and lower thresholds vary from species to species but have been measured for many insects of medicolegal importance.

Insects require a certain total amount of heat to develop to the next developmental stage. This is often referred to as the accumulated degree-days (ADD or °D) for development. Degree-days (or sometimes degree-hours) refers to the amount of total heat accumulated by a larva between its upper and lower growth thresholds, as shown in Figure 10.2.16. Different species require different ADD’s to complete their development. For example, the Greenbottle Fly (Phaenicia sericata) needs about 200 total ADD to complete its development while the Bluebottle Fly (Calliphora vomitoria) requires over 700 ADD to reach the same stage of development. The total accumulated degree-days can be calculated using a variety of methods but it is proportional to the area of the curve between the

![Figure 10.2.15. Development of fly stages as a function of temperature - called an isomophen diagram (from www.univie.ac.at/forensic-entomology/information.htm).](image)

![Figure 10.2.16. Accumulated Degree Days between upper and lower temperature thresholds (from www.univie.ac.at/forensic-entomology/information.htm).](image)
two thresholds (shown in yellow in Figure 10.2.16). For example, the lower threshold for the Blue Bottle Fly is 6°C. If the temperature were to be held at 7°C for 24 hours, the insect would experience one degree of temperature above the lower threshold for a total of 24 hours, on ADD would be accumulated (or 24 degree-hours). The Green Bottle Fly, which has a higher threshold of 10°C, would not accumulate any ADD at that same temperature of 7°C and, therefore, show no growth. This information is important in determining the age of specimens collected on the remains (see below).

Once it is known when the first opportunity for egg laying occurred, it is next important to determine the age of the oldest larvae on the remains – information critical to estimating a PMI accurately. There are two primary methods for determining the age of insect larvae found on a body. The first, and probably simplest, relies upon collecting live larvae at the scene and then rearing them to adults. By controlling the conditions in the laboratory and measuring the accumulated degree days necessary needed to raise the larvae to adulthood, it is then possible to estimate the ADD (and therefore the time) that was necessary for the larvae to have reached the stage found on the body. This is done simply by subtracting the ADD found from the laboratory rearing experiment from the total ADD needed for this species to develop from egg to adult, shown schematically in Figure 10.2.17. For example, if it requires 240 ADD to raise a particular collected Bluebottle larvae to adulthood in the laboratory, the number of ADD that occurred before collection can be calculated by subtracting 240 ADD from the total 740 ADD needed for the complete lifecycle of the Bluebottle. Therefore, about 500 ADD occurred before the maggot was collected. In general, this is the most accurate method for estimating the PMI but requires the obvious, but often overlooked, need for the collection of live specimens at the crime scene.

The second method of estimating the age of a larva involves the use of an isomegalen diagram. An isomegalen diagram is a plot that shows the relationships between the time necessary for a larva to grow to a given size and the temperature of its surroundings. An example of an isomegalen diagram

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**Figure 10.2.17.** Determining PMI from controlled rearing of insects using ADD measurements (JTS).

**Figure 10.2.18.** Isomegalen diagram for a species of blow fly Phaenicia (Lucilia) sericata (from www.univie.ac.at/forensic-entomology/information.htm).
is shown in Figure 10.2.18 for the Green Bottle Fly. In this method, the largest maggots are measured and the isomegalen plot is used to determine their age. The general assumption here is that the largest larvae found on the remains are also the oldest and, therefore, developed from the first egg-laying cycle that occurred on the remains. These larvae can be used, therefore, to provide a reasonable estimate of the amount of time that the body has been colonized by flies. For example, if the largest maggots on the remains were found to be 8 mm, the curve for 8 mm is first located on the isomegalen diagram (note there is a different curve for each maggot size). From weather estimates (often by interpreting data from a nearby weather station), the maximum temperature ($T_{\text{max}}$) and minimum temperatures ($T_{\text{min}}$) for the site are estimated for the period in question. From where the $T_{\text{max}}$ and $T_{\text{min}}$ lines intersect the 8 mm curve, the range of days necessary for the larvae to reach the observed size can be estimated. For this example, let's assume that the $T_{\text{max}}$ was 24°C and the $T_{\text{min}}$ was 16°C for the time period in question. By using the isomeglan plot for the correct species of fly collected, the $T_{\text{max}}$ of 24°C line intersects the 8 mm curve at about 1.5 days (red dotted line). This is the least amount of time necessary for an 8 mm larva to be formed. The $T_{\text{min}}$ of 16°C line intersects the 8 mm curve at about 4.5 days (blue dotted line) - the maximum amount of time necessary for a larva to grow to this size. Thus, a time estimate for this size of larvae to be found on the remains would be between 1.5 and 4.5 days, with an average of 3.0 days ± 1.5 days (green dotted line).

It is also very important to search the scene for and locate any pupal cases from larvae that might have migrated from the body in their quest for a dry and safe place to pupate. These pupal cases can help to both identify the species of insect present and help determine the time necessary to reach this stage of development (including how many "generations" of flies have been reared on the remains).

A variety of complicating factors have already been discussed in using these methods for determining the PMI including weather and insect accessibility to the remains. There are indeed additional factors that may limit the use of insect information in investigations.

For instance, in the decay stage there is an enormous increase in the maggot population on the remains that may lead to what is called a maggot mass. On the remains, fly larvae very actively convert the food value of the tissues into the nutrients they need for growth and metabolism. Some of the heat from this enormous amount of metabolic activity is released to the surroundings. As more and more maggots come together, the maggot mass formed may considerably raise the temperature around the larvae, up to 50°C higher than the surroundings, as illustrated in the thermal image shown in Figure 10.2.19. This increase in temperature results in significantly shorter development times and the continued

Figure 10.2.19. Thermal imaging of a larval maggot mass on a fetal pig showing the heat generated through maggot feeding.
development of the larvae even when the surrounding air temperatures drops below the threshold needed for growth. It is very difficult to accurately estimate the effect of the heat generated by a maggot mass on development, leading to relatively large uncertainties in PMI estimates. Neglecting the heat generated by the maggot mass, especially in cooler environments, may lead to a significant overestimation of the PMI.

Another complicating feature relates to the assumption that the largest larvae are also the oldest. Some species have a faster developmental growth rate than others, such that the larvae from one species may quickly overtake and grow larger than an older larva from another species. This makes species identification of each larva very important. Competition for food between species or the drying of the remains, reducing access to the moist tissues, also yields smaller larvae than would be expected, leading to underestimates of PMI times.

The presence of drugs or toxins in the body may also have a profound influence on the growth rate and even the mortality of the larvae. For example, a body containing cocaine (a stimulant) may greatly increase the development rate of the larvae while heroin may slow the growth. Poisons are often bioaccumulated within the tissues of the maggots as they feed and this may further slow the growth or even kill the larvae. This bioaccumulation, however, has also been used to good effect in toxicological studies to determine the presence of drugs or poisons in a decayed body since the concentrations of these substances may be much higher in the insects than in the body itself.

Additional complications in PMI estimates may arise from: (1) a need to better understand the activity of insect activity at night and during rainfall, (2) regional adaptation of insects to environmental conditions, (3) the effects of the maggot mass on different body sizes, and (3) the possibility of insect hibernation triggered by short days and cool temperatures.

While the focus in PMI estimates tends to be upon flies and beetles, a variety of other insect and arthropod species also visit remains and their presence can assist in PMI determinations. These species can include butterflies (Figure 10.2.20), moths, bees, and ants.

**Other Aspects of Mediolegal Forensic Entomology**

An understanding of the insect species that inhabit a particular setting can indicate whether the location where the body was found was the original crime scene or whether it had been moved after death. For example, identifying species of insects found primarily in a urban environment on a body that was found in the countryside indicates that the crime may have occurred in the city but that the body was later move to the country. It may also be possible to determine if the remains had been disturbed after death by looking for interruptions or unexplained changes in the development cycles of the insects.

Insects that feed directly upon dead flesh often make no distinction between fresh, decaying and even cooked tissue. These insects can also colonize unclean and infected wounds in living people and animals. Cases of abuse (harmful treatment) or neglect (failure to provide care or...
treatment) have been shown, especially involving the treatment of the elderly and young children, by noting insect infestation. This usually happens when blow flies are attracted to the smell of urine, defecation, or open wounds on a person and then lay their eggs in surrounding clothing and on the affected tissue. Young children and the elderly are often not capable of taking care of their bodily functions themselves and require others to provide this care. If a child or elderly person has not been cared for properly, blowflies can arrive and lay their eggs that will develop into larvae that feed on open sores and wounds, ultimately eating away the tissues. These irritated and wounded tissues then often become infected, a process known as myiasis (the disease arising from blow flies feeding directly on a host's tissues). These infestations can occur wherever conditions are suitable for insect development, especially warm and damp locations with dead tissue to feed upon. While blow fly larvae will feed only on dead tissue, and are therefore usually not an immediate threat to a person's health, other insect species may be present that feed on still living tissue. The presence of particular species and their stage of development can clearly indicate for at least how long the abuse had continued, possibly when it began, and the type of physical abuse inflicted.

Myiasis is not limited to humans but may also be involved in cases of animal cruelty. In these cases, insect evidence is often the most important type of evidence since animals are not able to communicate directly with us. These cases typically arise from an owner's failure either to treat wounds or provide a suitable state of cleanliness for the animal. In animals this condition is usually called "blowfly strike". Both domestic and wild animals are prone to this disease if not properly cared for.

Insects that do not feed upon remains may also have a story to tell. One suspect was caught when investigators noticed that his legs were badly bitten by small insects found near the crime scene that was know to be invested by these small biting insects. Another suspect was convicted of sexual assault by finding a cocklebur on the clothing of the suspect and identifying the insect inside as found only near the crime scene.

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### Larval Healers

Fly larvae (maggots) have recently been used to help heal difficult medical problems and wounds (larval biotherapy). In the past, when people had tissue that had died due to trauma or decreased blood flow (e.g., in the feet of diabetics), surgical amputation was a common treatment. Recently, however, the medicinal use of maggots to eat away the dead tissue without harming the living tissue has proven to be a very effective treatment for these types of problems - much cleaner than the surgeon's knife. Maggots have also been used to treat "super-bug" (Methicillin-resistant Staphylococcus aureus, MRSA) bacterial infections, with cures averaging 3 weeks instead of the typical 28-week conventional treatment course (Andrew Boulton, Univ. Manchester). This treatment, however, goes back to ancient times when people used maggots to keep infected wounds clean and to fight gangrene infections.
Insects have also been used to show that a body has been moved after death from the site of the violence to a secondary site, part of forensic taphonomy (see Chapter 9). For example, a body found in the countryside but containing insect species known to occur only in an urban setting suggests that the body was initially deposited in the city and then later removed to the rural environment. This can also be used to detect the transportation of remains over large distances where species and sub-species vary by geographic location. Finally, even small changes related to each insect species can be noted and used to develop evidence. For example, the green bottle blow fly colonizes remains in sunny and warmer locations while the blue bottle prefers shady, cooler areas. Finding primarily green bottle larvae on remains in a secluded, shady spot suggest that the remains were moved after the insect initially colonized the remains.

World War I Trench Entomology

An amazing story comes from a WWI soldier's diary describing difficult life in the trenches of France. The veteran, in describing the trench, wrote that on one sunny morning the top of the trench was a beautiful, shimmering, iridescent green color while in the darker, lower portions of the trench, the color shifted to a beautiful blue color. This is an excellent example of different species of blow fly seeking their own optimal conditions. The green bottle fly (*Lucilia illustris*, right) prefers sunnier and warmer locations (the top of the trench) while the blue bottle (*Calliphora vicina*, left) prefers the lower, darker portions of the trench (pictures from http://bugguide.net; story from Smith, K.G.V. "A Manual of Forensic Entomology").