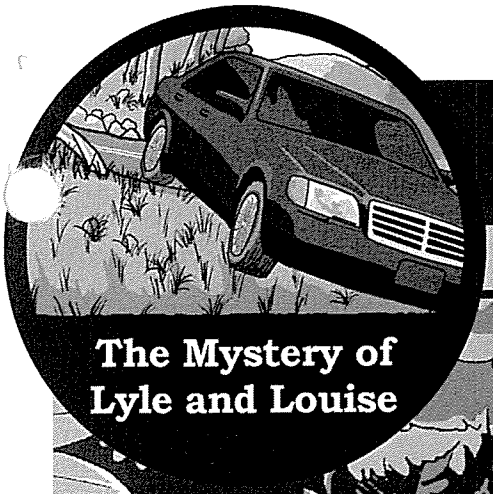


Nature's Witness

A Lab on Forensic Entomology

The Mystery of Lyle and Louise

Rev. 8/28/2012

Forensic Entomology

FORENSIC entomology is the application of the scientific study of insects to criminal and civil investigations. Forensic entomology experts testify in court in many diverse cases ranging from the origins of insects in food and termites in houses to murders and suspicious deaths.

In 13th century China Sung Tz'u, a traveling judge who investigated numerous murders, authored a book about his cases. In one of his most sensational cases Tz'u used the attraction of flies to fresh blood to identify a murder suspect. The victim in this case was murdered with a scythe, so Tz'u ordered all villagers to bring their scythes out for inspection. The murder weapon and suspect were revealed when blowflies flies swarmed on a single scythe coated with microscopic traces of blood.

Entomology was also used in 1855 to solve a murder in Paris. In this instance the body of an infant was found concealed behind the mantle in a house that had been plastered 5 years prior, thus preventing further insect contact after plastering. Dr. Bergeret d'Arbois, a Swiss entomologist, determined that the fly, *Sarcophaga carnaria*, had laid eggs which developed into maggots when the corpse was fresh. Mites, which prefer to lay their eggs on a dried corpse, were also identified. He estimated that this drying process would have taken at least a year, so the mites were dated from 1849 and maggots from 1848. This led the investigators to look for the occupant of the house from 1848, not 1849. Today, forensic entomologists use the same reasoning to estimate the time a cadaver has been exposed to insects since death.

Forensic entomologists collect and prepare insects for identification, provide accurate identifications of insects, and make inferences on the age of larval stages based upon the size and stage of larvae in the sample collected from a crime scene. Forensic entomologists rarely work alone. Instead, they draw from the expertise of many disciplines, including police detectives, pathologists, mathematical modelers and statisticians, meteorologists, and climatologists. Some cases have even used the expertise of forensic chemists to test the insects in a corpse, or even their discarded pupae, for the presence of drugs.

Forensic entomology rarely links a particular suspect with a crime or location. Rather, it provides data used to estimate the time that elapsed between the actual death and when the body was first discovered. This period is referred to as the post mortem interval, or PMI.

Adult insects are hard-bodied, segmented animals with six legs, typically one or two pairs of wings, and three distinct body regions – the head, thorax and abdomen. In contrast, larval insects are softer-bodied, often legless, segmented worm-like creatures that utilize a variety of habitats. Insects are the most numerous and diverse group of animals on earth, occurring in almost all terrestrial and aquatic habitats, with the exception of oceans. Approximately one million species of insects have been described and named, but most entomologists believe millions of new insect species have yet to be discovered, identified, and named.

The insects of most forensic interest are the flies (dipterans) and beetles (coleopterans). Within these two groups the number of species are massive, with over 300,000 species of beetles and 86,000 of flies in the world. In North America, however, only 30,000 beetle species and 16,000 fly species have been described.

Many organisms use "carrions", or carcasses, as a food source. Some fly species specialize in living on carrions. These carrion flies are the most important insects to the forensic entomologist. There are two families of carrion flies: the blowflies, in the family Calliphoridae, and the flesh flies, in the family Sarcophagidae. Adult calliphorid flies are easily identified by their iridescent blue, green, copper, or black bodies. Sarcophagid flies, on the other hand, are grayish, usually with three distinct longitudinal dark stripes on the dorsal thorax. Some species of beetles also live on carrion, but they are less common, and arrive later, than carrion flies.

Forensic Entomology

Carrion flies are attracted to dead bodies, often arriving within minutes of death. The flies lay eggs which develop into larvae in open, moist surfaces like eyes, mouths, and open wounds. Larvae become so numerous on the cadaver, they actually speed its rate of decomposition. This phenomenon is due to the fact that the large maggot mass has a high metabolic rate which can increase the temperature in the body above the ambient temperature. Entomologists measure the rate of carrion fly larvae growth and development; if a particular larval stage is present on a cadaver, and it takes three days for this stage to develop, then the cadaver must be a minimum of three days old.

Recent PMI (0-50 hours) is estimated by a medical examiner or forensic pathologist. Medical examiners rely on observation and measurement of physical changes in the body: cooling, muscular flaccidity, rigor mortis, lividity, skin pallor, and the condition of the cornea, which gradually becomes opaque. These physical changes take place before fly larval development in the body becomes evident.

If a body remains exposed to the environment for a longer period of time before being examined by a pathologist, the normal physical changes observed after death may not provide an accurate PMI estimate. Although most natural deaths are attended by families or medical personnel, often, when a person dies alone, the body goes undiscovered for several days. The well-known forensic anthropologist Dr. William Bass III reported that half of his cases involved maggot-covered bodies in an active stage of decomposition, therefore Dr. Bass founded a postmortem decomposition research facility at the University of Tennessee to improve the ability of forensic scientists to estimate the PMI. Forensic entomology is an important aspect of the research on decomposition and PMI at Dr. Bass' facility, commonly known as the Body Farm. Dr. Bass's research has shown that carrion insects can leave behind as little as a skeleton in less than two weeks in favorable weather.

Cadavers decompose in four stages: fresh, bloated, decay, and dry. The time the body spends in any individual stage will vary depending on environmental conditions; warm, wet weather speeds decay, while cold, dry weather slows it. Different insects are attracted to each of the four different stages of decomposition. The ordered series of insects attracted to the decomposing body is called a succession. The succession pattern is useful in estimating how long a cadaver has been exposed to the insects. For example, carrion flies are attracted to a bloated corpse, therefore they will only be present on a corpse once that stage is reached. Adult blowflies, however, are attracted to the fresh corpse and lay their eggs rapidly after death.

Forensic entomologists have developed succession databases for carrion insects found in different geographic regions. They perform experiments to determine the order in which various species of flies arrive at the cadaver and the times their larvae take to pass through the various stages through pupation. Then, when a crime scene is investigated, the forensic entomologist compares the insect species and their distribution of larval stages to the database to estimate the time of death. A key piece of data which must be experimentally determined is the time required for the different larval stages.

The adult female blowfly, for example, lays her fertilized eggs on the carcass in a single batch, but she may return to lay eggs several times during her reproductive life (two to three weeks). The eggs begin to hatch in 12 to 24 hours, producing small (approximately 2 mm) first stage larvae. Because the outer 'skin', or integument, of insect larvae cannot expand to accommodate growth, the larvae molt their outer covering to keep growing and developing. The first larval stage, or 'instars', become the larger second instars after they molt. The second instars feed and subsequently molt to become third instars. The feeding third instars are very active and grow rapidly to a length of 14 to 18 mm. They then develop into post-third stage larvae which stop feeding, migrate away from the cadaver, and burrow into the soil. They become inactive and the

integument hardens into a pupa. After six to eight days the adult fly emerges from the pupa, crawls to the soil surface, the wings harden, and it flies away to begin the process anew. Flies survive over winter in the pupal stage and emerge in the following spring when temperature conditions become favorable. The process for fleshflies is similar, with the exception that eggs hatch within the body of the female, and she deposits live first instar larvae.

Insect species are attracted to lay their eggs on a corpse at different times. The regular pattern of development of the larvae or maggots on the corpse can be used to estimate the number of days since the eggs were laid for each species. Each new species replaces an earlier species in this succession since the cadaver is going through a process of decay and attracts new insects able to use it as a food resource. A sign that this is occurring is the presence of younger larvae of one species (often flesh flies) with older larvae of another species (often blow flies) that colonized the cadaver earlier. Cadavers decompose as bacteria and the body's own cellular enzymes join forces to break down tissues, a process assisted by insects and other scavengers. Taphonomy is the science which studies the natural process of plant and animal decay.

In addition to succession, these larval development rates help forensic entomologists estimate the PMI. This is challenging since insects are cold-blooded animals and their larval growth rate increases as the environmental temperature increases until they reach a lethal point. Researchers rear insects at a constant temperature and calculate the time it takes for an insect to develop from one life stage to another. By comparing growth rates at a variety of temperatures, entomologists have calculated Degree Hours required for the insect to develop from one stage to another. The number of hours to reach a stage is multiplied by the standard rearing temperature during that time period. The Degree Hours needed to complete an insect's development does not vary. If larvae take 40 hours at 25 degrees C to develop to the next live stage, this is 1000 degree hours. If the larvae are kept at 20 degrees, they will take 50 hours to reach the same stage. When investigators can get accurate weather reports for an area, they calculate Accumulated Degree Hours and estimate the hour when larvae hatched from the eggs. The temperatures for the days preceding the discovery of the body and the growth and development rate of the fly species in degree hours must be known. By adding the incubation time for the egg, the entomologist can estimate the time of initial oviposition, which is an estimate of the time of PMI.

Additionally, there are exceptional circumstances which must be considered in forensic investigations. If a body is protected from flies by enclosure in a container, such as a car trunk, flies may not lay eggs until the body is exposed, or a stage in the succession may be bypassed. Also, the large mass of maggots present on a decaying corpse has a high metabolic rate which often increases the temperature in the body above the ambient temperature speeding decomposition. Furthermore, when two species colonize a cadaver at the same time, the pattern of development may differ from when each species colonizes individually. Finally, flies are generally inactive at night and during periods of rain. Thus, a corpse exposed at night or during a storm will not attract flies for several hours until conditions become favorable for adult fly activity.

Adult flies are very mobile and their age cannot be easily determined, so they are not commonly collected from a corpse. Ideally, samples of larvae are collected from several different areas of the carcass, such as nasal and oral cavities, open wounds, and from the hair and/or skin. A proper sample should contain 50 to 100 larvae. About half of the larvae should be processed immediately, on-site. This is best accomplished by dumping the larvae into a pan of boiling water for 15 to 20 seconds to kill bacteria in the intestinal tract, then quickly straightening out the larvae to allow for measurements to be taken later in the laboratory. The larvae are then transferred into a bottle of 70% ethanol for preservation. This bottle is labeled with the date, location and time of collection, and the name of the collector. Because adult flies are easier to taxonomically

identify than larvae, the remaining larvae are left alive and reared in the lab. When they develop into adults, a positive identification is easily made.

In addition to the succession of insects on the decaying cadaver, there is a succession of species of insects throughout the year, especially in a temperate climate. Some fly species are active in the early spring, different species are active in the fall, and others are continuously active. In regions with cold winters, bodies are often discovered when the snow melts in the spring, and investigators are called upon to determine in which season the death occurred. If an insect larvae which is more abundant in the fall is discovered, this can indicate the body was undiscovered for many months, while if larvae are found from spring flies, this could indicate the cadaver is more recent, or that it was recently exposed to the newly emerged adult flies.

Although human remains are best for forensic research, such research is often illegal or surrounded with regulatory issues. Dr. Bass's Body Farm research remains an exception to the type of research most forensic entomologists perform. Pig carcasses are frequently used in forensic entomology research to generate data for human comparison, as pigs have a similar body size and configuration to humans and lack most body hair.

It is critical to determine larval carrion fly development rates under different temperature conditions and for different species of carrion. As a result, there is a considerable need for forensic entomologists to engage in such growth and development studies, both in laboratory and field environments. As this research is performed and results published, forensic entomology is becoming an increasingly useful tool in crime scene investigations.

Forensic entomologists prepare for their career by obtaining an advanced college degree (either an M.S. or Ph.D.) in entomology, ecology, biology, or zoology. Certification by the American Board of Forensic Entomology requires completion of a minimum of three years of professional experience in forensic entomology casework, publishing at least one scholarly paper, and at least one professional presentation in the field of forensic entomology. A written and practical examination must also be passed for certification. Most forensic entomologists work for colleges or universities and are called in as consultants as needed.

Additional Reading

Catts, E. P. and N. H Haskell (eds.). 1990 (second printing 1997). Entomology and Death: A Procedural Guide. Joyce's Print Shop, Inc. Clemson, South Carolina

Goff, M. L. 2000. A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes. Harvard University Press, Cambridge, Massachusetts.

Bass, W. and J. Hefferson 2003. Death's Acre: Inside the Body Farm, the legendary forensic lab. Putnam Adult, New York NY.

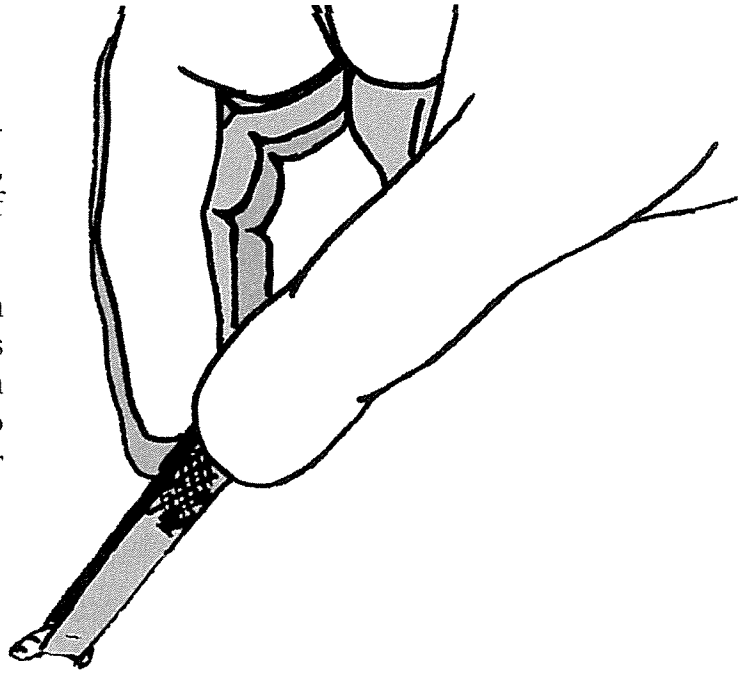
http://www.forensicmag.com/articles.asp?pid=112

http://www.forensicentomologist.org/certification.html

The Evidence

When the double homicide victims were discovered at the fishing cabin along Blackrock River, they were found to be in the advanced stages of decomposition.

In an attempt to determine the post-mortem interval and establish a time of death, maggots were collected from the face and wounds of both victims. These specimens were then placed into vials with 70% ethanol to preserve them for later identification.



Pre-Lab Questions

Background

1. What is the role of a forensic entomologist in a homicide investigation?

2. Why are insects important when determining post mortem interval (PMI)?

3. What is an instar?

4. What are some factors that may delay fly oviposition?

5. Flies develop at predictable rates. What measure is used to make this prediction?

Procedure

6. What should you record on your Species Separation sheet?

7. How many individuals from the sample collected from the decedent will you identify?

8. How will you determine the species and life stage of these individuals?

Fly Development Times

The following charts show the time in hours individuals of each species spend in each life stage at a standard temperature, 21 **°C**. Notice the species development times are somewhat different. It requires 21 hours for the egg from Species A to reach first Instar, while it takes 25 hours for species B.

Species A:

Temp °C	Egg	1st Instar	2nd Instar	Feeding 3rd Instar	Migrating 3rd Instar	Pupa
21	21	31	26	50	118	240

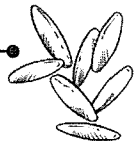
Species B

Temp °C	Egg	1st Instar	2nd Instar	Feeding 3rd Instar	Migrating 3rd Instar	Pupa
21	25	37	31	60	124	286

Fly Life Cycle Chart

Eggs

Off-white, translucent capsules, rarely more than 3 mm long.

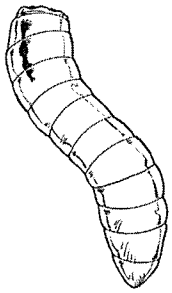


1st Instar Larva

Worm-like creatures between 2 and 4 mm long. Posterior spiracles (openings for breathing) are set apart in a darker area. One spiracle slit is present.

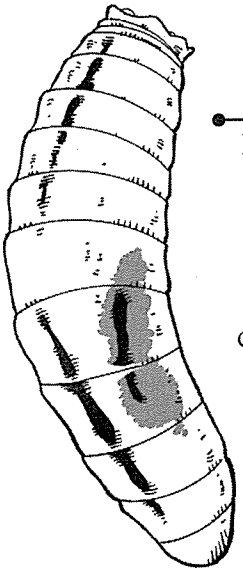
2nd Instar Larva

Intermediate in size between 1st and 3rd instar and approximately 4 to 8 mm. Posterior spiracle has 2 slits.



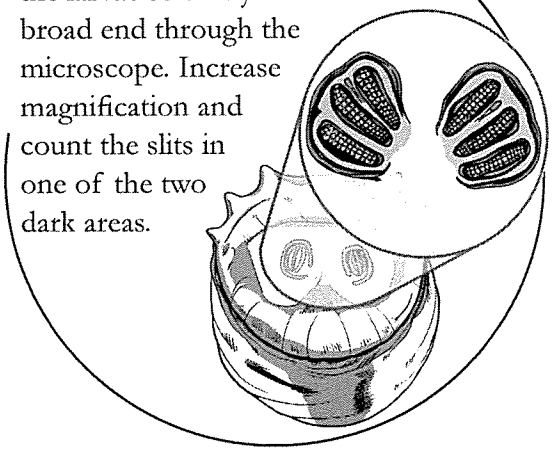
3rd Instar Larva

Much larger than 2nd instar larvae, between 10 and 15 mm, and noticeably thicker. Undigested flesh is observable through the outer cuticle as a dark area toward the tapered anterior end.



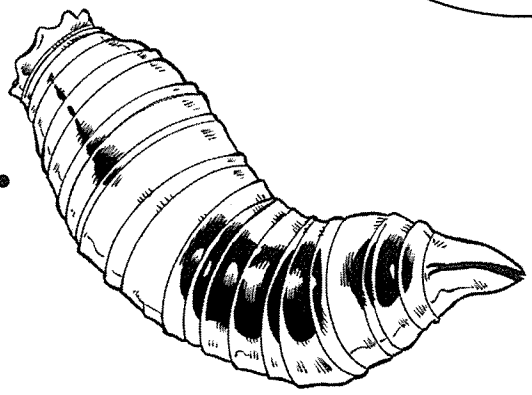
Posterior Spiracles

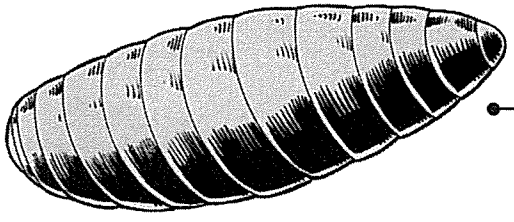
To locate posterior spiracles, orient the larvae so that you can see the broad end through the microscope. Increase magnification and count the slits in one of the two dark areas.



Migrating 3rd Instar Larva

Larvae have not changed in size, but flesh has been digested and is no longer observable through the cuticle. At this stage, larvae migrate to find a suitable site to pupate.





Pupa

Hard, dark, capsule slightly smaller than 3rd instar larvae.

Lab Procedure

Examination of Taxa

1. Look over your Fly Life Cycle Chart. Keep it nearby as you examine the life-cycle exemplars. Keep in mind not all the stages are shown on the chart.
2. Remove the earliest life stage vial from your exemplar collection. Place 1 or 2 specimens under your microscope.
3. Compare the sample under the microscope to the drawing in your Fly Life Cycle chart. Find the key identification features listed on the chart in the sample.
4. Place additional specimens under the microscope so that you have 3 or 4 samples. Note the amount of variation between individuals at the same life stage. Record your observations.
5. Return your samples to the vial.
6. Repeat this examination for each of the life stages for each of the species.

Separation of Taxa

1. Place 2 specimens of the same life stage from each species under the microscope.
2. Although species A and B have many characteristics in common, there are important characteristics that distinguish one from the other. Define one such characteristic.
3. Record this in your Species Separation Worksheet.
4. Return the specimens back to their respective vials, being careful not to mix up the samples.
5. Repeat this process with samples from each life stage.
6. You may not have two of the same life stage from each species. If this is the case, skip these stages.
7. Return all the vials to the exemplar collection.

Analysis of Evidence

1. Your instructor will assign you to analyze individuals from one of the six samples. Randomly collect 10 specimens from your assigned sample.
2. Place a specimen under your dissection scope.
3. Using your knowledge from examining different life stages and your Fly Life Cycle chart, determine the life stage of the individual.
4. Using your notes on species separation, determine whether the individual is of Species A or Species B.
5. Select a specimen of the species and life stage from the exemplar collection to which you believe your sample maggot belongs. Compare the two under the microscope to verify your analysis.
6. Record your results.
7. Repeat this process for each of the 10 individuals you removed from the collection vial.
8. Your instructor will have placed a grid corresponding to your victim on the board with columns for each species and rows for each of the life stages. Place a tick mark in the appropriate grid cell for each individual you analyzed. Everyone will record their data for this victim in this same grid.
9. When everyone has finished analyzing their individuals, copy the data from both grids onto your Data Collection Worksheet, recording the number of individuals of each species and life stage.
10. Using the charts at the beginning of this exercise, determine the minimum number of degree hours needed for the oldest life stage of each species to develop.
11. Choose the largest value for the minimum number of degree hours that the victim has been dead.

Lab Procedure 2: Data Analysis

Degree-Hour Determination

1. Review the Weather Service Data provided on the Excel document downloaded from the website. The bodies were discovered at 1:00 PM on June 20 and the insects were collected at 3:00 PM.
2. Determine the number of degree hours for each day using the weather service data. To do this, multiply the average temperature by 24 hours for each day. This can be performed in a spreadsheet.
3. Determine the number of degree hours required for each life stage of both species. To do this, multiply the number of hours by the degrees Celsius given in the table.
4. By adding all the degree hours for each of the six life stages together, you calculate the cumulative degree hours required for an adult fly to develop at 21 °C. Next calculate the cumulative degree hours required to reach each of the other five stages. Do this for both species.
5. Calculate elapsed degree hours for each of the days in the climatological data provided. To do this, multiply the number of hours by the average temperature that day. For example on day 20, there are 15 hours (since the insects were collected at 3:00 PM) times 18.4 °C for a total of 276 degree hours. For Day 19, add the degree hours for that day to the degree hours from day 20. Perform this task for each of the 20 days in the month of June.
6. Examine the Species A life stages collected as evidence and identify the oldest species A life stage in the collection for the adult male. Determine how many cumulative degree hours that life stage took to develop at 21 °C. Which day in the climatological data comes closest to equaling this number? This is an estimate of the day the adult insect laid eggs on the cadaver.
7. Repeat step 6 for Species B for the adult male collection. Is the number of degree days required for this stage to develop longer or shorter than for species A? What fact about the biology of carrion flies could explain any differences you have observed?
8. Based on the data from both species, estimate the earliest and latest time that each insect began developing on the adult male cadaver.
9. Repeat these steps for the collection from the adult female. Determine the earliest and latest time that each insect began developing on the female cadaver.

Data Collection Worksheet

Data Analysis

For each sample, record the species and life stage in the table below.

Sample	Species (A or B)	Life Stage
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

When finished, in the grid on the board corresponding to the victim from which you analyzed evidence, place tick marks in the cells corresponding to the species and life stage of each individual you analyzed.

Aggregated Data

After everyone has added their information to the tables on the board, fill in the two tables below with the total counts of all individuals analyzed by the entire class.

Adult Woman in Cabin:

	Species A	Species B
Eggs		
1st Instar		
2nd Instar		
3rd Feeding		
3rd Migrating		
Pre-Pupae		
Pupae		
Adult		

Adult Man in Cabin:

	Species A	Species B
Eggs		
1st Instar		
2nd Instar		
3rd Feeding		
3rd Migrating		
Pre-Pupae		
Pupae		
Adult		

Species Separation Worksheet

Record observations on the two species of flies provided at each life stage. You will rely on these notes later when identifying samples collected from the victims. You may not have all of the stages presented below.

Eggs:

1st Instar Larvae:

2nd Instar Larvae:

Feeding 3rd Instar Larvae:

Migrating 3rd Instar Larvae:

Pre-Pupae:

Pupae:

Adult:

Post-Lab Questions

Short Answer

1. What species was/were found on the male decedent? The female?

2. What was the latest life stage of which species found on the male decedent? The female?

3. For both victims, what were the minimum number of degree hours that passed between time of death and discovery?

4. What were the post-mortem intervals for the two victims?

5. What life stage did you find the most of on the male decedent? The female?

6. What reasons can you think of to explain why there were more of this life stage than any other life stage?

7. Review the climatological data. Which three categories of information do you believe will impact the development of insects the most and why?

8. What was the weather like on the day the PMI predicts the individuals died?