



Utilization of Forensic Entomology in Minimum Post-Mortem Interval (t_{\min} PMI) Estimation การประยุกต์ใช้นิติกีฏวิทยาประมาณค่าช่วงเวลาหลังเสียชีวิต

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ABSTRACT

Insects play an important role in crime scene investigations. This study portrays the importance of forensic entomology (the study of the life cycle, behavior, and habitat of insects) in post-mortem interval (PMI) estimation. When a body is discovered, determining the time of death is essential for investigation. Insects are often the first animals to colonize the body, making them valuable indicators of PMI. Forensic entomology is one of the fastest-growing methods used to determine the cause of death and time of death prediction. The author examined the maggots and pupae that were found on a body at a crime scene in Patanee province, southern Thailand. By collecting samples of maggots and pupae, we preserved them directly with 80% ethyl alcohol. Some maggots and pupae were placed in containers to feed them until they were adults and easily identified. The maggots were examined by sectioning the tip of posterior spiracle and visualized under a microscope. Study of the spiracle under a microscopic examination revealed 2nd and 3rd instars. The estimated time of death for the body was at least 30 days. This information is crucial for crime scene investigators as it helps to determine the minimum Post-Mortem Interval (t_{\min} PMI) and the decomposition stages.

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Keywords : Forensic science, Forensic entomology, Maggots.

บทคัดย่อ

แมลงมีบทบาทสำคัญในการสืบสวนการเสียชีวิตที่ผิดธรรมชาติงานวิจัยนี้ต้องการแสดงการใช้ประโยชน์จากการศึกษาวงจรชีวิต พฤติกรรม และถิ่นที่อยู่อาศัยของแมลง เพื่อนำมาประยุกต์ใช้ในทางนิติวิทยาศาสตร์ คือการประมาณระยะเวลาหลังการเสียชีวิต (Post-Mortem Interval, PMI) การทำงานด้านนิติวิทยาศาสตร์เป็นการปฏิบัติตามพระราชบัญญัติการให้บริการด้านนิติวิทยาศาสตร์ พ.ศ. ๒๕๕๙ การทดลองนี้เป็นการแสดงการตรวจสอบหนอนและดักแด้ที่พบบนร่างศพในที่เกิดเหตุ จังหวัดปัตตานี ภาคใต้ของประเทศไทย โดยการเก็บตัวอย่างหนอนและดักแด้ ผู้เขียนเก็บรักษาตัวอย่างด้วยเอทิลแอลกอฮอล์ 80% พร้อมติดฉลากรายละเอียด และนำหนอนและดักแด้อีกส่วนหนึ่งไว้ในภาชนะเพื่อให้อาหารจนโตเป็นตัวเต็มวัย หลังจากนั้นจึงทำการ identify โดยวิธีการ section ตัดส่วนปลายของหนอนด้านป้าน ที่เรียกว่า spiracle และตรวจด้วยกล้องจุลทรรศน์ (microscopic examination) ส่วนดักแด้ทำการตรวจด้วย X-Ray Computerized Tomography Scan (micro-CT scan) พบหนอนแมลงวันหัวเขียววัยที่ 2 และ 3 ปรากฏอยู่ ดังนั้น การประเมินระยะเวลาภายหลังการเสียชีวิตของเคสนี้จึงไม่ต่ำกว่า 30 วัน ข้อมูลนี้มีความสำคัญสำหรับการสืบสวนการตายเนื่องจากช่วยในการกำหนดช่วงเวลาหลังการชันสูตรศพขั้นต่ำ (PMI ขั้นต่ำ) ควบคู่กับการศึกษาการย่อยสลายของศพ

คำสำคัญ: นิติวิทยาศาสตร์, นิติกฎหมาย, หนอนแมลงวัน

INTRODUCTION

Forensic entomology was first used in 1235 in China by Sung Tz'u, who was a "death investigator". He wrote a medico-legal text book titled "Hsi Yüan Chi Lu" in Chinese, or "The Washing Away of Wrongs". This is the first book written for investigative purposes (Dorothy, 2007). In this book, Mr.Sung mentioned about the blow fly that likes to swarm on the open organs of the corpse, such as the eyes, ears, nose, mouth, anus, and vagina, especially wounds. During the 19th and early 20th centuries, forensic entomology began to gain popularity in death investigations. In 1996, the American Board of Forensic Entomology was established, which is a certification board for forensic entomologists (ABFE, 1996). In Thailand, the first case report on forensic entomology was published by a professor from Chiang Mai University in 2001. The body was found near a police station in Lampang province. On the corpse's leg, a honeycomb-like wound was found, with a large number of larvae in the wound. The maggots were sent to the Department of Parasitology at Chiang



Mai University for study, and they were found to be in stage 3. It's scientific name is *Chrysomya ruffifacies* (Macquart). From this information, it can be used to estimate the time of death (Sukontason, Kabkaew L., K. Sukontason, P. Narongchai, S. Lertthamnongtham, S. Piangjai, and J. K. Olson, 2001).

OBJECTIVE

To demonstrate the use of insect in estimation of the shortest possible time after death, or minimum Post-Mortem Interval ($_{\min}$ PMI)

LITERATURE REVIEW

Related Theory

The use of entomologists in crime scene investigations is normally and is becoming increasingly important as insects arrive at the scene quickly and are considered excellent first evidence and first witnesses (UiTM, 2014). Today, the use of insect and arthropod evidence to determine the cause of death is accepted in modern forensics. In Thailand, there are many reports of forensic entomology, such as the thesis "Insects Collected From the Late Stage of Corpse Decomposition: Case Report and Review." They studied forensic entomology, which is commonly used to estimate the post-mortem interval (PMI) (Lee, Goff M., 2000). Fly maggots have advantages for estimating PMI during the first three weeks of death; after this period, other insects could be considered for calculating PMI. They described four insect species collected from corpses during the late stage of decomposition (Payne, 1965). Black soldier fly (*Hermetia illucens*) larvae were collected from a floating corpse, and the PMI estimation was approximately three weeks. Adult hide beetles (*Dermestes maculatus*), red-legged ham beetles (*Necrobia rufipes*), and hisper beetles (*Saprinus sp.*) were collected from corpses hanging on forest trees; the PMI estimation was four to six weeks.

Understanding the life cycle of these insects would assist forensic personnel in estimating PMI more accurately (Pengsakul Theerakamol, Nantana Sirisup, Panuwat Chutivongse, and Padet Siriyasatien, 2008). Otherwise, in 2022, "Estimating the Age of *Chrysomya megacephala* Larval Stages From Morphology and Cuticular Hydrocarbon Analysis" by Miss Palita Sirisatien. She studied *Chrysomya megacephala* (Fabricius), which



is one of the most abundant forensic blowfly species in Thailand. Its growth and development patterns have great implications for the estimation of the postmortem interval (PMI) (Davis J. B. and Goff M. L., 2000). The objective of this study was to age fly larvae by morphology and analysis of cuticular hydrocarbons using Gas Chromatography Mass Spectrometry (GC-MS) (Richards S. Cameron, Thomas J. Simonsen, Richard L. Abel, Martin J. R. Hall, Daniel A. Schwyn, and Martina Wicklein, 2012). In this experiment, the developmental duration, larval body length, and morphological changes of *C. megacephala* were examined. The experiment was conducted at 30 ± 3 °C and ~70% relative humidity. The results showed that the mean width and the mean length of larvae varied according to their daily growth. Regression analysis was conducted to obtain equations of the variation in larval body width and length with time after hatching. The cuticular hydrocarbons were extracted with hexane and analyzed using gas chromatography-mass spectrometry. The specificity of the hydrocarbon compounds identified in daily larvae varied with their growth. The results from this work might be useful for estimating the age of fly larvae and could be applied in real forensic cases (Sirisatian Palita, 2022). Both studies are related to insects and have applications in forensic science.

Based on this review, insect variables remain so important that research studies may be conducted to investigate their various components. Therefore, insect research is considered to assess post-meal energy expenditure (post-mortem interval, PMI). Currently, in-depth studies in the field of forensic entomology cover a wide range of topics, such as the physical growth rate of insects. Order of growth of ecosystems, classification of insect species using molecular techniques or biological structural analysis, education, and promotion. Especially regarding the correct collection of evidence (Saigusa, Kiyoshi, Masataka Takamiya, Masatoshi Matsumasa, and Yasuhiro Aoki, 2006) from insects at the crime scene and in the autopsy room, to reduce errors in estimating the period after death. Therefore, it is important in the field of knowledge in forensic science and entomology. However, entomologists must still study, research, and search for new knowledge in order to put it to maximum benefit in solving problems that arise and providing equal justice.

Broadly speaking, forensic entomology is the study of insects applied to any sort of legal issue; as a general formula, one could use *insects + law = forensic entomology*. That means every time insects are involved in a situation that requires the intervention of the law, it could be a case where forensic entomology is applied (Gemmellaro Denise,



2017). Following this logic, forensic entomology is divided into three branches: 1) medical entomology (medo-legal entomology), 2) urban and in-house entomology, and 3) store product pests (Lee, 1989).

Medical Entomology (Medico-legal Entomology)

This section focuses on the criminal component of the legal system and deals with the necrophagous (or carrion)-feeding insects that typically infest human remains. Insects are related to humans and cause disease through contact with secretions, touching a wound, or eating infected food. The insects or arthropods that are related and easily contaminated are maggots, flies, cockroaches, mosquitoes, mites, ticks, etc. They are vectors and carriers of diseases in humans, such as dengue fever, malaria, elephantiasis, *typhoid fever*, etc.

Animal diseases, or diseases transmitted from animals to humans, are called "zoonosis." This includes diseases from humans that are transmitted to animals as well. Some people clearly call it "reverse zoonosis" or "anthroponosis." (Zoonosis, 2015).

Urban and in house

This aspect deals with the insects that affect man and his immediate environment. This area has both criminal and civil components, as urban pests may feed on both the living and the dead. The damage caused by their mandibles as they feed can produce markings and wounds on the skin that may be misinterpreted as prior abuse. Urban pests are of great economic importance, and the forensic entomologist may become involved in civil proceedings over monetary damages. Insects can also affect the interpretation of blood spatter pattern analysis. Roaches simply walking through pooled and splattered blood will produce tracking that may not be readily recognizable to the untrained observer. Specks of blood in unique and unusual areas (such as on ceilings) may mislead crime scene technicians unless they are aware of the appearance of blood-contaminated roach tracks.

Store Product Pests

These insects are commonly found in foodstuffs, and the forensic entomologist may serve as an expert witness during both criminal and civil proceedings involving food contamination (Byrd J. H. and Tomberlin J. K., 2020). Examples of this could be moths we

find in a rice box stored in our pantry or beetle-infested cereals in a warehouse (Russell W. C., 1947). In situations like this, it is important to find the source of the infestation to assess some responsibility. Are the moths in our rice there because we left the package open for too long? Or, is the box completely sealed, meaning the infestation occurred before that package got to our pantry, possibly in the supermarket or, even earlier, when the rice was being packaged? The entomologist, in this case, would be responsible for identifying the insects in question, determining their development stage, and, based on their knowledge about the insects' feeding behavior, typical habitat, and biology, giving their opinion on when and how the infestation might have occurred (Gemmellaro Denise, 2017).

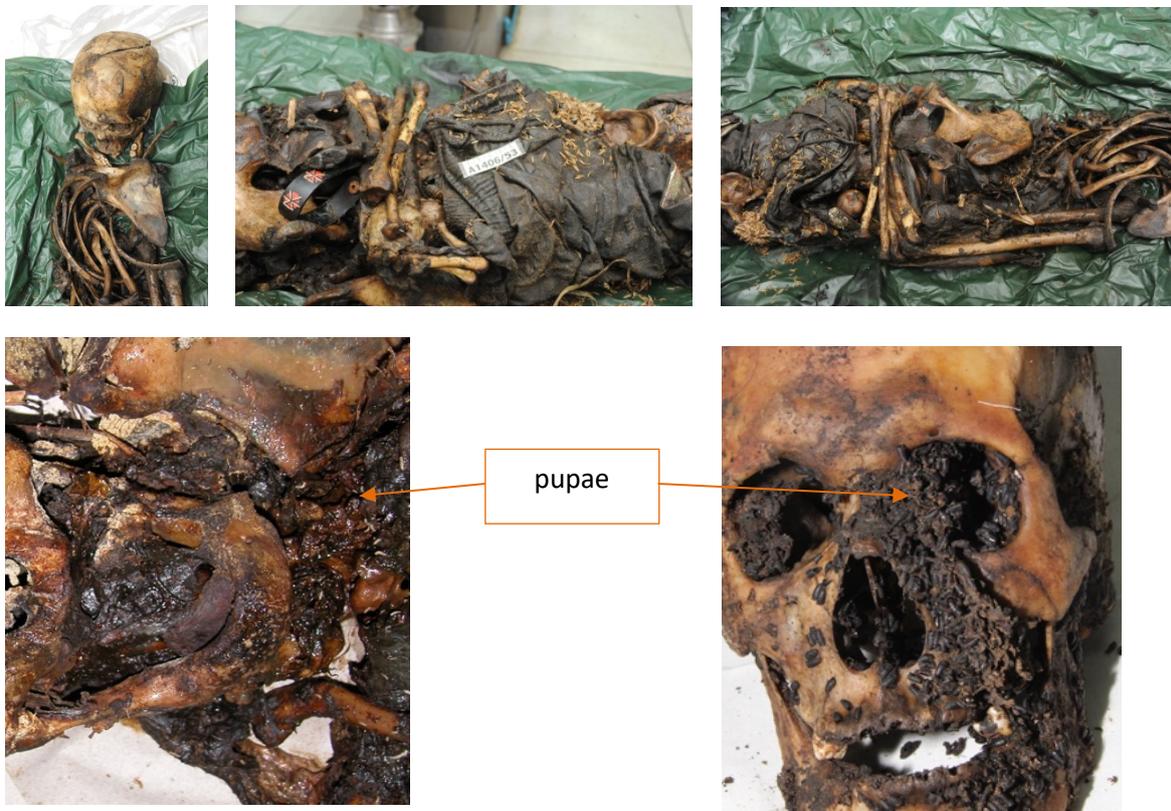


Figure 1 Body

CASE STUDY

The unknown body was found in the jurisdiction of Bang Sa-Rhong Police Station, Pattanee Province, in the south of Thailand. The body decayed and withered, leaving only the skin and bones; the hair fell off; and the skin disappeared; some turned brown and black. Pupae and maggots were found on the bodies as shown in Figure 1. In general, flies lay eggs, taking about 1-2 days to hatch from maggots, depending on many factors such as temperature (Deonier CC, 1940), light, humidity, etc. These factors are all interrelated and



are especially important in assessing the post-mortem interval (PMI). (Aballay, Fernando H., M. Cecilia Dominguez, and Florencia Fernandez Campón, 2012).

This research was done by a professional entomologist who worked and collected insects and arthropods for more than 20 years to make minimal mistake in estimating PMI (Matuszewski, 2021) or $_{\min}$ PMI (minimum post-mortem interval) (Sharma Ruchi, Rakesh Kumar Garg, and J. R. Gaur, 2015).

Study Limitations

1. Lack of scientific equipment, chemicals, and laboratories that will be used to identify insect species.
2. Lack of staff with the knowledge of properly collecting insect specimens from corpses at crime scenes and bringing them to the laboratory in order to further identify the species of insects.
3. Lack of entomologists with knowledge and skills in forensic entomology. The lack of forensic skills among entomologists can lead to data corruption, such as improper storage, photography, etc.

MATERIAL AND METHOD

The sample was taken from the corpse by the entomologist. Creamy-white maggots, and robust round-shaped pupae were found. During the collecting process, Rowe (2007) suggested that the following information should be recorded properly:

1. Details of the terrain: city, countryside, desert, forest, grassland, etc.
2. The place where the body was found: whether the ground condition is sandy soil, loamy soil, clay rock, hillside, plain, whether there is a road through it, etc. Type of plant found: Aquatic plants, endemic plants, etc. If you don't know, ask a botanist.
3. Date, time, and temperature
4. Species of insects (arthropods) found
5. Case number and Recorder's name

In taking notes on the collection of samples from the crime scene, photography is also an important part of helping researchers work more efficiently (Lee and W. D. Lord, 1994). In addition, photography plays a crucial role in forensic investigations, especially at crime scenes. Find below for important aspects for consideration:



1. **Documenting Evidence:** Crime scene photographers capture detailed images of evidence, including bloodstains, weapons, and other relevant items. These photographs serve as a visual record, preserving the scene's integrity and providing investigators with essential information.
2. **Precise Measurements:** Unlike sketches, which may lack accuracy, photography allows precise measurements of an item's location and position relative to other objects within the scene. This information aids in reconstructing events and understanding spatial relationships.
3. **Three-Dimensional Evidence:** Photography captures impressions (such as footwear or tire marks) in their original three-dimensional form. While photographs are useful for identification, casting these impressions provides even better evidence. Photographs are taken before casting to ensure documentation in case the cast fails.
4. **Overall Scene Layout:** High-quality photographs from various angles reveal the overall layout of the crime scene. This includes the positioning of evidence, bloodstain patterns, and other critical details. Such visual documentation assists investigators in piecing together what happened.

Specimen Collection Step:

Collection of entomological evidence at the case samples of insect live and preserved samples:

- Preserve samples include specimens that were placed directly into 80% ethyl alcohol: maggots and pupae (label the details).
- Collect live specimens of maggots and pupae from the same areas as the preserved samples (label the details too).
- Place approximately 50–75 maggots and pupae of representative sizes on a food medium (raw pork or beef) and wrap loosely in aluminum foil. Be sure that the container lid is ventilated with very small holes to avoid escaping maggots. Place the raw meat into a rearing container with ½ to 1” of vermiculite (pupae no add meat) (Saigusa, Kiyoshi, Masataka Takamiya, Masatoshi Matsumasa, and Yasuhiro Aoki, 2006).



- Separate the live maggots and pupae into additional containers and record the details as mentioned above.
- After collecting live maggots and pupae from the scene, feed them (in a temperature room) until they are adults to confirm the species of fly. It's useful for the entomologist to estimate, as close as possible to the truth, the t_{min} PMI.

Slide Preparation: Maggots

1. Take the insects (specimens) collected from the corpse and then bring them to the cutting laboratory and place them in the cassette. Bring the cassette to the automatic tissue processor for the preparation process, treated with a chemical solution so that the treated specimen is sufficiently hardened to be sectioned (Adams and Hall, M. J. R., 2003).
2. Prepare the chemical specimen through an automatic tissue processor and embed it in a liquid wax medium contained in a mold of various sizes according to the size of the specimen using a tissue embedding machine. The hardening medium will support the specimen in such a way that it can be easily cut into thin tissues 3-5 microns in size (making permanent microscope slides of insects) and also record the number of blocks in the embedding record. Insert the cartridge with the tissue embedding center (Alba-Tercedor and J., 2016).
3. Take the embedded specimen into the paraffin block and cut it into thin tissue (paraffin section) with a rotary microtome. Normally, we will cut the thin, flat tissue, 3-5 microns in size, with the tissue plate embedded on a glass slide. (Nelson Riley C. and Karin Gastreich, 2001).
4. Put the glass slides with paraffin sections into the hot air oven by adjusting the temperature into two ranges: 90–110 °C for 15-20 minutes and 60–65 °C for 30–60 minutes (STEMpedia, 2022). The adhesion of the paraffin section to the slide surface prevents the section from falling off easily during staining. (Slide Mounting Techniques for Very Small to Microscopic Animals) and recorded in a record of slide incubator drying (Nelson Riley C. and Karin Gastreich, 2001).
5. Glass slides containing paraffin sections were stained using conventional staining methods (hematoxylin and eosin staining) using an automatic slide stainer (prepared microscope slides: insect specimen).



6. Take a glass slide with a paraffin section for tissue staining and cover the slide. By covering it on the tissue, using a mounting medium to help and fix the cover glass firmly on the slide. To promote easy and convenient microscopic examination and a good way to preserve specimens.
7. Once the biopsy slides are completed, they are analyzed under a microscope.

Steps to Study Pupae by Micro-CT Scans

Principles of operation of an of an X-ray imaging machine with a small computer (X-Ray Computerized Tomography Scan), using techniques to photograph the sample material with X-ray radiation without destroying the sample and relying on computer processing to create three-dimensional images (3D) (Keklikoglou K. et al., 2019). Each pupa was preserved in 80% ethanol, dehydrated in ethanol, transferred into hexamethyldisilazane for several hours, and air dried for 24 hours. No stain was used. Pupa was scanned with a SkyScan 1172 microtomograph. To scan the whole pupa, the following were used: voltage (~ 80 kV) and current (~ 50 – 100 μ A) were customized for each scan based on pupa size and density to obtain maximum signal-to-noise ratio and optimal contrast. The reconstruction of the raw image dataset and “cleaning” were performed using the Bruker-Skyscan free software to reconstruct and process the images, permitting not only reconstruction but also virtual slices used to achieve the final volume rendering reconstructions. A detailed description of the procedure was published (Alba-Tercedor, 2014).

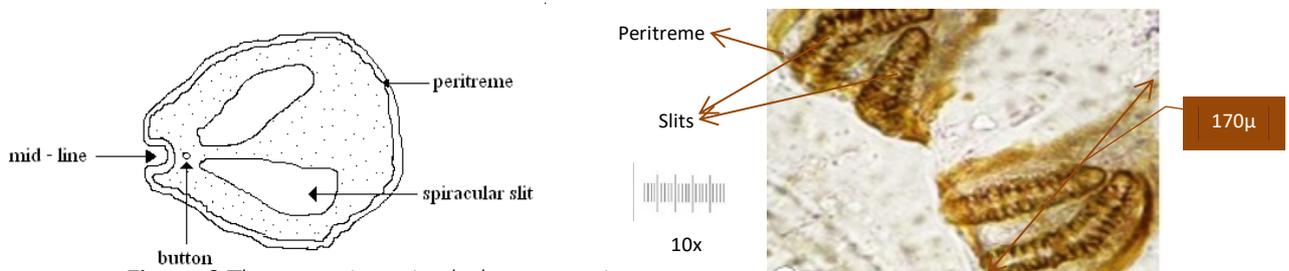
Raising Maggots to Become Adults

Raising maggots and fly pupae to become adults makes it easier to classify types. The process of raising in the laboratory is controlled at room temperature, humidity, and light. The optimum temperature is 30°C – 35°C (± 3 $^{\circ}\text{C}$), and the humidity is in the range of 70%–75%. The appropriate light period must not be less than 2,230 lux. In the laboratory, maggots and pupae are grown to adults successfully. They were classified according to taxonomy and systematic principles under a microscope (Natural History Museum in London).

FINDINGS AND RESULTS

Maggot

1. Studied morphology under the microscope of maggots. It is creamy white, the shape is slender, and it looks like a grain of rice. Body length is about 0.6 cm–1.2 cm. Classified as an Order Diptera (di = two, ptera = wings) refers to a group of insects with two wings, which are the flies.
2. The general body shape has a pointed head and an obtuse tail. The head has a mouth hook, and the tail has a pair of posterior spiracles (Jason H. Byrd and Jeffery K. Tomberlin, 2020).
3. Microscopic analysis of second- and third-stage maggots. Inside the posterior spiracle, two and three spiracular slits are found, respectively (Figure 2, Figure 3), and each piece is an oval fragment.
4. When examining by sectioning, to identify which family of maggots belongs to the family, specimens must be processed for at least 1 day for each specimen. A complete slide will be provided to study the life cycle, habitat, and behavior of each maggot species identified for the purpose of estimating the time of death (post-mortem interval = PMI) as close to true as possible (minimum post-mortem interval = min PMI)



Pupa

1. Studied morphology by micro-CT scanning found the pupae of fly brown color, long and slim-shaped size 2.5 mm–5 mm. in length.
2. The pupa has a hard skin, or obtuse term. Figure 4: The insect's appendages are fused or "glued" to the body wall as the exoskeleton hardens. Many obtuse pupae are enclosed within a cocoon (Hadley, D., 2019).
3. When the adult fly is ready to emerge from the pupa, the fly inflates a small bladder on top. This bladder presses against a weak point in the top of the pupa, causing a

circular rupture in its wall, which pops upwards to form a lid, allowing the adult fly to escape.

- The pupae that are found on the corpse have four stages. All of them are blowflies in the family Calliphoridae. It's used in forensic science at crime scenes to estimate the minimum period of time since death (minimum postmortem interval = min PMI).

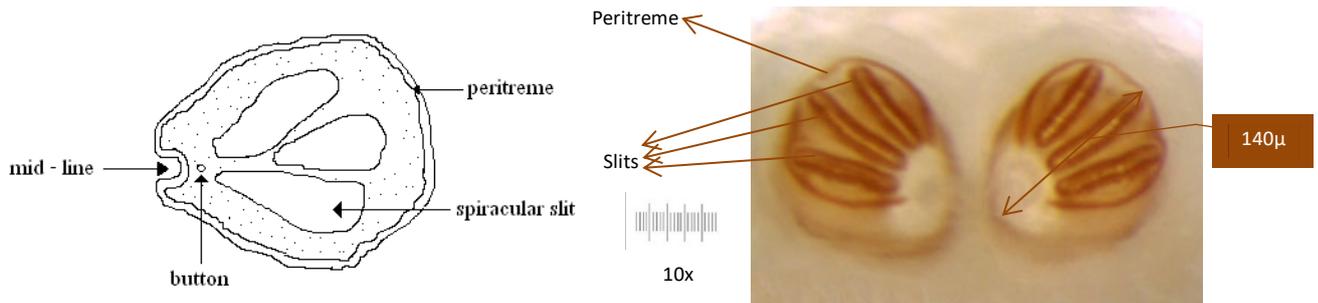


Figure 3 The posterior spiracle has three spiracular slits was shown third instar pupa.

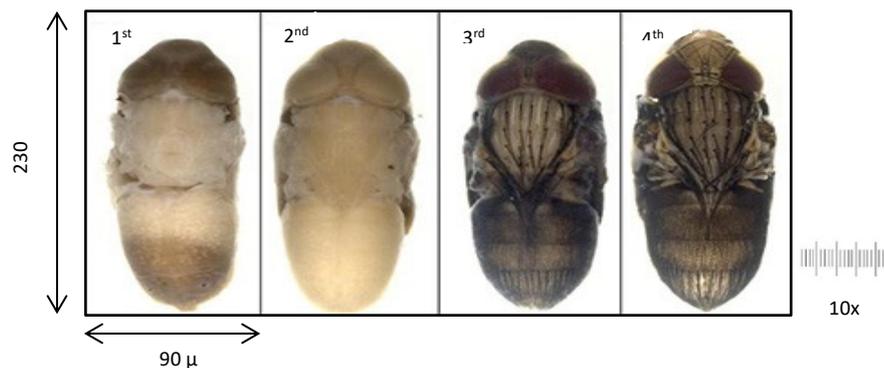


Figure 4 The Object terms of blowfly. From left to right; first instar pupa, second instar pupa, third instar pupa, and fourth instar pupa under micro-CT scanning.

DISCUSSIONS

By making a section, cutting off the tip of a maggot to make a slide, and looking under a microscope to find spiracles, which have a function in breathing comparable to the human nose, entomologists use this spiracle section to classify maggots. They have 2 and 3 spiracular slits, respectively. Spiracular slits have a straight-line shape, as shown in Figures 2 and 3. As for the pupa, it is an object; that is, the pupa has a clearly visible head, wings, and legs, as shown in Figure 4.

Both the maggot and pupa were raised in the laboratory until they reached adulthood and were found to be blue bottle flies, Order Diptera, family Calliphoridae (Davis J. B. and Goff M. L., 2000). Order Diptera have a complete metamorphosis (Davies L. and Ratcliffe G. G., 1994), with a total of 4 stages, namely egg stage, larva stage, pupa stage, and



adult stage. The average blow-fly life cycle is 6–8 weeks (Davies, 1994). The slower or faster life cycle of the blowfly depends on temperature, humidity, sunlight, and the flies' food (Deonier, 1940). Blow flies prefer to lay their eggs on carcasses or on wounds, especially rotten wounds or carcasses. Maggots are saprophytes; some live in dung; and some are parasites of vertebrates. Some species feed on the blood of birds (avian) or others may inhabit or cause ulcers in humans or domestic animals (myiasis) (Zumpt, 1965). Blow flies are highly attracted to odors, such as the smell of human or animal corpses or the smell of blood, lymph, etc.

However, to know what kind of fly it is, we still need to study it along with the decomposition of the carcasses. Which is easily divided into 4 steps as follows:

1. **Fresh:** at this stage, the corpse is usually intact and free of insects. The corpse undergoes algor mortis (reduction of body temperature to ambient temperature), rigor mortis (temporary tightness of joints due to chemical changes in muscles), and livor mortis (pooling of blood on the side of the body that is close to the floor). After death appears fresh from the outside, the bacteria that, before death, were feeding on the contents of the intestine begin to digest the intestine itself.
2. **Bloat:** the body's own digestive enzymes (normally in the intestine) also spread through the body, contributing to its decomposition.
3. **Decay:** bacteria break down tissues and cells, releasing fluids into body cavities. They often respire in the absence of oxygen and produce various gases, including hydrogen sulfide, methane, etc.
4. **Dry:** all the remaining flesh is removed over this period, and the body dries out. The surface of the body that is in contact with the ground becomes covered with mold as the body ferments.

In this case, it was found to be in the rotting stage (Decay). In order to diagnose the time of death as close to the $_{min}$ PMI as possible, in addition to using insects together with experienced entomologists, we also need to study the decomposition of the carcasses. So there will be accuracy to estimate deaths as close to reality as possible $_{min}$ PMI. All the information is beneficial to entomologists, forensic scientists, and investigators.

The presence of 2nd and 3rd maggots and pupae of blowflies on the corpse suggests that the corpse had been dead for at least 30 days, which is a forensic entomology estimate of mortality based on studying the life cycle, behavior, and habitat of insects. Relatively,



to predict the minimum time since death in the post-mortem interval (t_{min} PMI), which is very useful in estimating mortality (post-mortem interval = PMI) after death. There are key matters regarding findings, see follows:

1) **30-Days as a period:** According to ecological studies, the important breeding grounds for blowflies include:

- Animal manure: Garbage piles in these areas are moist and soft, suitable for the breeding of flies.

- Division of sewage and waste from food factories.

- Remnants of rotten organic substances, including various food scraps, garbage piles from the market and buildings. It is a suitable breeding ground for flies.

2) **Resting place:** Flies prefer to land on rough surfaces rather than smooth ones. In Thailand, it is found that flies hang everywhere outside of buildings such as markets, shops, restaurants, and grass or various weeds. But if it is very hot outside, it will stick in the shade. Especially during rainy and windy periods. Flies will stay in place.

3) **Spread:** Flies can fly with great flexibility. By nature, flies are rarely mobile, and will rarely fly long distances. It will fly around breeding grounds and food sources. It will rest within a radius of 100 - 500 meters from the food source.

4) **Life cycle:** Thailand is located in the equatorial region where is suitable for the growth of flies. From rearing, the life cycle from egg to adult takes about 15–25 days (± 5 days), depending on a temperature range of 27–35 °C (± 3 °C), the humidity is in the range of 70%–75%. The egg stage takes 12–24 hours to hatch into a maggot. There are a total of 3 ages: 1st, 2nd, and 3rd age maggots. The maggots take about 10 days (± 5 days) to pupate. After that, they will live for an average of 10–20 days (± 5 days).

CONCLUSION

The post-mortem interval (PMI) refers to the time that has elapsed since an individual's death. In forensic investigations, estimating PMI is crucial for determining the timeline of events surrounding a death. Insects play a significant role in this estimation. Here's what we learn from research on predicting the PMI based on insect evidence:

1. **Forensic Entomology and PMI Estimation:** Forensic entomologists use insects found on human cadavers to estimate the PMI. The accuracy of PMI estimation is



essential, as it directly impacts investigations. The goal is to reduce estimation inaccuracy by improving methods and understanding insect behavior.

2. Research Areas for PMI Estimation Improvement: In development and succession of Carrion insects, understanding the life cycles of carrion insects (such as blow flies and flesh flies) is crucial. Researchers study the growth stages, development rates, and succession patterns of these insects on cadavers. In thermogenesis in Larval Aggregations, insects generate heat during their life stages. Investigating thermogenesis in larval aggregations helps refine PMI estimates. For error rates of PMI estimation protocols, validating and improving existing PMI estimation protocols is essential. Researchers assess the accuracy and reliability of these methods. Lastly, educational and promotional challenges, collecting insect evidence requires proper training and awareness. Educating investigators and promoting best practices are ongoing challenges.

3. Laboratory Studies and Developmental Rates: Blow flies and flesh flies are particularly useful for PMI estimation. Scientists establish developmental rates of necrophagous species based on constant temperatures on laboratory environments.

4. Temperature-Driven Estimations: Temperature plays a crucial role in insect growth. The area under a curve between upper and lower temperature thresholds represents accumulated degree days. These degree days help estimate the time since death based on insect growth speed.

Recommendation

Ongoing research focuses on refining PMI estimation by studying insect behavior, validating protocols, and addressing educational challenges. Forensic entomology continues to evolve, contributing valuable insights to death investigations.

Conflict of Interest

The author declares that there are no conflicts of interest.

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