

THE HIDDEN WITNESSES TO MURDERS: AN INTRODUCTION TO FORENSIC ENTOMOLOGY

POORVA KASTURE

School of Molecular and Cellular Biology, University of Illinois Urbana-Champaign

ABSTRACT

Forensic entomology, the study of insects in legal investigations, plays a crucial role in determining the postmortem interval (PMI) and other factors that can help determine the time since death. For instance, forensic entomology has applications in identifying toxicological influences and helping reconstruct potential crime scenes. This article aims to provide an overview of forensic entomology in the context of how it is used to determine the postmortem interval, providing a general overview of the techniques used—such as successional waves and entomotoxicology—and highlighting its significance in its field. Furthermore, future implications and interdisciplinary collaborations will be explored, redefining the field with new technological innovation. However, potentially concerning impacts from climate change may arise as a result. By highlighting both the present and future, this article aims to showcase the nature of forensic entomology in modern criminal investigations as a valuable tool to bring justice to victims of violent crime.

SECTION 1: THE INTRODUCTION

WHAT IS FORENSIC ENTOMOLOGY?

Forensic entomology is considered the study of insects and arthropods in criminal investigations (Joseph, Mathew, Sathyan, & Vargheese, 2011). Often, a decomposing body may be past the point of when traditional techniques, such as rigor mortis (or body stiffness), liver mortis (the settling of blood in the lower parts of the body after death) can be used. These techniques may only work past a certain point after death, usually within 3-72 hours after death (Bucholtz, 2024; Eden & Thomas, 2025; Shrestha, Kanchan, & Krishan, 2025). After this point, insects that colonize the body in search of food and warmth may be used to estimate the time of death. Forensic entomologists may study the changes in insect population and developing larvae to estimate the postmortem interval (PMI), which is the time interval between physiological death and the examination of the deceased person (Munro & Munro, 2008).

IMPORTANCE OF FORENSIC ENTOMOLOGY

Deceased bodies can be thought of as mini ecosystems with insects and other microorganisms interacting with the abiotic, “deceased-body” environment. Because of these interactions, insects reproduce on the body and use the abundance of resources offered by a deceased body—such as warmth, food, and space. Given the complexity of crime scenes, forensic scientists try to answer the six W’s: why, who, where, how and what. Insects can provide a whole host of clues that can lead to answers to these questions (Viero, Montisci, Pelletti, & Vanin, 2019). For instance, they can help identify any evidence of abuse and/or neglect through the presence of maggots in open wounds and unclean areas or on clothing. Of note, the higher the number of maggots potentially found in wounds, the higher the likelihood of abuse/violence prior to death (Anderson, n.d.). Additionally, they may provide vital information about the location of death. The presence of a certain species of insect could show where a body was found after death, due to specific environments known to support specific types of insects (Anderson, n.d.; Wyland & Wyland, 2024). Insect evidence collected from the body may be used to then formulate further clues. Another important contribution from insects is their role in

identification through DNA analysis. A larvae’s stomach contents can be analyzed to, first, identify the victim and, second, link a potential suspect to the crime scene. One study suggested that short tandem repeat (STR) analysis of a maggot’s crop content can be used to associate maggots to crime scenes (Kondakci et al. 2019). STR analysis is a DNA profiling technique that compares the number of repeated sequences in DNA regions between samples, often involving secondary techniques (Barcelos et al., 2019). Combining this with further clues from the investigation may help direct detectives to the culprit or give an alibi to another suspect. For this reason, insects can be considered the tiny witnesses to crimes, and provide crucial evidence for catching the correct culprit.

SECTION 2: THE TECHNIQUES OF FORENSIC ENTOMOLOGY

THE USE OF INSECT LIFE CYCLES AND SUCCESSIONAL WAVES

Even though a multitude of different techniques are used for analysis, this article aims to shed light on some of the most common but crucial techniques that can help provide clues to investigators. Particularly, this article will emphasize two main techniques and their methods: Use of successional waves and insect life cycles and entomotoxicology and other chemical analyses.

It is also Important to note that every case is different: it is not necessary that investigators use the same techniques each time a body needs analysis due to the complex interplay of factors that may determine technique selection. For example, the stage of decomposition, presence of wounds, weather conditions etc can all influence what techniques will provide true evidence to point investigators in the right direction (Bambaradeniya, Magni, & Dadour, 2023).

Overall, this section will cover the methodology, applications, and limitations of the techniques listed above, and highlight their importance in uncovering crucial evidence that contributes to providing justice to victims.

As defined by the Oxford English Dictionary, the process of succession in ecology is “a process by which a plant or animal community

successively gives way to another until a stable climax is reached.” In this context, when one insect colonizes a body, it interacts with that body (e.g. through its consumption, or use of a body’s space as a breeding ground). As a result, it changes the corpse’s conditions, which after an insect’s death, becomes more suitable for another species to colonize. This cycle continues until a stable climax community is reached, a situation where one species may dominate, or several species may coexist. This technique is also related to the estimation of the postmortem interval and is applicable mostly to later stages of decomposition (Sharma, Garg, & Gaur, 2015).

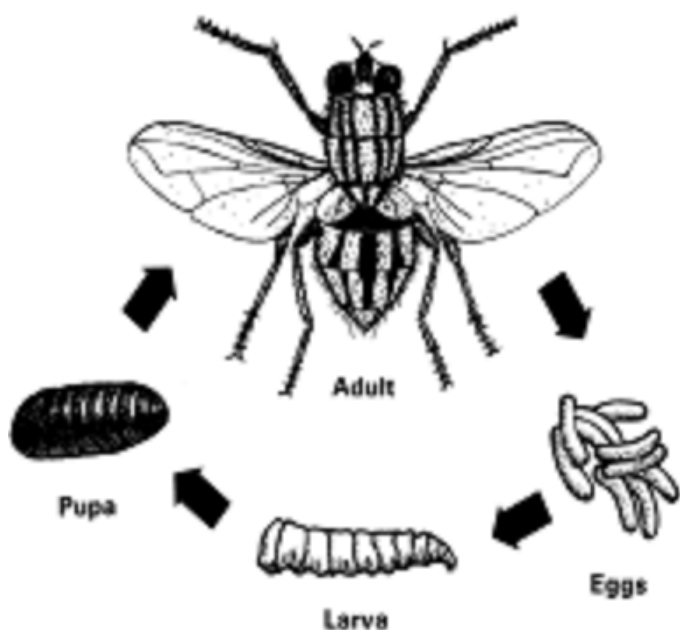


Figure 1: The life cycle of a fly (Illinois Department of Public Health, n.d.)

To use this technique, however, insect life cycles also need to be considered. For most insects, their life cycle consists of 4 stages: the egg, larva, pupa and adult. Typically, the greatest number of species will be attracted to a body during putrefaction, a stage of bloating that typically happens after days 2-7 of death. This leads to the first step of estimating the PMI: identifying the species of insect present. Next, the age of the larva can be estimated by measuring the length or dry weight of the oldest larva, comparing it with reference data found in a database. It is also important to note that the rate of development of a larva is dependent on the surrounding temperature. Thus, it is important to obtain a thermal history

(the history of temperature changes for that environment), which can thus be compared to the temperatures at the death scene. The PMI can then be estimated by backtracking the life cycle of the identified species (Sherlock Institute for Forensic Science, n.d.). The equation for this can typically be expressed by Accumulated degree hours (ADH):

$$\text{ADH} = (\text{temperature at crime scene} - \text{base temperature}) \times \text{time elapsed}$$

The base temperature corresponds to the minimum temperature at which the insect can develop, and time elapsed refers to the duration of each developmental stage at that temperature (Franceschetti et al., 2021).

Other factors also may affect insect activity—such as day length, oxygen levels and food quality. In terms of colonization sequence, flies arrive within a few hours of death, attracted to odors of decomposition. These flies are typically the most useful for estimating time of death. These are followed by carrion beetles, which typically arrive within a few days. Following this, carpet beetles, which consume the bone, hair and skin, may arrive during the dry stage of decomposition (North Carolina School of Science and Mathematics, 2013). Looking at this common colonization sequence, succession can be seen in action, as some insects prefer food sources more available during later stages of decay. These later stages are made possible through the interaction of earlier insect species, which make conditions more favorable for these later colonizers. As a result, using a combination of insect life cycles and cycles of succession can help estimate the time since death.

Another aspect to note is that obtaining live specimens on crime scenes requires no special training and minimal equipment, meaning that CSI personnel can learn to collect samples quickly and at a low cost (Volckaert, 2020). However, it is also important to note that environmental factors, such as humidity and temperature can affect the rate of development of insects, and thus the speed of successional waves, which may lead to an overestimate or underestimate of the time since death. Even though temperature is considered in the equation above, other factors, such as

humidity or wind, are not. These factors can also affect development, and therefore, successional waves.

ENTOMOTOXICITY AND CHEMICAL ANALYSES

Entomotoxicology is a relatively new branch of forensic entomology, where insects are used to detect drugs and other toxins in decomposing tissues. It also investigates the effects caused by drugs and toxins on arthropod development. The use of this technique may be especially helpful for the recent increase in drug-related deaths, connected to poisoning or overdoses (Introna, Campobasso, & Goff, 2001).

The technique works by using techniques such as gas chromatography (GC) and thin layer chromatography (TLC), which can help separate and analyze the components of unidentified fluids. These methods involve the use of a stationary phase (a starting point for any substance, which doesn't move) and a mobile phase (often a gas or solvent that can move throughout a column and help substances separate through their density, or solubility in the mobile phase). Often, to incorporate this technique, samples of insects will be homogenized and dissolved in a solvent to then go through GC or TLC.

However, this is not the only way that chemical analysis can be used: drugs and toxins can also influence the rate of development of certain insects. For instance, one study found that, in toxin-contaminated tissues, maggot development accelerated 36 hours after hatching. This was specifically observed on toxin-contaminated liver or spleen tissue. This pattern is abnormal as compared to normal maggot growth (Goff, Omori, & Goodbrod, 1989).

Additionally, another study demonstrated how these analyses can lead to real clues. In this study, a woman was found deceased in the early putrefaction stage (the bloated, discolored stage that occurs due to the build up of several gasses after death). Maggots found on the face and upper torso measured about 7.5 mm on average while just a single maggot from the woman's nostrils measured 17.7 mm, suggesting accelerated growth that was dependent on cocaine. A subsequent investigation revealed that the woman was a

cocaine abuser (Lord, 1990).

However, this technique does not only apply to the deceased. Chemical analyses can also be used to gather clues from cases of abuse or neglect. For instance, DNA analysis from the earlier blood feed of head lice can be used as markers for the length and frequency of abuse periods. One case found an elderly victim of neglect who had body lice present down to the torso, and the egg laying pattern suggested that the victim had suffered 2 years of continuous neglect (Lambiase & Perotti, 2019). Signs like these may help investigators protect the welfare of the elderly.

Additionally, pollen grains on corpses may also be analyzed as evidence. Even though it is usually thought of as a technique to identify climate change throughout history, by analyzing the pollen grains present (eg through identifying their shape, size and structure), and combining potential databases, pollen grains can be used to identify certain plant species (PaleoResearch Institute, n.d.). Thus, the original climate where the body may have initially been found can be identified. This is because different insects tend to prefer to inhabit different plants, and different plants grow better in some climates than others. This is especially helpful if the body is suspected to have been moved from its original place, again providing alibis to potential suspects, while connecting others to a particular location.

DRAWBACKS OF TECHNIQUES USED IN FORENSIC ENTOMOLOGY

One of the biggest drawbacks is environmental variability. Most insects require extremely specific temperatures and humidity levels to breed. Therefore, environmental variability can cause disturbances to the distribution and behavior of fly larvae (Bansode et al., 2025). As a result, forensic investigations relying on insect evidence might be compromised due to unpredictable environmental conditions that can alter larval growth, timeline of development, and overall patterns of distribution.

Additionally, the presence of non-forensically relevant insects can also make establishing successional waves and back-tracing patterns extremely challenging. Most of the time, these

insects may be present on a corpse because they are attracted to a corpse to feed, as predators, on the necrophagous insects (the insects consume the rotting flesh of a given corpse) that are already present (Amendt, Richards, Campobasso, Zehner, & Hall, 2011). However, these patterns do not always occur simultaneously on a corpse. They can also be predicted chronologically, but 2 conflicting waves of succession may be harder to differentiate as the cadaver further decays.

Deceased bodies may also be found in an extremely wide range of conditions – some cases have been found in dumpsters, while others have been found in places such as water tanks (Elisa Lam case), or inside of a dinosaur statue (Jones, 2021). Due to each case having the potential to be extremely unique, there may not be any previous data (e.g. temperature ranges or humidity estimates) available for certain regions, as making estimates relies on data that is region-specific. As a result, estimates of the PMI may be challenging to make in certain cases.

SECTION 3: FUTURE IMPLICATIONS AND CONCLUSIONS

IMPACTS OF CLIMATE CHANGE

As mentioned previously, environmental factors, namely temperature, can have massive impacts on insect development rate and potential speed of successional waves. However, climate change is now shifting the global distribution patterns of necrophagous insects, potentially shifting species that are typically found near the equator north due to global warming. This could affect their ability to colonize cadavers at crime scenes, complicating the calculations of the estimations of PMI. However, climate change is also causing a general decline in insect populations due to higher use of pesticides, deforestation, global warming and pathogens, to name a few (Amendt, 2021).

Rising global temperatures may also lead to faster insect development, and therefore an underestimation of the PMI if standard models are used (Amendt, 2021). Climate change can also cause unpredictable weather patterns, potentially resulting in patterns that lead to colder winters and warmer summers. These more unpredictable patterns may lead to an

inconsistency within models currently used to estimate PMI (Matuszewski, Szafałowicz, & Grzywacz, 2014).

TECHNOLOGICAL ADVANCEMENTS AND INTERDISCIPLINARY COLLABORATIONS

There are now several advancements within the field, which now has an even greater potential for interdisciplinary collaboration. For instance, soil may contain enormous potential as an indicator for PMI because succession in microbes is vital to decomposers and is provided with ammonia rich fluids from the body (News-Medical, 2020).

Additionally, newer AI models may be trained on genomic information containing information on entire microbial communities. One method that has been used is machine learning. Specifically, a method called an artificial neural network (ANN), which involves developing algorithms to enable computers to learn from existing databases without explicit programming, has proved useful (Wang et al., 2022).

However, the most common type of machine learning algorithm being used for microbial community studies is the random forest (RF) regression model, which may be able to predict microbial succession patterns on a cadaver through capturing non-linear patterns in the several variables that may impact PMI estimations. This means that it could incorporate multiple variables such as species specific growth rates, humidity, and effects of drugs all at once (Belk et al., 2018).

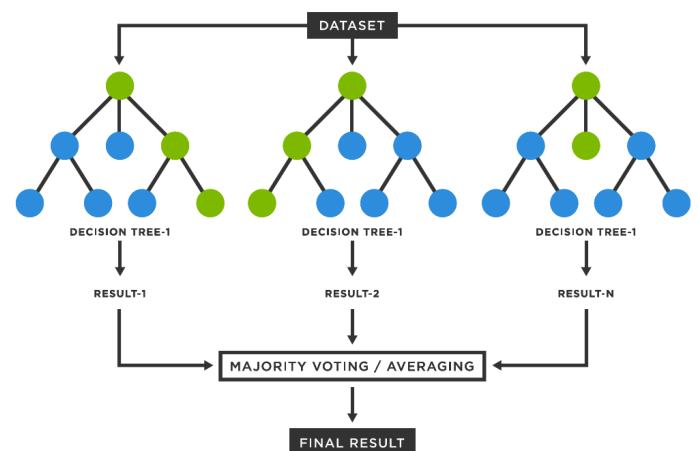


Figure 2: The mechanism behind the random forest model (Gunay, 2023)

Overall, the integration of RF models and microbiome analysis is a significant advantage over traditional regression models, offering greater accuracy and adaptability to diverse environments. However, it is important to note that these methods are still experimental and have not yet been officially approved as a standalone method for use in forensic entomology. Thus, further research may need to be conducted to validate their reliability.

CONCLUSIONS

Overall, advancements in techniques like entomotoxicology, DNA barcoding, and machine learning models such as random forest (RF) are helping forensic entomologists improve the accuracy and precision of estimating PMI and other clues that can find the true culprit of a crime. Collaboration between experts is also shown to be vital to make strides in the field, and can involve the collaboration of entomologists, CSI personnel, and even computer scientists. Despite earlier limitations, like environmental variability (which can affect insect development), advancements in technology such as the development of machine learning models have significantly reduced errors and the effects of limitations by increasing accuracy and precision of analysis, making entomology a more reliable tool in the justice system.

Additionally, interdisciplinary collaborations have the potential to play a vital role in advancing these methodologies. These collaborations have the potential to be much more thorough tools for analysis. Ongoing research and collaboration are vital pillars to keep up with environmental challenges, such as climate change and unpredictable weather patterns. Moving forward, continued innovation and refinement of techniques will be essential parts of keeping forensic entomology a reliable tool in the justice system.

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