DOI: 10.1478/AAPP.101S1A7

AAPP | Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Fisiche, Matematiche e Naturali
ISSN 1825-1242

Vol. 101, No. S1, A7 (2023)

THE USE OF FORENSIC ENTOMOLOGY WITHIN CLANDESTINE GRAVESITE INVESTIGATIONS

JASON H. BYRD[a*] AND LERAH SUTTON[a]

ABSTRACT. The use forensic entomology within clandestine gravesite excavations can provide valuable information for a medicolegal death investigation. Insect evidence can provide an estimate for specific portions of the *postmortem* interval as well as yield information concerning the possible treatment or movement of a body after death. Insect activity can even yield clues as to the possible wrapping of bodies in materials for concealment or during transport. In some cases, insect succession patterns can also indicate if the body was frozen or refrigerated prior to disposal in another location. Using techniques of forensic taphonomy to help locate clandestine gravesite better allows a forensic entomologist to identify, document, collect, and analyze evidence to help answer questions about the treatment, movement, location, and *trauma* of a body within a medicolegal death investigation.

1. Introduction

The history of forensic medicine, as we know it today, spans thousands of years. One of the earliest historically documented subfields within forensic medicine, that is still actively applied to medicolegal casework, is that of forensic entomology. Various translations list the year of publication between 1235 and 1247 *AD*, but it was in 13th century China that the first application of entomology to a death investigation was documented. An investigator named Sung Tz'u penned a volume entitled *The Washing Away of Wrongs* which detailed the case of a homicide of a local villager with the suspected murder weapon being a sickle. As this was a common tool possessed by many villagers, determining a suspect was difficult through traditional investigative means. Tz'u theorized that he may be able to use patterns of insect behavior to identify which sickle of the many in the village was used in the commission of the crime. He requested that all villagers bring their sickles to a common location during the heat of the day and lay their sickle down in from of them. Flies were attracted only to one sickle out of the bunch, due to the residual blood and tissue remaining on the blade. Confronted with this evidence, the suspect confessed to the crime, thus providing the first documented example of a homicide case solved using insects (Tz'u 1981; Benecke 2001).

Following the 13th century Chinese death investigation volume, little additional information related to the scientific and theoretical questions of entomology were published within the subsequent centuries. Through the 17th century, the concept of spontaneous generation was commonplace – likely due to limited understanding of ecological relationship between living organisms and their surrounding environmental conditions. One such example was the relationship between flies, maggots, and rotting meat; that is, many individuals during this time believed that flies and maggots – often called "worms" – were spontaneously generated from the process of decomposing meat. Francesco Redi, an Italian scientist – sought to disprove this theory in 1668 by devising a simple experiment. He set up 3 iterations of jars containing rotting meat, each with a different style of barrier between the rotting meat and the surrounding environment. In the first jar, there was no barrier between the environment and the meat – thus allowing flies unrestricted and direct access to the rotting meat. In the second, there was a thin gauze barrier over top of the jar that physically restricted flies from accessing the rotting meat but did allow smells to permeate. In the third jar, a tight-fitting seal was placed atop the jar to prevent both physical interaction and olfactory interaction between flies and the rotting meat. The premise of this experiment was that flies and maggots were not, in fact, spontaneously generated by decomposing meat but rather, flies were attracted to the smell of the rotting meat and laid their eggs on it. When the eggs hatched, they turned into maggots which is what many believed to be the process of spontaneous generation. After allowing several days to pass and consistently observing the interaction of flies with each jar, Redi recognized and documented that his hypothesis was correct. In the first jar, flies were able to directly access the meat which allowed them to lay their eggs on the surface of the meat and maggots eventually appeared on the surface after the eggs hatched. In the second jar, flies were not able to directly access the meat but were still attracted to the jar itself. They laid their eggs atop the thin gauze barrier instead and maggots eventually appeared atop the gauze, rather than the meat itself. In the third jar, due to the airtight seal provided, flies were not attracted to the jar as the smell of decomposition was inhibited. The meat within the third jar did continue to rot, but no insect activity was observed. Thus, this simple experiment using three jars with different barriers affecting the attraction of flies helped to disprove the concept of spontaneous generation (Redi *et al.* 1675; Byrd and Sutton 2020).

While the Redi experiment helped to understand the use of insects to answer theoretical scientific questions, the direct application of entomology to medicolegal death investigations did not reach Western society until the 19th century. Dr. Bergeret d'Arbois, a French physician, was called to investigate the death of an infant near Paris in 1850. The current owners of the home discovered the mummified body of an infant within the plastered wall of their home while they were undertaking renovations. An investigation was conducted to determine whether the responsible party was the current homeowners or the tenants that previously occupied the home. A pupal casing of *Musca carnaria* (Linnaeus) (Diptera: Muscidae) was discovered in association with the mummified remains. This casing was then used to create a timeline of insect colonization, explaining that the remains were likely placed in the wall many years prior to discovery, thus implicating the previous tenants in the death of the infant (Bergeret 1855).

2. Forensic Entomology in Medicolegal Investigations

2.1. Subfields of Forensic Entomology. Forensic entomology is traditionally defined as the application of the study of insects and their arthropod relatives to legal investigations.

The concept of forensic entomology is often used synonymously with medicolegal investigations that utilize entomological evidence, but more correctly there are three separate subfields within forensic entomology: urban, stored product, and medicolegal. Urban entomology focuses on insect interactions within modern human society and environments. Typically, it involves cases related to insect infestations of dwelling, both residential and commercial. These cases may involve commonly observed insects including roaches, bed bugs, or flies. More often than not, these cases are related to the control and eradication of insects viewed as environmental pests and result in civil litigation but in some cases, there may be a criminal overlap if insect pests are feeding or scavenging on living or deceased individuals (Brundage, Byrd, and Sutton 2017). Stored product entomology focuses on the infestation or contamination of food products by insects. This infestation could be accidental or intentional and may occur as a result of improper handling, packaging, or storage of food products. Most food products have an associated level of allowable insect contamination, and it may fall to a forensic entomologist to determine whether or not the amount of insect material contained within a food product meets or exceeds the published allowable levels (Center for Food Safety and Applied Nutrition 1995). The medicolegal aspect is the subfield that is most commonly associated with forensic entomology and involves the study of the succession, colonization, and development patterns of insects associated with decomposing remains. It is the medicolegal subfield of forensic entomology that will comprise the primary substance of this article.

2.2. *Postmortem* Interval Estimation. The most common analysis requested of a forensic entomologist by law enforcement is to help determine time since death within a medicolegal investigation through the use of entomological evidence. There is not clear consensus within the forensic entomology community on the most appropriate or standardized terminology as it related to time since death estimations (Tomberlin *et al.* 2012; Wells 2019). Many entomologists have utilized the concept of time since colonization to assist in answering this question (Catts 1990; Faris *et al.* 2016). This relies on the premise that immediately after death, decomposing remains begin to release chemical signals including putrescine, cadaverine, and methyl mercaptan (Cammack *et al.* 2015). Insects are sensitive to these chemical signals and will seek out the decomposing remains for colonization – a process which may occur as quickly as a few minutes after death. It is this insect colonization of remains that is often responsible for the bulk of soft tissue loss in cases involving decomposition (Early and Goff 1986; Kreitlow 2009). However, there are numerous circumstances that may inhibit or delay insect colonization in a way that would make a direct correlation between the time of death and the time of colonization inappropriate or unreliable (Anderson 2019; Lutz *et al.* 2022). With this consideration in mind, many entomologists instead choose to utilize the concept of a minimum *postmortem* interval (Mohr and Tomberlin 2015). This effectively means that – using information gleaned from an entomological analysis most often involving insect development data – an entomologist can provide an interval of time that represents the minimum length of time the individual in question has been deceased (VanLaerhoven 2008). Entomologists may attempt to establish the pre-colonization interval, which this the time period between death and insect colonization by eggs and/or larvae. This pre-colonization interval may not have the level of repeatability and verifiable data that is often associated with a period of colonization as determined from growth and development data provided from the immature insects collected as entomological evidence (Byrd, Sutton, and Brundage 2022), but entomologists are working on the research needed to build that dataset (Wells *et al.* 2021). While factors including environmental conditions or mechanical barriers may present challenges to accurate estimations of the minimum *postmortem* interval, approaching this concept carefully with appropriate consideration of all variables that may play a role in a time since death estimation will still allow a forensic entomologist to provide valuable input into a medicolegal death investigation, particularly when numerous methodologies are used in tandem (Goff 1993; Wells and LaMotte 2019).

2.3. Movement of a Body between Locations. According to recent statistical surveying tools assessing the faunal richness of the living species on earth, new estimates indicate that there may be as many as 5.5 million species of insects on the planet. This is in stark contrast to the approximately 1 million species of insects that are currently known and named within the current taxonomic identification system – indicating the possibility that upwards of 80% of unique insect species on earth have not yet been discovered (Stork 2018). Advances in DNA analysis and molecular identification research methods for insect identification have dramatically improved species identification beyond the traditional morphological identification methods, with the first such study being published in 1994 on some of the most commonly observed blowflies species in North America (Sperling, Anderson, and Hickey 1994). Provided the baseline understanding that the faunal richness of insects is far greater than that of any other living organism – with insects representing nearly half of all identified species on the planet – it stands to reason that their widespread distribution across the planet can provide meaningful information within a medicolegal death investigation. The natural insect fauna at a scene within a particular geographic distribution is often very unique and can provide identifying information on the location in which a crime has occurred. This is particularly valuable when the species of insects observed on decomposing remains is compared to the expected species within a geographic region. If insect species found on the remains are inconsistent with those typically associated with the location the remains were discovered – that is, there are insect species on the remains that are not natural to the surrounding environment in which the remains were discovered – investigators can infer that the remains were moved from a primary location to a secondary location (Weidner *et al.* 2021). This would further indicate that the remains were initially colonized in a separate geographic region than where they were discovered and that the differing insect species were transported to the location at which the remains were discovered in the process of moving the remains themselves. This type of evidence may be crucial within a medicolegal death investigation as it provides information on the location at which the crime initially occurred and may provide insights into that location based on the association of insect species found on the body that are not natural to the area in which the remains were discovered.

3. Methodologies within Forensic Entomology

3.1. Insect Succession. The presence, absence, and order of arrival of certain species of insects associated with decomposition is a method known as insect succession. In some cases, insect succession – when associated with specific stages of decomposition – may help investigators determine a minimum *postmortem* interval. This is accomplished by associating known or expected sets of insect species with each distinct stage of decomposition (Mégnin 1894; Schoenly 1992; Kreitlow 2009; Goff 2010). This is a method that has been used to estimate time since death for over 100 years, but it is not always considered a consistently reliable or applicable in all medicolegal death investigation cases involving decomposing remains. Insect succession is typically less reliable in the early stages of decomposition as compared to the later stages of decomposition. That is, insect succession is less useful when remains have been decomposing for days rather than weeks or months (Payne 1965; Goff 1993; Mohr and Tomberlin 2015). Despite the limitations for use within an estimation of the *postmortem* interval, insect succession can be extremely useful as supplemental investigative tool (Wells *et al.* 2015). When comparing insect assemblages observed on remains to those that would be expected given a certain state of decomposition, differences between the expected and observed assemblages may provide insight into peri- or *postmortem* conditions. For example, if a body is observed in a state of advanced decomposition but the observed insect assemblage is more consistent with the early stages of decomposition, an investigator could infer that conditions of the remains were such that insect colonization at the immediate time of death was inhibited in some way, perhaps by wrapping or other means of concealment (Smith *et al.* 1986). While insect succession can provide valuable insights into a medicolegal investigation, due to its inconsistent reliability in estimating the *postmortem* interval, it is not a method that should be used alone. Rather, it is most valuable when used in tandem with other entomological methods (Wells and LaMotte 2019).

3.2. Insect Growth and Development. By comparison to insect succession, the use of temperature-based methods associated with the growth and development of insects is considered to be far more accurate and reliable for determining a minimum *postmortem* interval. There exists within the published literature myriad articles on the subject of temperature dependent rates of growth and development of insects. Comparing the size and life stage of insects observed in remains to the known and published rates of development of insect species can be used to provide an estimation of a *postmortem* interval (Greenberg 1990; Catts and Goff 1992). Insects are poikilothermic and have a temperature dependent development rate. That is, in warmer environments they develop faster and in colder climates they develop slower. In order to correlate the development data associated with a particular species of insects to those observed at a death investigation scene, a mechanism of standardizing temperature against time must be used (Matuszewski and Szafałowicz 2013). This method of analysis is called the "accumulated degree-day model" and serves to correlate the temperature dependent development of an insect with an estimated time since death (Briere *et al.* 1999; Worner 2008; Byrd and Sutton 2020). This method helps to provide a baseline estimate of the time it would have taken the insects observed on the remains to reach the particular life stage in which they were observed on scene. This, in turn, can be correlated with an estimation of a possible minimum *postmortem* interval by associating the amount of insect development observed with the temperatures associated with the crime scene and standardizing that association against time (Greenberg 1990; Catts and Goff 1992; Byrd and Sutton 2020).

3.3. Methodological Considerations. While the use of temperature-based methods of insect growth and development can be more accurate and reliable than insect succession, it is also necessary to recognize that there are factors one must consider when expressing an opinion on time since death regardless of method used. One of the most critical factors is that of proper identification of the insects observed on and collected from the remains. This must be completed to the lowest taxonomic level in order to most appropriately correlate the species with either insect succession data or growth and development data. One of the first comprehensive texts published is *The Blowflies of North America* which details adult identification of calliphorids along with suggested methods of collection and preservation for identification, although many subsequent publications beyond this landmark volume have provided supplemental information related to morphological identification of insects using dichotomous keys (Hall *et al.* 1948; Marshall, Whitworth, and Roscoe 2011; Jewiss - Gaines, Marshall, and Whitworth 2012; Picard 2013; Whitworth 2019).

The importance of an accurate identification of insects recovered from a medicolegal death investigation scene cannot be understated, but proper collection and preservation procedures are integral to an entomologist's ability to analyze entomological evidence. While each death scene may present unique challenges, there are certain guidelines that must be followed at every scene. These include the types of information and evidence that must be collected and documented to provide critical context for an entomological analysis. The most critical aspects of documentations are photography and temperature. Evidence – including insects – should be photographed in *situ* with and without scale. Numerous temperature readings should be taken at the death scene to include ambient temperature directly over the remains and in surrounding areas, soil temperatures, temperature of the interface between the remains and the soil surface, and many others. If evidence is being recovered from a clandestine gravesite, record the depth at which evidence is recovered. Collect a representative sample of all insects present on scene. A good rule of thumb is to collect all insects that appear morphologically different in addition to insects from all areas of colonization on or around the remains. Each of these types of collections should be kept separate and labeled in accordance with area of collection either on or around the remains (Brundage, Byrd, and Sutton 2017). While there are many published recommendations regarding insect collection and preservation methods, there is currently only one published chapter that has been reviewed and approved by the American Board of Forensic Entomology. This comprehensive chapter by Sanford *et al.* in Byrd and Tomberlin's 2020 edition of *Forensic Entomology: The Utility of Arthropods in Legal Investigations* represents a standardized approach to the proper documentation, collection, and preservation of entomological evidence. It provides stepwise instructions including photographic examples of the detailed aspects necessary for proper collection of entomological evidence (Sanford *et al.* 2019).

4. Forensic Entomology within Clandestine Gravesite Investigations

4.1. Locating a Clandestine Gravesite. Many of the traditional applications of forensic entomology are associated with readily discoverable remains such as those that have been deposited on the surface in an outdoor environment. A complicating factor within medicolegal death investigation cases is that of clandestine gravesites wherein a suspect attempts to conceal the remains from discovery through burial. There are myriad challenges to completing a thorough investigation in decomposition cases, but this becomes more difficult when the location of the remains is unknown. Often, investigators must rely on informants to locate even the general area of a clandestine grave and pinpointing the exact location is a more complex task. One must first be able to identify a clandestine gravesite as such prior to conducting a thorough excavation and subsequent analysis of the remains and any associated evidence.

Locating recently buried remains (*i.e.*, days or weeks) within a clandestine grave often proves less challenging than locating remains that have been buried for a longer period of time (*i.e.*, months or years). In the days and weeks after a clandestine grave has been dug in an attempt to conceal the location of remains, the soil and vegetation will appear markedly different from the surrounding environment due to the immediate changes that occurred as a result of the gravesite being dug. These changes may include broken or disarticulated vegetation and disturbed soil. An area of overburden – that is, excess soil that has been displaced from the gravesite itself – may be visible atop or adjacent to the gravesite. It is critical for an investigator to have a strong foundational knowledge and understanding of what is normal within the environment in order to better identify abnormalities that may be associated with a clandestine gravesite.

This same principle applies to entomological evidence within or associated with a clandestine gravesite. Entomological evidence may help to determine if the remains were colonized before burial. If an individual died at a primary location and was then transported to a secondary location for burial, insect colonization may have already begun. Thus, investigators may be able to associate different species of insects on the remains with a location other than where the body was discovered (Smith *et al.* 1986). There may also be comingling of insects on or around the remains and some insects may be present within a clandestine gravesite itself. Differentiating the types of insects associated with a clandestine gravesite may provide valuable insights into the investigation. Some may be interred with the body as the larval forms are unable to burrow/dig, while other species have larval forms that can dig (Walsh-Haney, Galloway, and Byrd 2019). Identifying and analyzing insects within the grave may provide an interment interval which can help to determine how long the remains have been buried.

4.2. Insects Associated with Clandestine Gravesites.

4.2.1. *Surface Species.* The insect species associated with clandestine gravesites can provide valuable evidentiary information. The presence or absence of specific insect species, as well as their growth and development can provide information on portions of the *post-mortem* interval, as well as information on how long the remains have been buried. Many insect species must have some form of physical contact with the decomposing tissue before oviposition or larviposition will occur. However other species may deposit their eggs or larvae at the closest point accessible to the source of the odor. In these cases, the newly hatched larvae may be able to can access bodies place in containers such as refrigerator and freezers, luggage, and plastic bags by successfully navigating mechanical barriers such as door seals, zippers, knots in bags. In natural environments where the remains may be buried, it is important to note that many insect species do not have the ability to dig or burrow into the soil and would not normally be recovered from a clandestine gravesite in which the remains were not colonized before burial, and in which the remains were completely covered by a layer of soil. Therefore, the investigator should document if any portion of the body is exposed at the surface. Often, if shallow clandestine gravesites portions of the body are left exposed, such as the hands and feet. If any portion of the remains are about the soil surface, this may allow for insect access to the entire body once the skin of the exposed areas had been broken due to scavenging by vertebrate animals, or through the process of decomposition. Once insect colonization of the exposed tissues has occurred, this will allow for insect access to the portions of the remains that are buried. If the investigator can document that the remains were completely covered by soil and the insect species recovered from the gravesite at species that do not have the ability to dig, the entomological evidence would indicate that the body was colonized prior to burial, and the insect evidence may be used to determine the time of colonization.

4.2.2. *Sub-surface Species.* It is not unusual to recover entomological evidence from clandestine gravesites. The larval forms of some fly species, as well as the adult and larval forms of many beetle species, can burrow/dig to reach and colonize completely buried human remains. The presence of these species from within the gravesite can be useful in determining how long the remains have been buried. The recovery and collection of entomological evidence from the gravesite can be challenging as the number of insects present is generally far less than found on surface depositions, and the insects may be crushed or damaged from excavation tools such as shovels and hand trowels. Many of the insects may be recovered during the sifting process for the excavated soil, and if recovered during the sifting process, the depth of the soil the insect was recovered from should be noted.

4.3. Environmental Considerations of Entomological Evidence.

4.3.1. *Refrigeration/freezing.* Refrigeration of remains after recovery will slow, but may not completely halt, insect development. The refrigeration of remains prior to deposition, as a form of temporary concealment, may impact the species succession that may inhabit the remains, but this is dependent on the length of the period of refrigeration. Freezing of the remains may have a larger impact on altering species succession due to the damage done to the cellular structure of the remains by the formation of ice crystals. Frozen remains may decompose faster after thawing due to the cellular damage. The altered rate of decomposition may also alter the pattern of insect succession and time of colonization.

4.3.2. *Wrapping/concealment.* Human remains wrapped in natural fibers such as bedding, will likely not deter insect colonization due to the ease of odor permeation through the weave of the fibers, saturation from decomposition fluids, and due to difficulty in completely wrapping the remains as to prevent access. In most cases, the wrapping of human remains is done for concealment from view, or for ease of transportation. It is usually not conducted by the perpetrator to prevent insect colonization. Therefore, such attempts at concealment may not delay insect colonization (Magni *et al.* 2019). It can easily access gaps in the wrapping of materials such as bed sheets, and first instar larvae may be able to penetrate through the fabric weave. However, plastic wrappings such as trash bags present a more formidable barrier to insect colonization. Plastic bags are not a woven material, so there is no access though the weave of the fabric as with natural fibers. Carron flies and their larvae do not have mouthparts that will allow for chewing through intact plastic bags, however, adult beetles and their larvae may be able to easily chew through thin plastic bags to access and colonize remains. All wrappings should be photographed in detail prior to being removed from the remains and re-photographed once removed and inspected for any rips or tears in the wrappings.

4.3.3. *Seasonality.* The presence or absence of insect species may be dependent on seasonality. Depending on the season within which decomposition is occurring, there may be differences species of insects that are active during the decompositional period. Because of these differences in seasonality associated with insect activity, the season(s) during which decomposition occurs may alter the expected species of insects that are colonizing the remains. Additionally, the rate of growth and development of a particular insect species will be dependent on seasonal temperatures. The cold tolerance of insects is not consistent across all species – some are more cold-tolerant than others. Thus, the threshold temperatures necessary for development of a species will play a role in which species are able to colonize remains at various times throughout the year. Other seasonal factors such as humidity and precipitation play an important role in the decomposition process and the overall ability of insects to colonize remains (Donovan *et al.* 2006; Sanford 2017; Giles *et al.* 2020).

4.3.4. *Toxicology.* The field of entomotoxicology can provide useful information in legal investigations involving clandestine gravesites. The larval insects and pupae recovered from clandestine gravesites can be utilized as alternative samples for toxicological analysis either in supplement to or in the absence of traditional methods of sampling. Pupal casings that remain after the insects complete their development may remain within the gravesite and undisturbed for many years and can be useful for the detection of metabolites from medications and illicit drugs (Bourel *et al.* 2001; Chophi *et al.* 2019; Al - Khalifa, Mashaly, and Al-Qahtni 2021; Ugalde *et al.* 2022). It is important for an investigator to recognize any known or suspected drug use by the decedent and communicate this to the entomologist as *antemortem* and *perimortem* drug use can alter insect development rates which may consequently alter the estimation of a minimum *postmortem* interval.

5. Conclusion

The location of a clandestine gravesite can be a difficult task which may involve many replated scientific disciplines. Therefore, medicolegal investigations involving clandestine gravesites must be collaborative to ensure maximum success for the location of the site, recovery of the remains, and documentation and preservation of physical evidence. There are many methods utilized for estimation of the *postmortem* interval, and the success of those methods vary dependent on the *postmortem* interval. However, entomological evidence can provide useful information during the early stages of the *postmortem* interval, as well as in the late stages many years after death. Entomological evidence can be utilized in cases of clandestine burials to provide useful information into not only the *postmortem* interval, but in some cases, the time since burial. When dealing with clandestine burials in legal investigations, specific environmental factors – refrigeration/freezing, wrapping/concealment, seasonality, and toxicology – may play independent roles in altering colonization and resulting analysis. To properly account for the environmental, factors to be considered in clandestine burials, it is imperative that multi-disciplinary collaborations be implemented to yield the most successful evidentiary results.

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[a] University of Florida's College of Medicine,
Maples Center for Forensic Medicine,
4800 SW 35th Dr., Gainesville, FL 32608

* To whom correspondence should be addressed | email:jhbyrd@ufl.edu

Paper contributed to the workshop on "Advances and applications in geoforensics: Unraveling crimes with geology", held in Messina, Italy (26 September 2022) under the patronage of the *Accademia Peloritana dei Pericolanti*

Manuscript received 14 February 2023; published online 12 September 2023