A brief summary of Forensic Entomology

Content

Key Points

- The most common use of insects in legal investigation is the estimation of post mortem interval (PMI).
- Since insect growth over time, from egg to maturity, is dependent upon external temperature it is mathematically possible to calculate the PMI provided the species is identified and the ambient temperature recorded.
- Many variables can alter the calculated PMI, including body mass, wrappings, geological location, micro-climates and the presence of some drugs in the body.

Introduction

The use of insects to establish an approximate time of death (ToD) is well established and has been used in criminal investigation with increasing accuracy for over a century. Establishing ToD in this way is perhaps the best known use of insects in forensic investigations, but there are other applications, less frequently applied, some of which will be outlined later. These include the origin of grown drugs, whether a body has been moved, burial post mortem, confirmation of submersion, detection or confirmation of the presence of pharmaceutical or narcotic drugs, the effect of insects in insurance claims and even certain conservation or veterinarian situations. Therefore, although largely a subject dealing with human medicolegal events, forensic entomology may also include any interaction involving insects that is examined by the law such as: veterinary cases; misapplication of pesticides; conservation; import violations, food contamination and insurance disputes. This document will focus primarily on the human context.

From the onset, it should be clearly established that the post mortem interval (PMI) estimated using insect evidence is not necessarily equivalent to the time of death (ToD). Egg laying (or oviposition) and PMI are actually the estimation of the earliest oviposition date (EOD) based on finding the specimen at the crime scene with the most advanced state of growth.

There is a period between death and the laying of the first insect egg on the body (referred to as the pre-ovipositional period (POP)) that is difficult to estimate. Therefore, ToD = POP + EOD. Although POPs have been reported for some species, these are affected by environmental conditions making POP difficult to define, with the result that entomological reports usually provide a date after which the person could no longer be living, or the minimum time since death.

Keep in mind that we are thinking backwards in time from the time that the corpse was found. The earliest oviposition date, or EOD, is the time after death estimated that the first oviposition occurred. Prior to that, it is an unaccounted for period (the pre-ovipositional period or POP). Therefore, the estimated time and the unaccounted for time must be added together to get a time of death (ToD). Be careful, because in reality we calculate duration, not hours. Duration is then plotted onto clock/calendar hours to provide a time/date window of opportunity for the death.

Under ideal conditions for oviposition (an exposed body, in warm, dry and open conditions), mature adult blow-flies can lay the first eggs within as little as 15 minutes[1] of death, but the POP can vary from this period to a day. Each gravid female can lay 300 eggs in a batch. As soon as the first egg is laid, the PMI clock is set ticking and the hatching larvae become living evidence of a post mortem time sequence.

Where less ideal conditions prevail, such as a body hidden, buried, burnt, completely submerged, wrapped or concealed by some other means, blow-flies may not have immediate access, or may only have access after natural bacterial decomposition has set in. If the degree of decomposition has advanced beyond a stage that blow-flies find attractive, then other species of insect may become dominant.

The transition from one insect species to another (attracted to different degrees of decay) is called succession. The understanding of succession within a case can also be used to establish a less defined timeline for a case. Where prolonged decay occurs, the timeline becomes less certain, but still be very informative in criminal investigation.

Protocol

Protocols for collecting, handling and storing entomological evidence are as rigorous as any other branch of forensic science. General principles are outlined in Byrd and Castner (2009; chapter 3), while mandatory standard operational procedures (SOPs) and guidelines were established by Amendt et al. (2007). Although slight variations on these occur across the globe according to the different legal systems and requirements, there is a tendency toward standardisation.

Soft skinned insects require particular care in preservation, especially if these are required for PMI estimation or later DNA analysis. In this regard, blow-fly larvae taken from a fatal crime scene are of particular importance. For accurate measurements to be acquired, the living maggots must be dropped into near-boiling

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[1] established by actual experimental observation.

(>80ºC) for about 5-10 minutes water until hardened and then transferred to 70-95% ethanol. If dropped live into ethanol, then intersegmental membranes become flaccid, resulting in mis-measurement of body length that results in foreshortened PMI estimation. The additional benefit of using near-boiling water is that it destroys bacteria in the maggot gut, resulting in well preserved evidence; failure to utilise adequately hot water, results in black, poorly preserved maggots that may thwart later analysis.

Forensic insect species

Species of insect involved in forensic investigation are frequently those associated with various stages of decay from fresh body to skeletonised remains. In general these are saprophagous species feeding on decaying organic matter. Not all saprophagous insects feed on human (or vertebrate) remains, and consequently, those feeding on a flesh-based diet are sometimes referred to as sarcosaprophagous to distinguish them from other organic feeders.

The majority of forensic cases deal with sarcosaprophagous species. This reasonably small number of species (approximately <100 globally) nevertheless requires specialist identification, because the distinguishing features between many species (or even between the families in which these species reside) may be subtle and require detailed microscopic examination.

Approximately ten families of flies, 8 families of beetles (and a dozen or so insects and arthropods from other families) include species that are sarcosaprophagous and forensically useful. Notable are blow-flies (Calliphoridae) and larder or bacon beetles (Dermestidae).

The presence or absence of species from other families often encountered in the sarcosaprophagous context provides additional forensic information regarding the succession of species through the various stages of decay. In addition, species that are otherwise inconsequential (i.e. not obligate sarcosaprophages) and are simply sheltering at the crime scene, can be useful if they have specific or seasonal biology. The presence of species in specific instances can be important, for example, in legal cases involving spoiled foodstuffs, restaurant closures, insurance investigations etc.

Even though the number of sarcosaprophagous species is relatively small, the number of species potentially involved in legal circumstances runs into the thousands and it is beyond the scope of this document. A good initial outline for the interested reader is provided in Byrd and Castner (2009; chapter 2) and more detailed keys for identification of species across all geographic zones are scattered throughout the specialist literature (for example Greenberg and Kunich 2002). These usually require the interpretation and experience of a professional entomologist.

Thermal biology

Insects are poikilothermic - their internal body temperature varies with that of the ambient environmental temperature of the medium in which they are immersed. If the ambient temperature is known, then something can usually be said about the biology and developmental status of any insects present. Therefore, the local ambient temperature at a crime scene (or locus) is vital information, without which estimates of PMI cannot reliably be made.

Using regression mathematics, a series of temperatures recorded at the crime scene with a data-logger, can be related to those corresponding records from a standard meteorological weather station, which, if practical, should be no further than 10km distant. The regression equation that describes the trend line (or linear regression) through the scatterplot of the crime scene temperature against the meteorological data is used to extrapolate back in time to provide an estimated sequence of temperature conditions at the locus during the period leading up to discovery of the crime. That estimation can then be used to suggest a minimum period when the eggs were first laid (see below) and, by inference, provide a timeframe (or window of opportunity) for when the crime is likely to have been committed.

In cases requiring PMI estimation, the most advanced stage of the species that arrived first at the crime is of prime importance. Where blow-flies are the first colonisers, the first laid egg is usually already fertilised and partially developed prior to oviposition. Some flesh-flies (Sarcophagidae) larviposit rather than oviposit, in other words, hatching occurs in the adult female, which deposits a first instar larva (the instar describes the larval stage of development) onto the deceased. As a result of this pre-development in some flies, the first egg/larva has a head start, in terms of hatching and subsequent development time and ultimately reaches the greatest size. It is important, therefore, to evaluate species identification accurately and account for the possibility of early arriving (or faster developing), species such as the flesh-flies.

In an investigation, the most mature stage of development is sought from which to determine PMI. Depending on which stages are found at the crime scene and how long it is since the crime was committed the most mature stage of development is frequently the largest maggot, but it could also be the first egg to hatch under laboratory conditions (assuming only eggs are present at the locus and have been removed to the laboratory for rearing), or the first adult to emerge from it's pupa (assuming pupae are present at the locus and have been removed to the laboratory for rearing). On a case-by-case basis, it is often relevant to rear some of the evidence under secure laboratory conditions, especially if there is any uncertainty about identity of the species encountered or of the stage of development encountered (early stages of larvae development can be difficult to assess without a microscope).

The most frequently used methods of PMI estimation involve either measurement of the largest larva compared to known growth charts derived from laboratory experimentation; or the use of accumulated degree theory, which relies heavily on the regression statistics described above. Each has its own limitations. The former method assumes that the largest maggot can be found within the body, which may contain thousands of developing larvae. The latter method assumes that each species, or more precisely each geographic population of a species, will have a distinct and genetically preset period of development in degree-hours (see below) for each of the stages of the life cycle (in blow-flies, for example, that consists of: egg, larval instars 1 to 3, post feeding larva (or pre-pupa), pupa and adult). Specimens from high latitude or altitude cases may be cold tolerant and continue developing at temperatures well below the laboratory minima determined from more temperate examples.

Caution is required when transferring knowledge across geographic regions, because growth periods for differentially adapted populations may vary. Additionally, for obvious reasons, many laboratory studies use surrogate food sources, ranging from rodents, to dogs and pigs. Grow data from these food sources vary and may not be directly applicable to human forensic work. Pork is perhaps the closest of these surrogate foods to human tissue and yields the most reliable experimental results.

If the thermal conditions are known, then the insect's energy requirements are determined in accumulated degree-hours (ADH) and can be thought of as an energy budget. This is the number of degree-hours required for a particular stage of the life cycle to complete. Summed together, they provide an overall assessment of how long it will take to complete the entire life cycle from egg to adult, or parts of the life cycle, for example from egg to end of the third instar.

The number of degree hours at constant temperature can be experimentally determined for a species, thus providing an ADH value against which to compare the estimated temperature conditions at the crime scene. The value in ADH theory is that fluctuating conditions at the locus are taken into account by the accumulation technique. Thus, by comparing the known ADH reference value for the most complete stage of the species, to accumulated hourly budgetary values in degree-hours at the crime scene, a potential minimum time since oviposition can be assessed.

Clearly any circumstance that alters temperature should be taken into consideration. On the one hand concealment and wrapping considerably alters the microclimatic temperature, while on the other hand local climatic conditions such as wind-chill, the degree of shading or the amount of precipitation affects the final PMI estimate.

Blow-flies have a tendency to clump together (thigmotaxis) and under certain circumstances will form an aggregation or maggot-mass. The internal temperature maggot-masses can be considerably higher (>10ºC) than the surrounding body tissues, greatly reducing duration of the life cycle and having a significant affect on PMI estimation. Larvae within a maggot-mass optimise growth temperature by moving toward or away from the centre of the mass as their thermal requirement dictates. Therefore, the local ambient temperature fluctuations become minimally significant and it is vital to the investigation that the entomologist is aware of the presence of a maggot-mass and that temperature is recorded from its centre. Estimations of growth should be assessed (at least from second instar onwards) using the maggot mass temperature instead of the local ambient temperature.

Succession

Succession is the term used to describe the transition of colonising species from one insect species to another, which are attracted to different stages of decay. Succession is a less precise science, relying to some extent on the forensic entomologist's prior knowledge of species over a wide range of families and possibly also reliant on knowledge of local fauna. Various schematic interpretations of succession are available (e.g. Smith 1980), but there is no such thing as the standard succession - each case has to be dealt with uniquely. Nevertheless, we can speak of succession in general terms, as there are species that prefer newly dead bodies over well decomposed or mummified bodies and relative POPs have been recorded for some species. For instance scuttle-flies (Phoridae) can arrive on the fourth day after burial in some cases.

It is important to recognise that species may be present in the vicinity of a locus pre-mortem. Blow-flies, primarily insects of the first phase of succession, are relatively abundant outdoors and are readily attracted to a body over large distances by odour, arriving as early as 15 minutes after death and eggs may be laid in the first hours after death.

This first wave is sometimes shared with other families, such as house-flies (Muscidae), flesh-flies (Sarcophagidae) and sometimes sexton (or burying) beetles (Silphidae). Certain species of house-flies and lesser house-flies (Fanniidae) are attracted to faecal material if present at the locus. Ants are sometimes early colonisers in tropical locations and are also often present pre-mortem. If ants or sexton beetles do arrive before blow-flies, this can prevent blow-fly colonisation altogether. Ants actively carry off blow-fly eggs and first instars, whereas sexton beetles carry phoretic mites (which travel on the host beetles between food or breeding sources without causing any adverse effect to the host), which in turn feed on the eggs.

Once butyric fermentation sets in (i.e. the formation of butanoic acid, which is a carboxylic acid), a corpse is generally no longer attractive to blow-flies and other smaller flies such as scuttle-flies colonise the body, intermixed with a variety of beetles dominated in many cases by larder beetles (Dermestidae). Knowledge of the local fauna can be most valuable, since the knowledge that a particular species is only seen in spring for instance, can help set a timeline.

Entomotoxicology

Recovery of heavy metals from bodies in the 1970s and 1980s led to the development of forensic research at the boundary between entomology and chemistry: this became known as entomotoxicology. The subject can be divided into two main categories:

1. Entomological analysis of substance induced changes to insect development rates and subsequent effects on PMI estimation.
2. Toxicological analysis of drugs within insects: substances may be more easily detected because of bioaccumulation in insect metabolism, resulting in concentrations of substances higher than in the surrounding tissues.

Substance induced changes can be caused by substances external to the body (for example fire accelerants) or those internal, such as ingested or injected drugs. Probably most important in the context of this book is the presence of pharmaceutical or narcotic drugs in the deceased and the differential affect these can have on insect growth, depending on where in the body these drugs and their metabolites concentrate and whether some (or all) of the maggots have fed on those tissues.

We know for example that over-the-counter medicines (OTC) such as paracetamol (4-acetamidophenol), is one of the foremost drugs used in suicide cases in the UK and elsewhere, typically resulting in a level of 250mg/kg drug to body weight concentration. At this concentration, growth period of blow-fly larvae is differentially increased by up to four days, having most effect during days 2-4 of development. This critical piece of research suggests that the growth pattern diverges from the normal linear relationship we more commonly use. In contrast, co-proxamol (i.e.

paracetamol combined with dextropropoxyphene), appears to have no effect on insect development, even at human lethal doses.

A commonly prescribed drug for anxiety and sleep disorders, Nordiazepam (and Oxazepam, its metabolite in the human body) causes an increase of at least 24 hours, especially on or around day 4 of drug accumulation. Amitriptyline (a tricyclic antidepressant) prolongs the post feeding and pupa stages, resulting in death during either, thus preventing complete development to adulthood. Another prescription drug, Zopiclone (for the short term treatment of insomnia) prevents pupariation, the process by which blow-fly maggots turn into pupae (personally observed at crime scene). Zopiclone belongs to the cyclopyrrolone group of medications specifically listed as a non-benzodiazepine, which acts on brain receptors (GABA receptors) causing the release of gamma amino butyric acid (GABA). In humans GABA is an inhibitory neurotransmitter, acting on the Central Nervous System (CNS), but the effect on insects is quite different. Acting on the Peripheral Nervous System (PNS), it has the effect of seriously disrupting the pupation process.

Among the narcotics, cocaine seems to have little effect, but ecstasy (MDMA, i.e. 3,4-Methylenedioxymethamphetamine) speeds up growth and reduces both the larval and pupal growth periods, sometime resulting in insect death; while methamphetamine on its own appears to have little effect other than terminating insect growth at the pupa. On the other hand, human lethal dosage of morphine results in a 24-hour increase in development, while heroin at human lethal doses, increases the larval growth period and dramatically alters the pupa duration by 18-36 hours. Organophosphate poisons, such as Malathion, do not necessarily result in death of the flies breeding in bodies containing a human lethal dose; instead Malathion increases larval growth by about 72 hours.

Clearly, such alterations of the growth in different stages of the insect life cycle impact on the assessment of PMI. It therefore becomes critical that the entomologist is informed of drug or alcohol usage in cases requiring PMI assessment.

Entomological DNA

Species determination and assessment of human DNA from the crops of haematophagous (blood feeding) species are two aspects of DNA studies particularly important in the forensic context. The crop is the portion of the insect digestive tract, forming the bulk of the foregut and capable of considerable expansion. It is distal to the oesophagus, serving as a storage vessel, retaining undigested food prior to its movement through the proventriculus (valve) into the midgut for digestion. Wells & Stevens (2008) discuss the merits of different techniques and point out that there is no single agreed upon locus for DNA-based identification of forensic insects. In general terms mitochondrial DNA (mtDNA) sequence data (as distinct from nuclear DNA) is examined, There are two specific genes which are looked at primarily, coding for the cytochrome c oxidase subunits one and two (COI+II); these are involved in biochemical respiration processes. The analysis either directly compares the sequences of the genes or uses a technique called restriction fragment length polymorphism (RFLP), which exploits variations in DNA sequences).

These techniques may also be useful in detecting geographic variation in cases where evidence or a body has been moved significant distances, but it is also important to take into account that geographic variation may possibly be observed between specimens taken as evidence and the reference data from genetic databases (e.g. GenBank). In other words, the reference databases do not yet contain sufficient data to account for the possible range of variation expressed over broad insect distributions. Furthermore, Wells & Stevens (2008) point out the possible pitfalls in using BLAST (Basic Local Alignment Search Tool) searches for DNA sequences in GenBank without any background knowledge of basic insect taxonomy, because the method uses nearest match technology and cannot account for species or variation not yet included in the data bank. In this instance, a BLAST search will locate a 'nearest approximation' species identification based on the data in the database and will not accommodate the possibility that the species found as evidence does not yet have profile included in that database. Without any taxonomic background, such an error could lead to inaccurate PMI estimations.

The prevalence (20 - 70% infection rate) and effect (horizontal gene transfer) of cytoplasmically inherited rickettsiae, Wolbachia, transmitted through egg cytoplasm, further confound DNA based insect identification. Four phenotypes have evolved various mechanisms for manipulating the reproduction of their hosts, but more important here is that clearly morphologically different taxa sometimes share similar cytochrome c oxidase I gene sequences, as a result of horizontal gene transfer by Wolbachia. In such circumstances, the use of DNA bar-coding to distinguish insect species (especially in the absences of taxonomic knowledge) becomes questionable.

Purified and PCR amplified human DNA can be extracted from the guts of species feeding on human remains. In addition, within a given post-feeding period, human DNA remains viable and can be isolated from blood-meals of haematophagous insects including: lice, bed-bugs, assassin bugs, mosquitoes, fleas, non-insect arthropods such as ticks and mites, and other invertebrates, such as leeches. Species with limited dispersal (such as bed-bugs) and longer post feeding storage of crop contents offer the most promising results.

Further considerations

On the fringe of criminal investigation using insect evidence, sits accident insurance investigation. On occasion insects cause accidents, fatal or otherwise. While not all instances are medical in nature, those of value in death inquiries, assessment of accident events caused by insects and investigations of fraudulent insurance claims are of relevance here.

Stings from bees and wasps are common and can result in panic or anaphylaxis, in turn, the cause of fatal accident, particularly vehicular death. Fluno (1961) classified reaction to insect stings into three degrees of severity:

- hymenopterism vulgaris: the majority of cases. Painful, but not serious or lethal although accidents can result from loss of concentration or panic.
- hymenopterism intermedia: usually non-lethal, but caution is required when swelling of the tongue, neck or throat results in impairment of swallowing and breathing.
- hymenopterism ultima: lethal or near- lethal reactions to stings, usually as a result of anaphylactic shock.

Summary

The use of insects in criminal investigation is well established. Usually, the knowledge and experience of a professional entomologist is required, to establish species identity and to understand and explain the biology associated with those species.

The most frequent application of insects to criminal investigation is the estimation of a post mortem interval (PMI): the minimum period since the first eggs were laid. Considerations affecting this estimation include the temperature, weather, time of day, presence of drugs, amount of clothing or concealment or attempts to destroy evidence or prevent investigation.

Other important applications of forensic entomology include the assessment of drug use and the extraction of human DNA from the crops of haematophagous species. Significant developments are expected in both these areas of research.

In the wider forensic context, insects are used in many aspects of forensic investigation, sometimes of less medical significance such as: veterinary cases, misapplication of pesticides, conservation, import violations, food contamination & insurance disputes.

Definitions: ADH - accumulated degree-hours.

EOD - earliest oviposition date (=PMI).

OTC - Over-the-counter medicines.

PMI - post mortem interval (= estimated EOD).

POP - pre-ovipositional period.

ToD - time of death (= POP + EOD).

SOP - standard operational procedures.

REFERENCES AND FURTHER READING

Amendt, J., Campobasso, C.P., Gaundry, E., Reiter, C., LeBlance, H. and Hall. M., 2007. Best Practice in Forensic Entomology: Standards and Guidelines. International Journal of Legal Medicine 121, pp.90-104.

Byrd, J.H. and Castner, J.L. eds., 2009. Forensic Entomology: The utility of Arthropods in legal investigations. 2nd ed. Boca Raton: CRC Press.

Fluno, JA., 1961. Wasps as enemies of man. Bulletin of the Entomological Society of America 7, pp.117-119.

Greenberg, B. and Kunich, J.C., 2002. Entomology and the Law. Flies as Forensic Indicators. Cambridge: Cambridge University Press.

Smith, K.G.V., 1980. A manual of Forensic Entomology. London: British Museum (Natural History).

Wells, J.D. and Stevens, .JR., 2008. Application of DNA-based methods in forensic entomology. Annual Review of Entomology 53, pp.103-20.