Review article

A review of the estimation of postmortem interval using forensic entomology

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Abstract

The postmortem interval (PMI) is a crucial factor in death investigations. For PMIs exceeding 24 h the forensic pathologist must turn to other specialties that focus on decompositional ecology of animals, including humans. Primary among these specialties is forensic entomology. Here, we review the importance of forensic entomology in estimating the PMI, and we examine the factors that influence these estimates. Among key concerns are environmental factors, especially temperature, and aspects of insect biology. Additionally, we examine current methods used for calculating PMI based on insects and their development.

Keywords

Postmortem interval, entomology, taphonomy, degree days

Introduction

In all homicide investigations, the postmortem interval (PMI) is a crucial factor in establishing the sequence of events in the crime and in excluding or including potential suspects. Shortly after death, typically up to 36 h but occasionally up to 48 h, a forensic pathologist can offer estimates of a PMI through such criteria as livor mortis, rigor mortis, algor mortis, and putrefaction. Unfortunately, these criteria provide a relatively coarse estimate of the PMI. Many methods have been proposed and evaluated to offer more precision in estimates of the PMI, but to date none have proven superior. For postmortem intervals beyond 36–48 h, our focus must turn from forensic pathology to other forensic disciplines, primarily forensic entomology, and forensic taphonomy. Here, the authors review some of the history and current status of estimating the PMI estimation in relation to forensic entomology.

Forensic entomology

The classical book by Mégnin (1894), titled *La faune des cadavres*, was published in France and can be described as having largely laid the foundation for the application of entomology in forensic science.[1] It recognized the so-called invasion of the dead body by waves of insects, arriving at pre-determined "succession." That succession theory suggested the possible use of these necrophagous insects in the estimation of the postmortem interval (PMI). Von Hoffmann in 1898 published data on the development of blow flies and correlated this with the PMI, following their colonization of cadavers.[2] Of course, the claims of succession have long been challenged by other scholars who drew attention to developmental variations that resulted from varying changes in the environment, the carrion, as well as, in the insect themselves.[3–5] Some of the factors that alter the responses and development of the insects include, variations in species population, geographical and latitudinal locations, feeding behavior, presence of wounds on the body, ambient temperature, and nature of the clothing. All these variables will affect the developmental rates of the necrophagous insects and consequently any attempt to accurately determine the PMI.[6]

Blow flies are known to be immediately attracted to a dead body and consequently are currently considered the most accurate tool for the determination of the PMI.[7–9] Oviposition is encouraged by the odor of ammonia and hydrogen sulfide produced during putrefaction, as well as, the presence of water (moisture).[10–12] While the female

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fly will use moisture as an ovipositional cue, it is more important for when the first stage maggots emerge from the egg. Their thin cuticle makes it very easy for them to dry out. Hence, the female flies will not oviposit on dry or mummified bodies. The carrion may also act as a source of protein for reproductive organ development in both male and female blow flies. Beyond moisture, there are many factors that can impact how and when blow flies are attracted to, oviposit on, and maggots develop in decomposing bodies. Some flies will avoid oviposition where the risk of parasitism by competitors is envisaged.[13]

Decomposition

Decomposition is relatively slow in a newborn or fetus, apparently because of the absence of endogenous bacteria. Contrastingly, the process is accelerated in obese persons with abundant fluid that will encourage bacterial growth and successful fly oviposition. Where death was associated with wasting caused by sepsis or malignancy, decomposition is hastened. Asphyxia is generally accompanied by fluidity of blood and this also aids decomposition. Other factors include the integrity of the body such as the presence of injuries with open wounds and exposure to bacteria in the soil or environment; the flies are attracted to such open and moist wounds which encourage oviposition. A heavily clothed body will not lose heat quickly and the warmth will expectedly hasten putrefaction. Other important factors include the ambient temperature, humidity, ventilation, existence of a windy and dry environment that will favor mummification, and the accessibility of the flies to the cadaver. Blowflies are known to have the capacity of invading carrions buried up to 30 cm below the ground.[14]

Myiasis

Myiasis is a condition in which flies oviposit and develop in the skin of a living person or non-human animal. Such person dying will obviously exhibit maggots of advanced ages that will not correspond to the PMI.[15] Thus, myiasis must be distinguished from postmortem fly infestations because of the risk of overestimation The diagnosis of myiasis at autopsy can on addition raise concerns about child/adult neglect/abuse.[16,17]

Activity based on light

Blowflies are generally inactive at night (because they have a difficult time seeing in the dark). So, finding dipteran eggs predawn indicates oviposition (and death) occurred the day before during daylight hours. This suggestion has been queried by some scholars based on experimental laboratory studies,[18,19] but other experimental evidence contradicts these findings.[20]

Diapause

An interesting observation in developing necrophagous flies is diapause.[21] Diapause is a neuro-hormonally regulated state of suspended development. If several eggs fail to form larva, or there is no progression to pupation from the existing larva, individuals may have entered diapause. A transient developmental arrest can be caused by prior exposure of the adult females to extremes of temperature; development is greatly reduced when the temperature exceeds 35°C, and at 39°C or above, thermal death will usually occur.[20] Similarly, diapause could be observed below 5°C.

Precocious development

Some eggs referred to as being precocious, mature to larvae quickly and consequently there is an accelerated development, again resulting in a wrong estimation of the PMI.[22] While precociousness can occur, it does not happen in a large enough number to significantly impact on PMI in a representative sample.

Aquatic environment

With respect to bodies found in water, the rate of decomposition has been observed to be reduced, when compared to bodies on land, because the insect population is reduced and largely different in terms of the dominant species.[23] Furthermore, cold temperature, limited body exposure, submergence or a combination, contribute to the reduction in the rate of decomposition.

Using forensic entomology

As previously indicated, the science of entomology has continued to be applied by forensic pathologists involved in criminal and civil cases, to determine the PMI, in addition to detecting the presence of drugs in the decedent (entomotoxicology), and also determining the presence of antemortem injuries.[24–31] The ability to correlate the insect activities with the stage of decomposition allows for an estimation of the PMI. The forensic pathologist must seek the assistance of the forensic entomologist in respect of this determination because of the recognized impact of such factors like body size and clothing,[32] co-invasion by different species of insects,[33] effect of temperature on insect growth rate,[34] and the consequent variation in the rate of decomposition. This is complicated by the differences in the developmental biology of the different invading insects coupled by the different times of invasion.[35–41] It therefore appears that the life cycle, possibly the larval size (including length and weight), temperature, and morphology all contribute to the estimation of the

PMI.[42–47] These necrophagous insects are affected by a number of variable factors.

Effects of abiotic and biotic factors on insect development, and the estimation of the postmortem interval

The abiotic factors that affect insect development, and consequently impact on the estimation of the PMI include temperature, humidity, light (photoperiod), insect population (crowding with attendant risk of hyperthermia), accessibility, and geographical location (including aquatic environment). The biotic factors include genetic characteristics of the species (including olfactory and chemotactic sensitizations, as well as diapause), food (nutrient) availability, and predation (which can be aggravated by overcrowding).

Abiotic factors

Temperature

Perhaps the most important modulating factor is the temperature to which the different stages of the insects are exposed. To appreciate the significance of temperature in the development of these necrophagous insects, some terminologies must be defined.[48]

Terms

Isomegalen diagram. This is graphical plot of the larval size (length, weight, or width) after hatching, against the ambient temperature. This does not correlate temperature with all the developmental stages; it focuses only on larval dimensions. However, it is noteworthy that some other scholars have expressed doubts about the use of size as a reliable indicator of age, particularly because size varies with nutritional status.[49]

Isomorphen diagram. This is a scatter plot showing the time between the hatching of eggs with the emergence of the pupa, and the emergence of the adult fly. The time spread between oviposition and termination of the larval stage, or between pupation and emergence of the adult fly can be plotted against temperature.[48]

Mathematical models

Thermal summation (linear) model. This is the most common model for blow fly development. It employs the use of a linear regression analysis in the positive correlation of the development with temperature.[28,50,51] The reciprocal of the development time (1/day) is plotted against temperature; this would usually produce a sigmoid-shaped curve.[52] Earlier studies had shown that insect development is limited by extremes of temperatures and could in fact be halted.[53] However, even when studying the less variable range, it must be remembered that the observations are species-specific and undue generalization must be avoided. In using this model, the usual practice is to rear the insects obtained from a cadaver/scene at a controlled and constant temperature until the time of eclosion. The time recorded is then subtracted from that normally required by that particular species when grown to eclosion in the laboratory at specified and comparative temperature. This model is limited by temperature variations, especially when extremes are involved.[54–56]

Curvilinear model. This model appears to give a better assessment of development by providing for the curvilineal characteristics of temperature extremes and for the ability to generate error rates.[51,57–59] However, it has not been consistently found to be superior to the linear model.[60] This model could potentially incorporate more of the variability that inevitably exists between species, temperature, and geography.[61–68] The observed geographical variations have been attributed to possible genetic and environmental variations within the population of a species.[66–68] It is common to have temperature fluctuation at death scenes (especially outdoors) and this can constantly alter the development of any species.[42,69–77] Further studies are needed to determine the effect of temperature variation on specific species of defined geographical origin.

Humidity

Humidity affects decomposition, starting with the preference of some necrophagous insects for moist environment for the purpose of oviposition.[78,79] The influence of humidity on insect development has been observed in the adult and larval forms of some arthropods that are known to invade plants of economic interest.[80–82] Generally, increased oviposition and enhanced development is observed with increased humidity; the longest time for development is observed at relatively low humidity.[83,84] It is noteworthy though, that the influence of humidity is further modified by the temperature with the latter having a more dominant effect.[84]

Even within the same genus, variability in response to humidity is possible. For example, Sharif found that three species of rat fleas in the genus *Xenopsylla* demonstrated variability in their reaction to humidity.[85] While *X. cheopis* larva can tolerate a fairly wide range of humidity, it is reduced in *X. brasiliensis*, and very narrow in *X. astia*. Interestingly their adults also display a reduction in size with reduced humidity.

Light

It is recognized that necrophagous insects, particularly the larval forms respond to light with consequent variability in their development.[86,87] The period of exposure to light

or darkness (L:D) in a 24-h period impacts on their growth rate. This photoperiod affects the insect species differently.[88,89] The effect of the photoperiod on the development rate of *Phormia regina, Cochliomyia macellaria,* and *Calliphora vicina* under four light regimes (0, 12, 16, and 24 h, at 20 °C and 26 °C), varied among the larvae of the three insects.[89] The effect of light period can be magnified in some larvae at low temperatures (~20 °C) causing an extended development time. These development changes suggest a strong relationship between temperature and light, particularly since the rate of development is increases as the photoperiod increases, regardless of temperature.

Some larvae have eyes that are genetically primed to respond to light stimuli due to the presence of photoreceptor neurons.[90] Many times, the larvae are attracted to certain locations due to olfactory stimulation, but most of them are repelled by light as they seek dark places. It was observed, working with *Drosophila melanogaster*, that the larvae are initially photophobic, but they later become photoneutral, and eventually photophilic. Of course, there is a lot of interspecies variation, and for most diptera it is generally accepted that the larvae of all developmental stages, move away from light. The phenomenon of diapause also must be borne in mind as this can by itself further complicate larval growth despite the impact of light.[67]

Population density (crowding)

Flies, particularly Calliphoridae, are usually the earliest to arrive on the carrion, but are quickly followed by an array of other insects. Usually, these insects arrive in a seemingly overlapping succession, although the pattern can change based on temperature, geography, and carrion type. With time the insect population increases with increase in temperature and, interestingly, depleted food availability.[9,25,79,91,92] The carrion becomes populated by larvae derived from necrophagous larvae, predators, parasitoids, and some incidental insects.[9,93] The ultimate effect is that the developmental stages being viewed by the forensic entomologist would have been greatly modified, but by taking all these variables into consideration, a near accurate estimation of the PMI might be made.[94] Where a wide range is considered, the error might be reduced.

Population effects also include potential competition between blowfly species (including interspecies predation, like *Chrysomya rufifacies* larval predation on other blow fly larvae), which is likely to be associated with the evolution of differences in oviposition times on the carrion.[95] In contrast, mutual benefits from interspecies maggot assemblages of *Calliphora vicina*, *Calliphora vomitoria*, and *Lucilia sericata* have been noted.[96]

Accessibility

Estimation of the PMI will be variable when a body is not easily accessible. Examples include immediate burials, placement below floorboards, locking up of the body in a house, and heavily wrapping the body.[9,79,97–101] Experiments involving heavily wrapped pigs revealed a delay in decomposition, and it is not uncommon to observe adipocere formation due to the high humidity.[100] An interesting observation in a study involving pigs, done in indoor and outdoor settings in North Carolina, and spanning Spring, Summer, and Fall, revealed striking differences in the dominant arthropod.[99] There was delayed colonization across all seasons (36–768 h) depending on the extent of concealment, but the predominating insects showed seasonal variation. Bodies concealed indoors showed greater presence of beetles, while dipterans predominated outdoors. *Lucilia illustris* dominated during Spring, *Chrysomya megacephala* were commonly observed during Summer, while Fall witnessed more of *Calliphora vicina* and *Calliphora vomitoria*. Overall, there is a tendency to underestimate the PMI. The whole intention of concealment is to prevent, or at least delay, oviposition. This situation will affect the final PMI calculation if the expert is not aware of the relevant information. Further complication is introduced when dealing with submerged bodies.[9,86] There are few known aquatic necrophagous insects, and their population varies depending on whether the body was found in fresh or salt water.[23,102,103] Bodies located near river banks might have an invading large population of the usual land-based insects.

Geographic location

The microenvironment includes open and covered locations, indoors and outdoors, moist and arid environments, and still or windy places. The macroenvironment will include temperate and tropical locations; the former will be associated with winter and summer periods while the latter might have wet and dry seasons. Geographic location will continue to have an impact, particularly as climate change allows insect groups to move into areas they were not previously found or established. These, and intra-species variations based on geographical origin have been previously identified.[45,66–68,79,104–106] There is a complex interaction involving these insects, season, and oviposition.

Biotic factors

Genetics

A strong biotic factor is the genetic priming of necrophagous insects and their immature forms which allows them to adapt to a changing microenvironment. Genetic differences have been suggested as the reason for developmental variation within a species in different geographic locations.[66–68] As previously mentioned, the odor of ammonia, hydrogen sulfide and the rancid odor associated with butyric acid fermentation, are strong chemotactants

for some insects.[8,9,79] The phenomenon of diapause (mentioned previously) observed in immature offspring is derived from the genetic information transferred from parents who had experienced some changes in the microenvironment, especially the extremes of temperature.[21]

Nutrients (food availability)

On being attracted to carrion, the female blow fly will oviposit in moist areas and open wounds. In humans, oviposition areas include the eyes, nose, mouth, ears, anus, and the vagina. Blow flies will also lay eggs in open wounds found in other parts of the body. The emerging larvae digest the surrounding tissue through enzymatic action apart from directly burrowing into tissue. The larvae feed on the carrion and undergo the various stages of larval development as long as there is abundant carrion biomass and appropriate temperatures.[107–111] This natural course of events is subject to factors like overcrowding, excessive heat generation, light, humidity, deleterious effects of drugs that might be present in the decedent, age of the substrate, diet, and the depletion of the biomass.[112,113] As previously mentioned, overcrowding can be accompanied by predatory activities. Also, food depletion can induce larval biochemical changes, ultimately leading to the emergence of smaller adults. All these alterations can potentially affect the estimated PMI.

Entomotoxicology

Any drug present in the tissues and body fluids of the decedent will be consumed by the feeding larvae. Forensic entomotoxicology has shown that while some drugs might not affect the growth and development of the insects many drugs are capable of accelerating or decelerating the rates of larval growth and development, or extending the intrapuparial stage; in very large doses, death of the developing insect could occur. It is thus obvious that these drugs will significantly alter any calculation of the PMI.[114–118] Methamphetamine produce increased larval growth rate in *Calliphora vomitoria* while it induces puparial mortality in high doses in the same species. Non-dose dependent accelerated larval development is observed in *L. sericata* with ingested codeine, but it does not affect the pupal stage. Diazepam produces acceleration of larval development with extension of the puparial stage in *Chrysomya albiceps and Chrysomya putoria*. Similar effects are observed in *Sarcophaga peregrina* and *L. sericata* in association with heroin. There is acceleration of the larval stage of *C. vicina* in association with ingestion of paracetamol. Nicotine has no effect on the larval stage of *C. vomitoria*, but at high doses, it produces intra-puparial death. It is obvious from the above that the different drugs produce varying effects on the necrophagous insects, and these effects are themselves species-specific. Generally, xenobiotics that accelerate growth/development (accelerants) include cocaine, heroin, diazepam, ketamine, amphetamine/methylamphetamine, codeine, and hydrocortisone. Xenobiotics that act as retardants include methadone, ethanol, phenobarbital, and morphine. One interesting effect of morphine is that it causes retardation of larval development in *L. sericata*, while it produces growth acceleration in *Chrysomya megacephala*. Flunitrazepam and phencyclidine do not affect larval development, but they cause delays in the onset and duration of the puparial stage.

Tools used for age estimation

The tools available for developmental age estimation of the blow flies include morphology, optical tomography, hyperspectral imaging, and molecular studies (steroidogenesis, genetic expression, and cuticular hydrocarbons). Various methods can be used based on the life stages found. Generally, those stages will fall into one of the following categories: eggs, larvae (instar), pupae, and adult. However, the different methods vary as to their accuracy, reproducibility, and overall reliability.

Morphology

Developmental stages (Figure 1) exhibit characteristic morphological features that are species-specific, and are modulated by the previously described abiotic and biotic factors. While the egg might not show much change, morphological alterations are observed during the larval and pupal stages, with the latter accounting for >50% of the total developmental period.

Size-related changes insignificantly vary in the eggs. Studies have utilized sizing parameters such as the larval length, width, and weight to assess the developmental stages in an attempt to calculate the PMI.[109] These measurements are subject to various factors like sampling and preservation methods, nutrient availability, the type and population of reared larvae (because of the effects of overcrowding and attendant heat production), presence of drugs in the tissue, and errors of measurements.[119] All these are

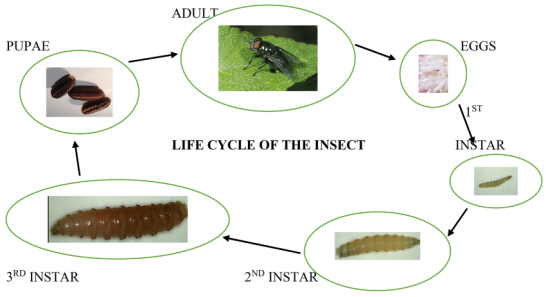

Figure 1. General life cycle of an insect.

pre-analytical and analytical errors that are pertinent for consideration.[30,120–122]

In contrast to the eggs, the larvae show non-size related morphological changes involving the posterior spiracles. The latter are on the last abdominal segment; each spiracle has 1–3 slits (corresponding to first to third instar stages) surrounded by a peritreme or border.[123]

The pupal stage occupies a significant portion of the overall life cycle.[124] Physiological categories like the digestive, reproductive and nervous systems are developed during pupation. Anatomic characteristics that develop during pupation include the wings, eyes, and legs. During this period, the immature insect is immobile and it feeds on its larval glycogen and fat stores. The developmental changes observed at this stage are used to estimate the PMI.[125] Though the hardened and darkened cuticle makes it difficult to determine the age of the pupae research has shown some promising results with imaging techniques.

Optical tomography

This method of assessment is non-destructive. It is a direct visualization of intra-puparial development, though there is some degree of opacity caused by the absorption of part of the transmitted light by the puparium. The maturation stage and morphological developments in the brain, mouthparts, and limbs have been observed using this method.[126]

Hyperspectral imaging

This is also a non-invasive and non-destructive method often employed in medicine and agriculture, to view an object by combining spectroscopy with imaging. An object can be both spatially visualized in three dimensions while also being able to analyze the chemical composition.[127] The method is able to determine the age of the pupae, as well as, differentiate between closely related species.[127]

Steroid (hormonal) evaluation

Hormones are produced by insects throughout their development. These are essentially ecdysteroids (polyhydroxylated steroids) hormones which control the process of molting and metamorphosis. A correlation is known to exist between the assayed hormonal level and the developmental age.[128]

The results are subject to the method of preservation, temperature, and stability of the hormone. The maximum level is seen between 36–96 h after pupation; more studies are required to cover the entire length of pupation.

Genetic studies

Developmental changes are triggered by genes which themselves are switched on and off by the DNA present in the cells. The DNA acts by first being transcribed to RNA (transcription) and the latter then stimulates the production of proteins utilized in development. By evaluating the level of transcription at a time in the course of development (at a known age), a database can be generated for the transcription pattern from egg to hatching or larva to pupa. The best correlations are obtained if multiple genes are studied.[129,130]

Cuticular lipid wax (hydrocarbon)

The cuticle of the insect contains a protective lipid wax layer that prevents drying, apart from guarding against invasion by microorganisms, and possibly producing sex attractants such as pheromones. The lipid waxes are believed to be species-specific, contain either saturated or unsaturated lipids, and exhibit geographical distribution. Their production has been associated with age, sex, and geographical location.[131–133]

Calculation of postmortem interval using temperature

The preceding sections provided background information about the abiotic and biotic factors that could influence the growth and development of necrophagous insects, particularly blow flies. These factors essentially affect their highly complex biological behaviors, and explain in part why they could be unpredictable. Understanding these variables have allowed forensic entomologist to calculate with some degree of accuracy, the PMI.[134,135] The task of attempting this calculation is premised on some assumptions which are itemized as below[51,59,136]:

(a) the adult female flies do not oviposit or larviposit on the live host; in other words, the forensic entomologist must avoid confusion with myiasis;
(b) the necrophagous insects (including their developmental stages) used for the estimation of the PMI must have been exclusively feeding on the remains under consideration; that is, there is the probability that the pupa seen in the vicinity of the cadaver could have come from insects that had earlier fed on a dead animal in that same location;
(c) the necrophagous insects cannot regulate their body temperature, and must keep adjusting to the ambient temperature; they require heat from the environment (poikilothermic);
(d) there is a linear relationship between the ambient temperature and the development of the insect;

(e) the developmental stage of the insects can be fairly accurately determined using its morphology;
(f) the tissues and body fluids of the decedent are devoid of drugs and toxins that are capable of affecting the growth and development of the insect; in other words, there is no drug-induced alteration of the recognized developmental rates of the insects;
(g) there are no other operating factors like diapause;
(h) the nearest weather station has temperature recordings that accurately reflect that of the scene from where the body was recovered.

Baseline recordings: correction factor, base temperature

Calculation of the PMI requires the initial identification of the insect(s) present on the cadaver, as well as recording the temperature of the body at the location where it was discovered. Ideally, the temperature at the scene would be recorded over the next 3–5 days after removing the body. The nearest meteorological station should be able to provide the data for the average daily ambient temperature (preferably maximum and minimum) over the preceding 3–6 months, in addition to providing similar information over the next 6–8 days. A graph (scatter plot) is then plotted using the scene temperature (in Celsius) on the *y*-axis against the temperature recordings from the meteorological station (in Celsius) on the *x*-axis. From this a regression equation is calculated, and this is used to determine a *correction factor* that will be subsequently used to correct the meteorological data (Figure 2). This factor will be used to calculate what the ambient temperature would have been at the location before the discovery of the body. This temperature is then used to determine how many hours or days it would take the identified flies to develop from the stage of the egg to the full adult stage.

Each insect species develops optimally within a known temperature range, referred to as the upper and lower limits; the eggs/larvae will die at a lethal upper temperature and will not develop below a specific lower temperature.[137] A typical illustration of insect development across a complete temperature range is presented for the egg stage of *Lucilia sericata* in Figure 3. Specific temperature requirements vary by species and stage. For example, larvae of some *Calliphora* sp. stop development when the temperature reaches 39°C, while the upper limit for some *Phormia* sp. is 45°C. Some African and Australian species are known to have upper limits of, or exceeding 45°C.[63,138]

The import of this is that the more the temperature at the scene approaches the lethal level, the less the tendency for the insect to develop, this will lengthen the time taken to reach the adult stage, and consequently, affect the estimated PMI. The same is true for the lowest temperature (*base temperature*), below which no development or growth of the insect will occur.[51,59] These base temperatures are specific for the insect species and (possibly) locality. Davies and Ratcliffe (1994) reported a base temperature of 3.5°C for *Calliphora vicina* in the North of England,[139] while Donovan et al. (2006) reported 1.0°C in London[140]; Marchenko (2001) had reported 2.0°C for the same insect in Russia.[141]

The base temperature is calculated by plotting a graph of the developmental rate (inverse of the total number of days required to develop, that is, the time required for the larva to emerge from the egg, or that time taken for the adult fly to eventually emerge from the pupa) against the temperature.[51,134,140] This is also referred to as the *Linear Approximation Estimation* (Figure 4).

Higley and Haskell (2009) emphasized that the graph is actually curvilinear (Figure 3), though the most reliable range for the estimation of the PMI is the linear (mid) zone.[51] There are research compilations on the peculiarities

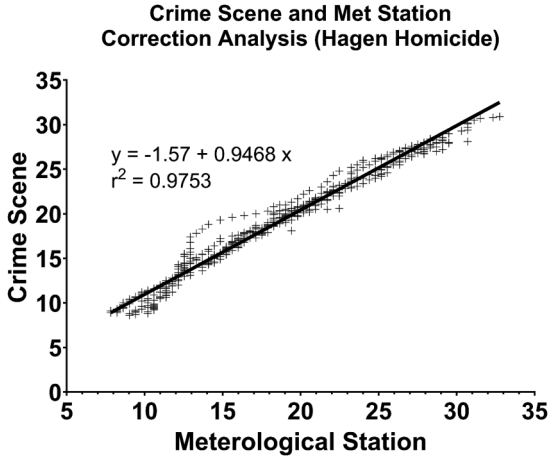

Figure 2. Plotting the temperature at the death scene against that from meteorological station based on an actual homicide (the Hagen murder).

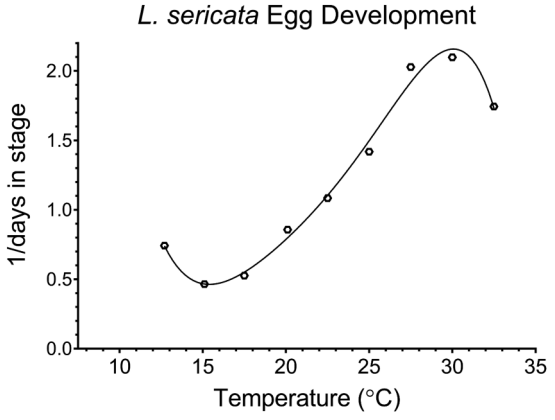

Figure 3. Plotting temperature against development time (1/days in stage) to demonstrate the upper and lower temperature limits at which insect development will occur (after data by Roe).

of many insects with respect to their growth and development at different temperature ranges.[142]

Degree days/degree hours

Temperature is the major abiotic factor involved in the growth and development of the pre-adult insect. Each species requires a specific amount of thermal energy per hour/day to grow or develop; this is can be described as the thermal unit. It represents the total degrees of heat above the base temperature that the insect must accumulate (in order to grow or develop) over one 24-h period (Degree days; DD) or over 60 min (Degree hour; DH) (Figure 5). The sum of the thermal units accumulated over specific number of days is called the *Accumulated Degree Days* (ADD) while the total sum of the thermal units accumulated over specific number of hours, is called *Accumulated Degree Hours* (ADH). These are derived as below:

$$\text{ADD} = (\text{Average temperature over 24 h} - \text{Base Temperature}) \times \text{Number of Days}$$

$$\text{ADH} = (\text{Average temperature over 1 h} - \text{Base Temperature}) \times \text{Number of Hours}$$

The average temperatures above are the corrected temperatures originally obtained from the meteorological stations, using the correction factors, after plotting the scatter diagram. It is this corrected value (using the regression equation) that will give an accurate temperature of the location where the body was discovered. It is the ADD and ADH that give the best estimate of the PMI, provided the correct base temperature is applied.[39,143,144] It is noteworthy that many laboratory studies are dependent on the type of substrates used to provide nutrients when the larval stages of the insects are being reared.[113] The rates of their development are dependent on the maggot mass and the initial size of the maggots used for the experiments. An interesting question not yet fully answered is whether the date to be recorded for the adult eclosion should be when the first adult emerges from the pupa, or it should be an average of the time taken by all the pupae studied.

If possible, when recording the temperature at the scene, it is better to use that obtained from the maggot mass, as opposed to the ambient temperature. The former is supposed to reflect the temperature of the early third instar/late second instar larvae.[51] If the puparia are retrieved from the scene, the soil temperature at 5.0, 10.0, and 20.0 cm depths should be recorded and averaged, to derive the soil temperature. Some workers advocate that, in calculating the ADD, a mean scene temperature is better derived by using the average maximum and minimum temperature.[145]

The forensic entomologist must for legal reasons understand the possible complexities involving the different species of necrophagous insects usually present on cadavers, and the various sources of inconsistences or contradictions that might affect the calculation of the PMI.[146] It is also pertinent to note that the application of forensic entomology in the calculation of the PMI has been based on experimental studies using a number of animals, with the preferred animal being the pig – *Sus scrofa*. The choice of this mammal is premised on many similarities to humans with respect to body mass, skin, and genetic compatibilities; the animal is relatively cheap, available, the experimental results can be replicated, and there are minimal ethical considerations.[147] However, these pig

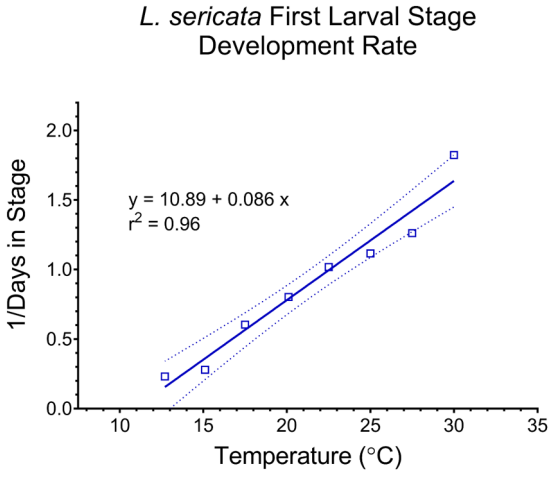

Figure 4. Graph (linear regression) to determine the base temperature for first stage *Lucilia sericata* (here the base is 10.89°C). This base temperature is used in calculating degree days but is NOT necessarily the same as the biological base temperature.

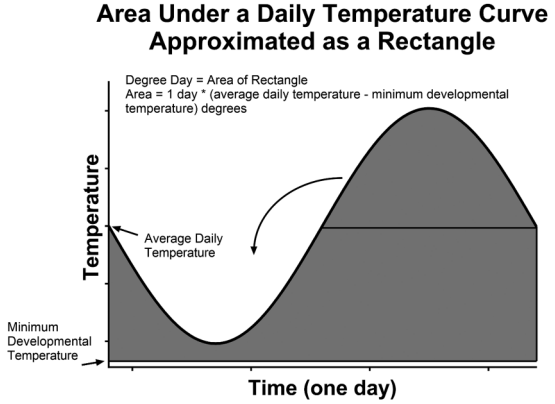

Figure 5. Curvilinear graph showing the base temperature and how the area under a daily temperature curve can be approximated by a rectangular area (the "rectangle method" for calculating degree days per day).

models are not without limitations and the experienced forensic entomologist will exercise some care in making direct extrapolations in Court. Studies during the Summer and Winter months in Australia, showed that pigs were colonized postmortem, faster than humans and thus decomposed faster. The pigs exhibited a greater population of the necrophagous insects compared to humans, and the latter tend to undergo desiccation while the former exhibit more of skeletonization.[147] All these differences are attributed to variations in the body in mass, diet, medical history, microbiome constitution and biochemical differences in the emitted volatile organic compounds, postmortem.[147]

The forensic pathologist and law enforcement agents must recognize the role of the forensic entomologist and seek early assistance where indicated.[148]

Conclusion

The use of forensic entomology in estimating the postmortem interval is complicated by environmental factors and aspects of insect biology. However, these complications can be addressed by trained forensic entomologists, and in most instances do not prevent the estimation of a PMI. Research in forensic entomology continues to improve the scientific basis for PMI determinations and relatively sophisticated models are emerging that offer improved accuracy in PMI estimation.

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