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Review

# Forensic Entomology: A Review on Post-Mortem Interval and the Need for Statistics

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## Simple Summary

Various methods have been established and proposed for post-mortem interval estimation. We review some of these approaches and explore how statistical robust entomological data could significantly improve the reliability of the insect evidence in forensic investigation.

## Abstract

Forensic entomology involves the utilization of insect and other arthropod colonizers in approximating the time since death, i.e., time interval between death and discovery of the cadaver/carcass or carrion/remains. This is frequently referred to as postmortem interval (PMI). The calculation of PMI can be useful when certain assumptions are met. However, determining a true PMI without proper statistical analysis has been a challenge for most entomologists. This review offers some concise overview of PMI determination methods principally based on two types of entomological development, i.e., thermal summation and insect succession. It also includes an appraisal of existing literature in forensic entomology concerning statistical applications, summarizing the forms of statistical analysis that have been, and should be utilized, with entomological evidence. The inclusion of statistical evaluation/statements of entomological data in most forensic entomology investigations have the potential of increasing the robustness of the evidence when presented to the court. Nevertheless, this requires comprehensive and accurate reference data, which the criminal justice system needs to understand in allusion to both the statistical assessment and methodologies employed to generate entomological data from laboratory and field studies. Furthermore, there is clear need to standardize terminologies and improve on the unsurpassed procedures of estimating PMI taking into account the various assumptions discussed in the literature.

**Keywords:** forensic entomology; cadaver; carrion; insect succession; postmortem interval; thermal summation model; statistical use/analysis/methods; experimental design

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## Introduction

Forensic entomology constitutes a specialized division of forensic sciences in which insects and related arthropods (specifically arachnids, mites, ticks, scorpions, spiders and macroinvertebrates such as freshwater shrimps) are employed as tools in legal investigations [1–5]. In modern practice, this scientific discipline is routinely split into three areas: urban entomology, stored product entomology and medico-legal entomology (also sometimes referred to as medicocriminal entomology) [4,6].

Urban forensic entomology focuses primarily on legal cases encompassing termites, cockroaches, and other urban pest species of insects that are commonly found human environments such as dwellings or other structures, and agricultural pests that may invade occupied buildings [7]. Stored product entomology deals with disputes concerning arthropods and their fragments that may contaminate a wide range of commercial foods (e.g., processed cereals, seeds, dehydrated fruits, nuts, and other types of dry food produces) and infest food storage facilities and production sites [2,4,6,8,9]. Akin to its urban counterpart, this grouping frequently involves civil lawsuits or litigation [6].

Medico-legal entomology is the most widely recognized aspect of the forensic sciences involving entomology that is most utilized by law enforcement agencies. It encompasses insects and related arthropods implicated in legal investigations pertaining to living or deceased individuals, domestic animals, wildlife, and livestock as well as other violations such as animal and human neglect or abuse, contraband trafficking, illegal trade in live animals, and wildlife trophies [6,10–16].

Entomological evidence can provide crucial information about a potential crime scene and its surrounding circumstances including geographic location, possible conveyance of a body or remains and locations of trauma on the corpse or carcass [1,16–19]. This entomological evidence can also be instrumental in recognizing deaths resulting from automobile accidents where the victim experienced a severe anaphylactic reaction following an arthropod bite or sting [19] or where loss of vehicular control resulted into an accident.

A constituent of medicolegal entomology that has garnered renewed attention is forensic entomotoxicology. This field involves examination of insect tissues in order to detect toxicants or their role in the death of victim(s), and/or the utilization of insects' gut contents as sources of human/animal DNA. Furthermore, the use of entomological evidence for genetic testing in the field of veterinary, livestock or wildlife entomology may fall within these forensic entomology subdivisions [4,19–21].

The objective of this paper is to review recent forensic entomology literature specifically focusing on post-mortem interval (PMI) estimations using insects, and more precisely on their statistical significance. This paper will review the various methodologies employed to estimate and calculate PMI, including minimum PMI (mPMI), time of colonization (TOC) and pre-appearance interval (PAI).

## Postmortem Interval (PMI) Estimation

In medicolegal investigation of human and animal fatalities, the determination of PMI is essential, and forensic entomology often plays a fundamental role. The PMI may be defined as the duration between the time death and discovery of the carcass, cadaver, carrion or remains. PMI estimation is central in addressing inquiries such as the conceivable time of death (especially in unwitnessed deaths) and in some situations, the cause of death. However, it is vital to remember that the PMI typifies an estimation of the most probable time of death and its calculation influenced by various biotic and abiotic factors surrounding the organism as well other biochemical and physiological factors within the organism [22,23]. For the most part, it is an estimation rather an exact determination; however, it remains an essential and a fundamental process in medicolegal death investigation for specialists in forensic medicine, pathology, and entomology

Diverse methodologies have been employed and novel promising protocols proposed for PMI estimation. The employed techniques have encompassed both early and late postmortem changes. The early postmortem changes include supravital reactions (post-mortem excitation-induced reactions of tissue), *livor mortis*, *rigor mortis*, *algor mortis*, and dryness of skin and visible mucosa with development of *tache noire* in the eyes [24–26]. *Livor mortis* typically involves gravitational settling of blood based on gravity, which commences immediately subsequent to death. During life, blood continuously circulating within the vast network of blood vessels (arteries, veins, and capillaries). Conversely, upon death, circulation ceases, and the blood pools and this causes skin discoloration in certain areas especially those closest to the ground. *Rigor mortis* or rigidity characterizes the

postmortem stiffening of body muscles and joints a sequel of chemical alterations in their myofibrils. *Algor mortis* (cold death) denotes the postmortem cooling of the body that typically occurs after death; the body temperature equilibrates with the ambient temperature. *Tache noire* (French for “black spot of the sclera”) is a reddish-brown discoloration that develops transversely across the sclera. It appears when the eyes are not absolutely closed so that the sclera is exposed to air. Late post mortem changes comprise decomposition, which include autolysis and putrefaction [26–29], biochemical variations (thanatochemistry) [30,31] as well as the analysis of degraded nucleic acids, both DNA and RNA [26,29,32–35].

The precision and accuracy of the aforementioned techniques diminishes over time. Beyond 48–72 hours, ecological methodologies, especially entomological evidence, seem to be the most precise and the superior method of determining PMI and other pertinent information surrounding the circumstances of death [1,16,22,23,36,37]. Nonetheless, in situations where insect evidence is non-existent, taphonomic indicators may also play a significant role [27,38–42].

Forensic entomologists have developed numerous methods for calculating the entomological-portion of the PMI [19,23,43–48]. Several methodologies that have been suggested or utilized in estimating insect age mostly based on the developmental stages of eggs to larvae and to pupae. The age estimation procedures have characteristically concentrated on the larval stage, integrating morphological change such as length, weight, and the color of pupal casing for specific species [3,49]. Nonetheless, it is crucial to note that the insect age is not capable of estimating the exact time of death but preferably a minimum PMI [50].

Currently, the most established methods for estimating larval age rely on the measurements of larval length and size (weight) augmented with other morphological characteristics of the larva [23]. These have been developed experimentally as reference models for a given insect species through growth curves and include three developmental models. The first is the isomorphen diagram (involving a simple scatterplot illustrating the duration of developmental events on the X-axis against temperature on the Y-axis) [17,51]. The second is the isomegalen diagram (encompassing a 3D contour plot simulating larval size [length, weight or width on the Z-axis] as a measure of age against temperature {Y-axis}, and time on X-axis) [17,52]. The third one and arguably the most sophisticated and advocated model for PMI estimation is thermal summation model [1,16,17,23,53,54]. It has been demonstrated that in such curves, length is most accurate during the incline stage, i.e. the growth phase. The major limitations with the determination of age by length or weight is that the data is frequently collected at established temperatures in the laboratory and thus do not actually mimic natural conditions, which can have fluctuating temperatures amongst other variables.

Some of these methods, when combined with other internal morphological features, can be adequate for PMI estimation [3,55,56]. Insect life cycles (through egg, larval, pupal and adult stages) are characterized by complex developmental processes involving many genes and their expression functions can serve as a contemporary tool in age determination [49,57–59]. Other molecular methods include analysis of cuticular hydrocarbon [3,49,60–64], cuticular banding pattern [65] and volatile organic compounds [66–68]. The usefulness, impartiality and consistency of these methodologies vary and have not yet been fully refined for use in the courts at the present moment.

The most commonly recognized method for assessing PMI primarily relies on two types of entomological development [48,69]. The first is based on temperature-dependent arthropod development [19,47,48,70] while the second involves insect succession on carrion or remains [16,23,43,44,48,71,72]. The latter can be termed the succession interval and typically involves the period since oviposition or larviposition, i.e., insect age, or the period where the carcass, cadaver or remains has been accessible to carrion insects [73]. Insect succession can be employed for several weeks to months in the post-mortem period. Although temperature-dependent arthropod development is frequently considered more accurate than species succession models, its applicability as an estimation of minimum PMI (mPMI) can be very restrictive due to the arduous undertaking of data retrieved for certain environments, amongst other factors that include the accessibility of growth rate data for precise necrophagous species and geographical locations [19]. Nevertheless, the two

methods are established on presuppositions that are subjected to influence by various biotic and abiotic factors.

As discussed earlier, law enforcement detectives are routinely concerned with the chronology of death that a forensic entomologist calculates from insect evidence. In the literature, various terminologies exist for PMI divergences generated from insect evidence [13,14,19,74–79]. The so-called “death-interval terminologies” used in the field of forensic entomology are presuppositions linked to the analysis of collected evidence [77,78]. These may lead to confusion and assumptions where the empirical facts governing insect presence and development are overlooked or are unidentified. Other terms include, period of infestation (POI) [74], postcolonization interval (PCI) [80], time of colonization (TOC) [14] and period of insect activity [75]. In some instances, PMI has been divided into minimum and maximum PMI ( $PMI_{min}$  and  $PMI_{max}$ ) since it can more precisely describe a collection of values as opposed to a single point of estimate [23,81,82].

Other intervals preceding the presence of an insect taxon on a carcass comprise time of oviposition, preappearance interval, and precolonization interval [76,83,84,144]. Recent publications have suggested that to advance this field and resolve disagreements regarding terminologies utilized in forensic entomology, “all these terminologies can be conscientiously submitted to court of law if they are induced as null hypotheses with a congruous list of presuppositions” with a proposal ensuring that forensic entomologists commence conducting research and casework with this approach in mind [78,79,144].

## Insect Succession

During decomposition, a carcass, remains, or cadaver undergoes significant physical, biological, and chemical transformations during decomposition [85–88] which attract a varying assemblage of insects and associated arthropods. This succession leads to an alteration in the entomofauna over time [89,144]. There are two orders of insects that predominantly occupy the carrion resource; Diptera (true flies) and Coleoptera (beetles) [1,90,91].

Diptera have a predilection for reaching ultimate profusion at the earlier stages of decomposition, whereas Coleoptera normally take over remains in the later stages [1,92]. Both use carrion resources as: (1) a foundation for nutrition, (2) a breeding location, (3) an oviposition/larviposition location and (4) prey habitat (for parasitoids) [1,23,37,93–95].

Diptera that often arrive within minutes of death are the blow and bottle flies (Family: Calliphoridae), although in some regions, flesh flies (Family: Sarcophagidae) have also been identified as early colonizers. The true necrophagous flies are either enticed to feed directly on the carcass, cadaver or remains, on the other hand utilize the carrion resource for egg oviposition, whereas other species are drawn by the large congregation of other insects that they utilize as a sustenance supply [1,5,16,22,23,37,83,88,91,93,94,96] This insect succession typically follows a relatively predictable sequence of successive waves, referred to as *seres* of organisms, each comprising diverse species drawn to a specific stage of decomposition.

From a practical outlook, when the order of insects inhabiting carrion is established for a certain region, an insect succession studies on a carcass, remains, or cadaver can be utilized to assess the duration of their presence and thereby providing an estimated of time of colonization relative to the time of death, or PMI. This is depende[3,5,16,19,75,88,96–98,144]nt upon appropriate entomological collections being done, persons (non-expert vs expert) undertaking the collection and preservation procedures, along with adequate historical climate data present and available [3,5,16,19,75,88,96–98,144].

Nonetheless, insect succession on carrion is influenced by numerous determinants including those that impact the decomposition process. Such circumstances typically include geographic region, habitat type, weather/seasonality, and conditions where carcass, remains, or cadaver are located possibly in enclosures, vehicles, wrapping, burials, presence or absence of clothing, protective integuments and burnings, and aquatic submersion. Beyond affecting the rate of decomposition, these can also modify insect succession patterns. Furthermore, insect succession patterns can be

further complicated by characteristics such as individual species traits, presence of precocious eggs in some species, myiasis, maggot mass, food type, and drugs/toxins, as well as geographic region, preservation method and antemortem colonization [23,43,86–88,96,98,144].

## Insect Temperature-Dependent Development/Thermal Summation Model

The thermal summation model is grounded on the principle that insects have a positive relationship between their development and temperature, which is typically represented by a linear regression analysis [17,54,99]. Temperature is the foremost abiotic factor regulating development of insects (as all insects are 'cold blooded' or poikilothermic) making it the supreme density-independent factor for insects [5,16,23]. Temperature is known to directly modulate the estimation of TOC, which in most circumstances can be equate or be a component of PMI. Insect development is fundamentally linked to ambient temperatures such that each insect species has its own thermal activity and death thresholds and characterized by thermal tolerance range with minimum and maximum parameters, outside of which growth or function ceases [5,16,23,53,100]. Amid these two points, growth rate of the immature insect is contemplated to have a linear/curvilinear relationship with temperature and is commonly exemplified by a sigmoidal shaped development curve [53,54,99,101]. Near the temperature extremes (either high or low), insect development exhibits reduced growth, eventually reaching a point where no growth occurs [3,99].

As documented in the literature, the development time of a specimen can be predicted from the thermal history. The linear regression can be utilized to ascertain an x-intercept (lower developmental threshold, T) and inverse of the linear regression's slope (thermal summation constant, K) which enable the prognostication of development time from a specimen's thermal history [3,23,101,102]. Under this model, it is evident that heat is pivotal for flies to progress through their developmental stages and this heat is referred to as the '*physiological energy budget*' [3,23,99,103,104].

This energy budget can be quantified as thermal units designated to degree days (DD) or degree hours (DH), where one degree day equates to one degree above the lower developmental threshold over 24 hours and one degree hour represents one degree above the threshold over one hour [3,16,53]. The duration required for either ametabolous, hemimetabolous or holometabolous metamorphosis typically depends on the aggregate number of thermal units accumulated and this is termed as accumulated degree days (ADD) or accumulated degree hours (ADH). The ADD is calculated using the following formulae [16,23,53]:

$$ADD = \text{time (days)} \times (\text{temperature} - \text{base temperature})$$

$$ADH = \text{time (hours)} \times (\text{temperature} - \text{base temperature})$$

Data necessary for the PMI calculation comprises identification of the fly genus and species and developmental age, experimental development information at relevant temperatures for collected insects, base temperature or developmental threshold for each species (obtained from published data such as life-tables or developmental rate of specific insect species), temperature data from the crime scene and a nearby weather station, calculation of accumulated degree data (hours or days) representing relevant stages of insect development, and calculation of accumulated degree days for the crime scene [3,16,23].

Recently, a curvilinear model named ExLAC has been recommended as a preference to linear thermal summation modeling [3,105,106]. ExLAC determines larval age in forensic entomological investigations and relies on exponential functions that define the precise development period for each larval stage until eclosion (adult emergence), as these are influenced by time and temperature [3,105,106]. This prototypical is designed to integrate variation in the reference dataset for development time and measurement error in the temperature representation. Furthermore, ExLAC also approximates an error value (RMS = root mean square) for larval age calculation, which is a statistical quantity similar to a standard aberration [106]. However, since ExLAC has not been extensively appraised prior to its enactment in forensic practice, the linear thermal summation prototypical remains the preferred method for estimating specimen age and PMI<sub>min</sub> [3,107–110].

## PMI and Statistical Use

Forensic science professionals and experts routinely present their findings and opinions in court by using words such as “to a reasonable scientific certainty” or to a “reasonable degree of (discipline) certainty.” In the United States, forensic entomologists often offer “expert testimony” under several Federal Rules of Evidence (FRE) or their State-level equivalents or counterparts [111,112]. As specified by Faigman [113], Tarone and Foran [114], and Faris et al. [45], the admissibility criteria for expert or scientific testimony are entrenched by the 1993 U.S. Supreme Court case *Daubert v. Merrell Dow Pharmaceuticals, Inc.* where Rule 702 of the Federal Rules of Evidence has become the benchmark. This “Daubert Standard,” warrants that, the testimony must be founded on appropriate scientific evidences or data; authenticated, accurate and dependable methods and principles (supra 584 – 587).

Following the National Research Council (NRC) report [115], several forensic specialties encompassing fingerprints handwriting, fingerprints, tool marks, hair, tire marks, and bite marks, were censured and criticized for lacking scientific rigor, and admonished for deficient peer review practice exemplifying by major scientific fields [45,80,116]. Even though forensic entomology escaped scrutiny by the NRC report and its evidence is still admissible in court; however, recent courtroom challenges and critiques amongst some in the forensic community have surmised that the field could gain reputation from ongoing research efforts required to fully meet the criteria of the Daubert standard and avoid the criticisms identical to those presented in the 2009 NRC report for other disciplines [80,117,118].

As an inferential science, forensic entomology depends on extrapolations from experimental data from aspects such as the PMI determination as well as responding to other insect-related interrogations; therefore, it is essential that it adheres to definite standards of excellence and thoroughness in connection to logical reasoning and other requirements [45,80,117,118]. Therefore, PMI estimation could substantially deviate from the actual interval due to the environmental settings surrounding the carrion and the variable assumptions that are used when calculating the same. These variable assumptions include variability in aptness of egg-laying, night activity of the fly, meteorological conditions, influence of maggot mass heat on development, disruption of decaying carrions, and antemortem circumstances of the dead animal or human [36,45,81,86,87,117,118]. In final analysis, PMI estimation generated from entomological evidence is directly linked to the quality of available data, which ultimately is expected to yield a result that is not far from the definite PMI. When estimating PMI by means of development and succession studies, forensic entomologists largely depend on information derived from peer-reviewed scientific literature and experimental data (laboratory and field base). If the experimental designs and outcomes impossible to validate, the result attained may be inaccurate.

Michaud et al [117], in a review of experimental study designs in forensic research, established that a large amount (78%) of field studies possessed design shortcomings and statistical discrepancies that could preclude usable inferences of relevant information for impending legal proceedings. Notably many entomological studies, are usually subjected to courtroom challenges through case study reports containing inaccuracy rates, interval estimates, probabilities and unsubstantiated suppositions [17,36,43,83,117–119].

In reviewing these inadequacies, Moreau et al. [118] proposed that in the biological, behavioural and physical sciences, sufficiently robust inference is contingent on well-articulated propositions and extrapolations, methodical-designed experiments, and comprehensive analysis of statistical data. Some researchers have incorporated null models and randomization methods in hypothesis testing [120–122], while others have recognized the need to develop experimental designs coupled with improvement PMI estimation and statistical models that can supplement data during the courtroom proceedings [44,46,104,117,119,123–127].

This perspective is gradually transforming the way in which researchers are embracing well-designed experiments, consistent representation, and acceptable analysis of statistical data in conducting forensic entomological work and discarding the previously preconceived assumptions in

this field. Since the early 1990s, a few researchers have embraced various statistical analysis and computer models with the conviction that forensic entomology needs to amplify its inference strength, statistical power (including computer modelling) and ecological (and empirical) foundations [19,75,80,117,118,122,128].

Several methodological solutions have been proposed to reinforce inference strength forthcoming casework, exclusively those concerning PMI estimation [117,118]. These can be summarized as outlined. In experimental design, it is recommended the forensic investigator should allow for proper extrapolations coupled with robust inference strength. Inferences is an act of rigorous thinking where deductions e made from existing data may be influenced by random deviation. Strong inference strength is necessity during court validation and have to comply with certain edict;

- (a) *Acceptable Reproduction*: Experiments should be repeatable with several set or units. Insufficient repeatability resulted in limited comprehension of the inherent variation in insect succession. Moreover, in particular instances, to enhance inferential power of a succession dataset a collection of larger samples over a period of time and ensuring consistent methodology at the a similar point or contemporaneously sites at comparable seasonal periods [117,128,129]. In cases, where there is insufficient sampling effort, pseudoreplication may develop and this diminishes the inferential strength. Pseudoreplication occurs when there is the lack of adequate treatment replication and such results render the comparison invalid.
- (b) *Independence of Experimental Units*: This guarantees that there is no discernible correlation (i.e. interdependence) among experimental units; a fundamental supposition of most statistical tests. Experimental establishment dealing with carrion-insect succession, suggest a minimum distance of 50m for spatial independence which prevents cross-colonization of insect cohorts especially larvae, though this may be inadequate in halting actively vagile adults. One study designed to evaluate carcass independence; this was done by placing 32 domestic pig carcasses at least 30 m apart for over two years. The analyses of succession intervals and community-similarity indices established that this distance was sufficient to ensure each carcass functioned as an independent sampling unit [95].
- (c) *Capturing a Representative Range of Natural Variability*: This is a prerequisite that is often flouted in forensic entomology field studies. It necessities the use of diverse study locations in order to justification for prospective locational effects and integrate site-to-site variation. Relying on a single study location reduces exterior cogency and limits inferential influence that specific location.

While statistical and computer modelling coupled PMI algorithms are present, the forensic entomology professionals have been tardy in conniving investigations that can advance these models and reduce inaccuracies. Schoenly [120] and Schoenly et al. [130] were the pioneers who laid the foundation for these studies. Schoenly [120] established some guidelines for assessing carrion-arthropod succession (occurrence matrix) in forensic entomology studies. This procedure interpreted data in three techniques: (1) succession of arthropod engagement (non-reoccurring taxa =80% and recurrent taxa =20%) on nonhuman remains; (2) chronological alterations in the taxonomic conformation of the carrion-arthropod community considered by enumerating the gradation of taxonomic likeness and (3) use of randomized trials (via Monte Carlo simulation) to community-wide insect visitation times [131].

Schoenly et al. [122] further incorporated the null models and randomization methods in testing hypothesis by means of two computer-intensive sampled randomization tests (the Jackknife and Bootstrap) and information from three studies of carrion-arthropod succession. They examined the extent to which the PMI was influenced by presence and absence of taxa and deduced that the randomization methods were promising instruments in forensic entomology jointly for the verification and scrutinization of arthropod successional data as well as evaluating statistical improbability of entomology-derived PMI estimates. Prior to this study, no succession-based research had utilized mathematical techniques to compute statistical confidence intervals or

acceptance region for a PMI estimate or incorporated sensitivity assessments of baseline data to define accuracy of repetitive PMI estimates under varying ecological and methodological conditions. To this end, a vital assessment is to ascertain whether the 95% confidence intervals, obtained from the mathematical techniques, represented the factual PMI in 95% of the samples retrieved from successional data. Perez [95] employed similar methods to those of Schoenly et al. [122] by refining statistically arthropod succession models appropriate for PMI. This model covered temporospatial and sample-based variability on carrion insect communities; guidelines for creating huge forensic datasets; the establishment of least carcass separation space for forensic entomology field procedures and assessing the hexapod species' utility for computing a confidence interval and exactness/accurateness of succession-based PMI estimates by means of a physiological and an absolute time measures. Statistical methodology involving inverse prediction (IP) was used to calculate the PMI.

Successively, Byrd and Allen [131] pioneered the procedure of computer modelling of insect species' life cycles in forensic entomology. Utilizing MATLAB® and Simulink® software, these researchers were able to predict thermal development including life cycle of various forensically dominant Calliphoridae from their research laboratory data. The model is determined by input from both temperature and oviposition submodels. They were able to exhibit computer visual outputs of program profiles of relative abundance of each life stage (egg through pupa and adult) along a time axis. The benefit of this classic model is that it allowed the participant to alter preliminary situations and envisage modifications in life-cycle development (i.e., PMI) for computer-generated crime scenes) [117,118].

Numerous physical, chemical and/or biological alterable that influence function of PMI have been suggested as "representative time cues" for PMI estimation [132]. Construing period subsequent to death is a normal objective of a death investigation and the interrogations characteristic in such interpretations are statistical and the chance-based aspects of the statistical processes must be understood to correctly account for uncertainty. Forensic entomological field data that is utilized for this purpose frequently prove unpredictable, with surrounding crime scene conditions unpredictable and prior knowledge often limited.

A thorough examination of the entomological PMI estimation methods is vital, necessitating the development of practical, reliable and accurate studies. It is imperative that the development of reliable statistical procedures with an acceptance region for PMI estimation exist for use by the legal system. Until the concerns of unpredictability, unpublished data, and inability to provide reliable conditions during modeling and explanatory procedures, accurate estimates of the PMI may not be achievable.

Thus, statistical approaches are crucial for generating the accuracy for a PMI estimation and assessing any controversy between experts (i.e., diverse PMI estimates is not likely, fundamentally different). When statistical methods are employed, each may give diverse dimensions of importance or certainty. As previously stated, methods have been suggested for working out lower and upper limits for an insect specimen's age centred on a continuous quantitative response(s) such as body length or weight [123,126,127,133,134,144].

In order to improve quantitative standards anticipated of statistical approaches especially the confidence sets on carrion insect age from development stage, a number of researchers adopted the inverse prediction (IP) or calibration [46,73,82,123,124,134–137]. The computations obligated for IP were performed using PROC MIXED in SAS [46,132]. Fundamentally, IP can be utilized to develop confidence sets on  $x^*$ . These authors explain IP's mechanism: Within a well-regulated situation, developmental stages are tested at each  $x$  over a variety of standards, and the answer  $y$  is measured on each developmental stage. The resultant data set is termed the training data (TD). A model connecting  $y$  to  $x$  is appropriated to the TD, typically encompasses the specification of class models for first and second moments, (means and variance-covariances of  $y$ , in terms of  $x$  and other covariates (e.g. temperature). In discussing this inverse logic calibration, using an unknown age insect (a mystery specimen, MS) with response  $y^*$ , its answer is equated statistically to the model at

each of a grid of probable values of the unidentified  $x^*$  from which it created. This yields a probability statement, a p-value, for each probable  $x^*$ . All ages whose p values are not small (conventionally equal or  $>0.05$ ) constitute a confidence set for the true age. These authors asserted that these techniques are widely relevant and eagerly expect to see IP being assimilated into all technical disciplines involved with PMI estimation.

PMI estimation methods based on ADD indices have been employed constant in logistic regression to projection insect visitation trends [125] and decay stages [104]. Moreau et al. [118] outlined that this approach could produce statistical likelihoods and acceptable ranges for PMI estimates with a handful of carcasses. Furthermore, the development of ADD indices permits for the utilization of inferential methods unaltered by climatic patchiness (in terms of seasonality and year-to-year changes), incorporation of statistical data and other variability (microbial succession, species abundance data, carcass temperature) [104]. Nonetheless, ADH remains unapplied in place of absolute time in a succession model [73,95]. Its primary disadvantage is that it necessitates superior statistical adeptness than other methodologies.

The PAI is an interval before the any insect taxon arrival on a carcass, cadaver or remains. PAI estimation of carrion insects from temperature is an innovative and promising upgrading of entomological approaches for PMI particularly where carrion-frequenting beetles are studied [76,98,138,139]. These studies have demonstrated substantial inverse relationships connecting PAI and temperature in most studied beetle species paralleling the development of PMI estimation procedures for fly taxa. This methodology may be widely acceptable in case examination related to human remains uncovered in more advanced stages of decomposition [118] and in circumstances where insect evidence is lacking [84,118,140,141,144].

As demonstrated, numerous research articles addressing different aspects of forensic entomology and statistical application focusing on introducing new statistical analyses and advanced statistical models for the validation of methods used in court proceedings [127]. The 2015 study by Moreau et al [118], in which estimations of insect age, and by extension PMI are calculated using linear regression, isomegalen diagrams, isomorphen diagrams and thermal summation models is one example. However, in some circumstances, age estimates can be distorted and gene expression profiles altered as a consequence of variations/factors including growth differences between laboratory and natural conditions, coupled with the non-linear growth of some flies [58,114,142].

Furthermore, such settings, can predictably lead to differential growth rates for the same fly species thus culminating in less precise age and inconsistent PMI conclusions based on the similar entomological evidence contingent on type of growth data utilized [23,114,127]. In those particular circumstances, the linear models fail to provide absolutely dependability results as the development rate of blow flies is only linear during brief developmental periods and within specific temperature ranges [23,114,127]. A major drawback of linear prototypes is the fundamental supposition of autonomous observations and equal variance of errors or homoscedasticity [127,143]. This implies that interpretations within an experimental setup must be taken as autonomous from each other. If the interpretations exhibit dependent structures, the supposed replicates are simply pseudoreplicates or false replicates. Consequently, the procedure of linear models with a solitary unit of variability turn out to be inappropriate.

Baqué and Amendt [127] compared three statistical procedures (linear regression, generalised additive modelling-GAM and generalised additive mixed modelling-GAMM) for evaluating and testing forensic entomological datasets. They were the first to demonstrate that generalised additive mixed (GAM) models gave the regression parameters that adequately reflect the data used. Additional details are discussed concerning ecological data, however these principles are applicable for forensic entomological data in circumstances where there is heterogeneity and nonlinearity problems [143].

Recent reviews [128,144], have emphasized that experimental studies in forensic entomology must adhere to a series of guidelines that produce precise expectations regarding casework and fulfil established experimental procedures that yield probabilistic inference of quality and robust

validation of the accuracy of PMI estimations. The first author further emphasizes that scientists to need to employ more robust and highly powerful statistical tools and prevent the dissemination into the courtroom of any forensic evidence that fail to meet strict experimental conditions because such evidence can generate erroneous positive outcomes that risk wrongful convictions or unwarranted acquittal [128]. Furthermore, the second author stressed that ongoing research need to prioritize areas that can be used to decrease the characteristic inaccuracy of PMI estimations [144].

In conclusion, the most critical real implementation of forensic entomology is the estimation of carrion insect age, which can subsequently be use to sustain a forensic investigation into determining the age of an insect or its developmental stage on carrion. To gain the right determination or estimate, appropriate methods need to be employed, coupled with the right mathematical values or powerful statistical tools. Forensic entomologists are continuously striving to refine these techniques including the modification of the appropriate terminologies or language. As discussed by many authors, forensic entomologists must embrace more advanced statistical (and other mathematical) modelling as they perform repeatable experimental work devoid of experimental errors. Therefore, incorporating of probabilistic declarations or additional statistical assessment of entomological evidence and related research findings in their casework would significantly enhance the robustness of the evidence in court. Nonetheless, this necessitates suitable and detailed baseline and benchmark datasets and it is crucial to ensure that the criminal justice system stakeholders (judge, jury prosecutors and other relevant players) comprehend the statistical evaluation and how entomological data is generated from the laboratory and /field studies. Forensic entomologists too, need to agree on the various terms and the best methods of estimating PMI (with the ultimate goal of reducing the estimation inaccuracy), taking into consideration the various assumptions that have been advocated in the literature. Until all these groups are empowered in coming up with rational, fact and probability-based decisions, they will remain pliable to or manipulable by persuasive plaintiff litigants and other expert witnesses cognizant of the fact that human discernment is uncertain is unpredictable.

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