Predicting Post mortem Interval using Discriminant Function Analysis of the Succession Pattern of Necrophagous Insects

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ABSTRACT: Estimation of postmortem interval (PMI) is an integral part of most death investigations. Succession pattern of insects associated with decomposing animals is one of the methods used for PMI prediction. This study aims to design a model with which PMI may be predicted in Lagos and Kaduna States using the successional pattern of insects on decomposing carcasses in the two regions. Seventy percent of the data on the succession pattern of insects collected from decomposing pigs were fed into discriminant analysis to generate a classification model while the remaining 30% of the data was used to test the model. Twenty six variables were used to classify time of death into nine groups (0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8 and over 8 weeks). Discriminant function analysis revealed that empty calliphorid puparium around carrion (0.675), Tenebrionidae (0.598), Histeridae (0.582), adult Calliphoridae (-0.509) and Scarabidae (0.50) were the five most discriminating variables for predicting postmortem interval. The model (LD₁) generated was tested with blind data to time of death with an upper accuracy limit of 79.7%.

Keywords: Postmortem interval, Succession, Discriminant analysis.

INTRODUCTION

The most commonly used and required information that can be provided by forensic entomological work is the estimated minimum time of death also known as the Postmortem Interval (PMI). In forensic entomology, it is generally accepted that there is actually no scientific way to precisely determine the exact period of time since death, hence, an estimation of the Period of Insect Activity (PIA) on the body is determined. Period of Insect Activity reflects the minimum period of time since death or Postmortem Interval (PMI) but does not precisely determine the time of death. In most cases, arthropods are used as biological

indicators of PMI and have been shown to offer more reliable estimations for longer PMI intervals. From 72 hours after death, forensic entomology is usually the most accurate and in some cases the only method that can be used for estimation of PMI (Kashyap and Pillai, 1989; Tempelman-Kluit, 1993).

In recent years, entomological techniques used in postmortem interval estimation have developed and become increasingly important thereby resulting in their successful application. The calculations of PMI by entomological techniques fit well with the time intervals established by other methods (Smith, 1986; Goff *et al.*, 1988; Catts and Haskell, 1990). Kashyap and Pillai (1989) examined and analysed 16 insect-infested cadavers to evaluate the

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reliability of the entomological method in estimation of time elapsed since death in relation to other medico-legal approaches. Their results revealed that the entomological method was statistically more reliable and superior when compared to other prevalent methods.

Insect scientists are increasingly being called upon to apply their expertise in criminal and civil proceedings and even constitute part of forensic laboratories and medicolegal investigation teams (Lord and Rodriguez, 1989).

An entomologically derived PMI is based on two general principles which may be complementary, although each may be better suited for use during different stages of carrion decomposition. The first method involves using developmental data of certain species commonly found on decomposing carrion in a region to estimate the age of a carrion, or the post-oviposition interval (Wells and LaMotte, 2010). This method is used in initial decomposition stages, with Diptera species from Calliphoridae family as the main indicators. The ages of insect immature stages found on a dead body can provide evidence for the estimation of a minimum PMI ranging from day one up to more than one month, depending on the insect species involved and the climatic conditions at the death scene.

The second method is to predict PMI based on the succession of arthropods present on a carrion (Anderson, 2001; Wells and LaMotte, 2010). This method can be used in long postmortem intervals (Catts and Goff, 1992) weeks, months or even years after death (Anderson, 1995). It is based on the premise that insects found on decomposing animal remains so in a predictable manner as decomposition progresses. Hence, it requires detailed records of the time at which each species arrives on carrions as they decay and not the development records.

Succession studies, usually made with animal models such as pigs provide the reference data used to compare with the taxa collected on human remains (Wells and LaMotte, 2010). Ecological successions are however dynamic and less predictable than

insect development (Braack, 1981; Wells and LaMotte, 2010). There are many parameters that can affect the timing and species composition of the carrion fauna, therefore research is highly required to develop a geographical database of insect succession on carrion in a variety of habitats and scenarios for forensic investigation.

The proper identification of the insects and arthropod species of forensic importance is the most crucial element in the field of forensic entomology. In order to apply entomological evidence to the estimation of the postmortem interval based on the stages of succession, it is essential to precisely identify the species of insects attracted to the remains and also note the succession patterns typical of seasonal periods (Goff, 2010; Sharma *et al.*, 2015). It is the species identification that allows for proper developmental data and distribution ranges which is to be applied to proper investigation procedures. If the species determination is incorrect then the estimated postmortem interval will be invalid (Byrd and Castner, 2010).

Watson (2004) established a statistical technique for distinguishing between deer, bear, swine and alligator carrions using the insect taxa collected during their decomposition. The study identified the insect taxa having the highest discriminating powers for each animal type using discriminant analysis which showed that bear, deer and swine were accurately classified 66.67%, 69.29% and 74.71% of the time respectively while alligator carrion was inaccurately classified.

Using generalized additive model of gene expression, Foran (2007) precisely determined the age of *Lucilia sericata* as a method of PMI estimation. Nine genes were assessed for their informative level using quantitative Polymerase chain reaction (PCR). The model was tested in a blind study to predict the age of 90 flies in which results revealed varying level of accuracy with more gene expression information provided.

The aim of the present work is to establish a statistical model with which PMI would be predicted using the succession pattern of insects.

MATERIALS AND METHODS

Study Sites

The study was conducted during the wet and dry seasons in Lagos and Kaduna States, representing two different biogeoclimatic zones of Nigeria using 24 pig carrions, of known PMI.

Data Collection Procedure

All carrions were monitored immediately after death occurred, for insect activities and arrival pattern. Insects were collected using three sampling methods: Aerial sweep net, handpicking off carrions and surrounding soil and pitfall traps. Twenty insect larvae were collected from each pig carrion into pots containing hot water and thereafter transferred into vials containing 70% ethanol to prevent the larvae from shrinking.

Average weight of dipteran larvae found on decomposing pig carrions were determined by collecting 30 larvae randomly from each pig carrions daily which were killed by immersion into hot boiling water for 30s and thereafter removed into 70% ethanol. Ten representative larvae per pig carrion per location were placed on filter paper, air dried, and they were thereafter weighed using a Mettler®PM 400 (g) digital weighing balance with 0.01g accuracy.

Larval lengths were measured in pixel and with the aid of a calibrated hand held digitized microscope Zarbeco® with magnification of x40 and x140 and then converted into mm.

Larval length in mm =

$$MPI \times 2.54 \div DPI$$

Where

DPI (Dots per inch) = 300

MPV (Measured Pixel Value)

(www.dallinjones.com)

Insects were identified by comparing them with type specimens at the insectary of the

Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria and using keys from previous studies. Confirmation of the identified insects was carried out at the museum of the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria.

Statistical Analysis

All Postmortem interval was predicted using Discriminant Function Analysis (DA). A classification model used to predict group membership based on a linear combination of the interval variables was established for PMI prediction using the succession pattern of insects collected in the study.

The hypothesis tested was that there is a linear combination of variables which when appropriately weighted will maximally discriminate between members of nine groups and permit new cases to be classified into the groups. The variables used in the prediction were based on the occurrence pattern of selected insect families on pig carrions at different stages of decomposition. The independent variables are the predictors (26 different variables based on the occurrence pattern of insect families on the carrion) (Table 1) and the dependent variables are the groups (weeks of decomposition) which are categorized into nine classes: Weeks: 1, 2, 3, 4, 5, 6, 7, 8, and over 8 Weeks.

The data used in the analyses contained the following information for each sampling event: season, location, stage of decomposition, day (i.e., day of decomposition), week of decomposition and number of specimens collected. The variables $X_1 - X_{17}$ as well as variables $X_{20} - X_{26}$ are a function of numbers or quantity observed (equation 1), while variables X_{18} is a function of size (length) (equation 2) and variable X_{19} is a function of weight (equation 2). The variables were assigned with classification score C_i ranging from 0 – 5, depending on the number, length and weight of insect life stages observed on the carrions.

Table 1: Predictor Variables

No	Variables	Experimental Premise
x_1	Presence of dipteran larvae	f (n)
x_2	Presence of prepupa	f (n)
x_3	Presence of pupa	f (n)
x_4	Larval aggregation on surface	f (n)
x_5	Larval migration for pupation	f (n)
x_6	Empty Calliphorid puparia	f (n)
x_7	Larvae buried under carrion	f (n)
x_8	Presence of adult <i>Musca domestica</i>	f (n)
x_9	Presence of adult <i>Chrysomya</i> sp.	f (n)
x_{10}	Presence of adult <i>Sarcophaga</i> sp.	f (n)
x_{11}	Presence of adult Clerid, <i>Necrobia</i> sp.	f (n)
x_{12}	Presence of adult <i>Nicrophorus</i> sp.	f (n)
x_{13}	Presence of adult <i>Dermestes</i> sp.	f (n)
x_{14}	Presence of <i>Dermestes</i> larvae	f (n)
x_{15}	Presence of <i>Dermestes</i> pupa	f (n)
x_{16}	Presence of adult Formicidae	f (n)
x_{17}	Empty <i>Dermestes</i> puparia	f (n)
x_{18}	Average length of Dipteran larvae	f (l)
x_{19}	Average weight of Dipteran Larvae	f (w)
x_{20}	Presence of <i>Carabidae</i>	f (n)
x_{21}	Presence of <i>Scarabidae</i>	f (n)
x_{22}	Presence of <i>Histeridae</i>	f (n)
x_{23}	Presence of <i>Staphylinidae</i>	f (n)
x_{24}	Presence of <i>Melyris abdominalis</i>	f (n)
x_{25}	Presence of <i>Coccinelidae</i>	f (n)
x_{26}	Presence of <i>Tenebrionidae</i>	f (n)

Discriminant function analysis is a 2-step process, which involves:

- (1) Testing significance of a set of function and
- (2) Classification.

Subsequently, the function with the highest proportion of trace was therefore used to predict membership in naturally occurring groups using the formulae:

$$y = PMI = f(x_n) \text{ --- (1)}$$

$$y = C_{x_1}X_1 + C_{x_2}X_2 + \dots + C_{x_m}X_m \text{ --- (2)}$$

Location= Lagos=1 and Kaduna =0

Where $x_1 - x_{17} = f(n)$ and

n=0 for nil,

n=1 for ≥ 3 .

n=2 for $\leq 3 \leq 10$,

n=3 for $\geq 10 < 20$

n=4 for $\geq 20 \leq 30$

n=5 for ≥ 30 .

And

$$y = C_{x_1}X_1 + C_{x_2}X_2 + \dots + C_{x_m}X_m \text{ (2)}$$

Where $x_{18} = f(l)$ and measured in mm

l=0 when length of larvae is 0

l=1 when length of larvae is $\geq 0 \leq 5$

l=2 when length of larvae is $\geq 5 \leq 10$

l=3 when length of larvae is $\geq 10 \leq 15$

l=4 when length of larvae is $\geq 15 \leq 20$

l=5 when length of larvae is $\geq 20 \leq 25$

and

$$y = C_{x_1}X_1 + C_{x_2}X_2 + \dots + C_{x_m}X_m \text{ (2)}$$

Where: $x_{1w} = f(w)$ in μg

$w=1$ when weight of larvae is $\geq 0.001 \leq 0.004$

$w=2$ when weight of larvae is $\geq 0.005 \leq 0.008$

$w=3$ when weight of larvae is $\geq 0.009 \leq 0.012$

$w=4$ when weight of larvae is $\geq 0.0013 \leq 0.018$

and

$$x_{20} - x_{26} = \int n$$

$n=0$ for nil,

$n=1$ for ≥ 3 ,

$n=2$ for $\leq 3 \leq 10$,

$n=3$ for $\geq 10 \leq 20$

$n=4$ for $\geq 20 \leq 30$

$n=5$ for ≥ 30 ,

* C_k is the classification score for group k

* C 's are the coefficients in table

Cross validation of the first discriminant function was carried out using 30% of the total succession observations (as blind samples), and accuracy of

the result was tested using specificity and sensitivity of classified data, where Specificity is the probability of true negatives and Sensitivity is the probability of true positives. Analysis and presentation of results was carried out using Microsoft excel, SPSS version 21 and 'R' version 3.0.2. Significant differences were established using ANOVA @ $p < 0.05$.

RESULTS

Forty-two insect families were identified out of which 22 were of forensic importance and these insects belong to three insect orders; Diptera, Coleoptera and Hymenoptera. These insect families of forensic importance were collected from pig carrions as they decompose through five stages, namely; Fresh, Bloated, Active decay, Advanced decay and Skeletonized stages. Some of the insect families were common to the two study sites with certain insects found exclusively to either of the sites (Table 2). The

Table 2: **Forensically important insect families collected from pig carrions in Ojo, Lagos and Samaru, Kaduna State**

Order	Family	Ojo	Samaru	Status	Stage of occurrence
Diptera	Calliphoridae	*	*	N	All stages
	Drosophilidae	*	-	N	Fresh
	Fanniidae	*	-	N	Fresh
	Muscidae	*	*	N	All stages
	Rhinnidae	*	*	N	Fresh
	Tachnidae	*	*	N	Fresh
	Tephritidae	*	*	N	Fresh, Bloated
	Sarcophagidae	*	*	N	Fresh, Bloated, Active
	Sphaeroceridae	*	-	N	Fresh
	Carabidae	*	*	P	Advanced
Coleoptera	Cleridae	*	*	P	Bloated, Active, Advanced
	Coccinellidae	*	*	P	Active, Advanced
	Dermentidae	*	*	N,P	Bloated, Active, Advanced
	Histeridae	*	*	N,P	Active, Advanced
	Hydrophilidae	*	*	P	Active, Advanced
	Melyridae	-	*	N,P	Active, Advanced
	Tenebrionidae	*	*	N,P	Active, Advanced, Skeleton
	Scarabidae	*	-	N,P	Active, Advanced
	Silphidae	*	-	N,P	Active, Advanced
	Staphylinidae	*	*	P	Active, Advanced
Hymenoptera	Formicidae	*	*	N,P	All stages

N= Necrophagous, P= Predators

Table 3: Proportion of traced for discriminant functions

Function	Proportion of trace	% of trace	Singular value decomposition
LD1	0.5648	56	11.176
LD2	0.2379	24	7.102
LD3	0.0895	8.95	4.51

Table 4: Number of observations and percent classified into weeks determined using first discriminant function

WEEKS	Observation	% Classified	Sensitivity %	Specificity %
1	16	93.75	93.9	97.2
2	6	66.67	66.67	95.65
3	8	42.85	37.50	100
4	7	85.71	85.7	95.6
5	4	66.67	25.00	97.92
6	2	100	100	94.00
7	2	0	0	98.00
8	4	75	33.33	93.88
Over 8	3	66.67	33.33	93.88
Total	56			

Overall Accuracy 52.6 % - 79.7%

* Overall statistics: Accuracy= 0.673; P-value 6.46e-08; 95% CI= (0.529, 0.797). Specificity is probability of true negatives and sensitivity is the probability of true positives.

necrophagous insects which feed directly on the carrion and the predators that feed on the necrophagous insects and/or on the carrion itself were all included as predictor variables for PMI prediction (Table 3). The total number of observations recorded during the five stages of decomposition (i.e the total number of events captured throughout the studies which involves the predictor variables, N=173. More observations were recorded during active and advanced decay stages of decomposition which coincides with the 2nd, 3rd, 4th and 5th weeks of decomposition. During these stages the carrions attracted higher diversity of forensically important insects in greater abundance especially members of the order Coleoptera. The later stages of decomposition was characterized with less insect diversity hence fewer observations were recorded.

In the first step of the analysis, eight linear discriminant functions were generated from the nine groups to distinguish between the events of each week. The first linear discriminant

function (LD1) determined more than half (56%) of the observation and hence has the highest proportion of trace and was therefore accepted as a suitable model for PMI prediction (Table 4).

The model identified five observable variables having the highest predictive power for estimating PMI which were assigned with coefficient rating powers viz: presence of empty calliphorid puparia (0.675), presence of Tenebrionidae (0.598), presence of Histeridae (0.583), presence of adult Calliphoridae (-0.509) and presence of Scarabidae (0.50). The variables that had the lowest predictive powers were presence of adult clerid beetles (-0.01366) and presence of *Dermestes sp.* puparia (0.00449). Location (0.484) also had higher predictive value than season (0.046).

The model (LD1) which specified that PMI was a linear combination of location, season, presence and/or absence of some insect species and their abundance level on pig carrions is shown as:

$$\begin{aligned}
 PMI = LD1 = & location \cdot 0.484 - season \cdot (0.046) - (0.042) \cdot x_1 - (-0.085) \cdot x_2 \\
 & - 0.037 \cdot x_3 - 0.047 \cdot x_4 + (-0.031) \cdot x_5 - 0.675 \cdot x_6 - 0.142 \cdot x_7 \\
 & - (-0.465) \cdot x_8 - (-0.51) \cdot x_9 - 0.336 \cdot x_{10} - (-0.014) \cdot x_{11} \\
 & - (-0.22) \cdot x_{12} - 0.195 \cdot x_{13} + 0.296 \cdot x_{14} - 0.004 \cdot x_{15} - [-0.277] \\
 & \cdot x_{16} - [-0.483] \cdot x_{17} - [-0.278] \cdot x_{18} - [-0.091] \cdot x_{19} + 0.202 \cdot x_{20} \\
 & - 0.505 \cdot x_{21} - 0.583 \cdot x_{22} - 0.399 \cdot x_{23} - 0.315 \cdot x_{24} - 0.416 \cdot x_{25} \\
 & - 0.599 \cdot x_{26}
 \end{aligned}$$

DISCUSSION

The purpose of this study was to provide a statistical model for the prediction of PMI, using the succession pattern of insects on the carrions. The succession pattern of insects on the carrions followed the usual arrival pattern with the dipterans arriving earlier than the coleopterans and this was similar for both dry and wet seasons. The changes in carrion decomposition and succession trend of insect species is similar to observation of other works (Smith, 1986; Shi *et al.*, 2009). The active decay stage of decomposition attracted more necrophagous species and hence predictions during this stages were more accurate as evident in the specificity and sensitivity levels of the predictions. Earlier studies have also shown that the active decay stage attracts higher diversity of insect species especially the Coleopterans (Smith, 1986; Anderson, 2001).

Although variations was observed in the species diversity between the two regions, this factor was considered in selecting the variables included in the model. Similar variations have been reported by previous researchers from studies conducted in different geographical regions. It is general knowledge that the geographical region determines the types of insect species that would be found in an area and their seasonal availability.

All tested variables which included presence of calliphorid, puparia migration and average length of larvae were significant predictors of PMI in at least one model, though some exerted more influence on prediction than others. The inclusion of as much as 26 variables in the model helped to improve on the prediction performance. This finding is consistent with report of Wells and LaMotte (2017), who suggested that the importance of a covariate or

the practical value of a response can be assessed based on predictive model performance.

The accuracy level of PMI predictions using training data was higher (83.6% - 94.9%), than the test data set (52.9% - 79.7%), these shows that the procedure adopted in the discriminant analysis which involves using part of the observation is also a means of improving the prediction performance and eliminating bias. This method has been reported as an appropriate unbiased means of statistical analyses (Wells *et al.* 2015).

Presence of empty Calliphorid puparium (X_6) around the carrions was identified as the most informative predictor variables for estimating PMI with coefficient power of 0.675 while presence of adult clerid beetles X_{11} (-0.01366) and presence of *Dermestes* puparium X_{15} (0.00449) were the variables with the lowest predictive powers. Generally, several reports have documented the importance of fly puparia as entomological evidence in investigations not only to estimate the PMI but also to analyse for presence of drugs or poisonous substances. Greenberg and Kunich (2002) reported that presence of amber coloured puparia of *Phaenicia* (*Lucilia*) *sericata* on a body was used as the critical evidence in determination of PMI of a double murder in Chicago. Campobasso *et al.* (2004) also reported that accumulation of amitriptyline in the puparia of *Megaselia scalaris* was used as important information in determination of cause of death. This implies that since the pupa usually have a longer duration on carrions than the eggs and larval stages combined, the presence of empty puparia indicates that the adult calliphorid flies had visited the body and laid eggs and also shows that the eggs have developed to larvae and hatched to adults.

The presence of clerid and dermestes beetles consistently at all stages of decomposition except

during the fresh stages show that their degree of contribution to PMI prediction was reduced. This study has shown potentials for the success of further estimates of PMI using the model proposed.

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