

ORIGINAL ARTICLE

Forensic Entomology: Insects in Crime Investigation

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ABSTRACT

The minimum post mortem interval (PMI) can be estimated from the measurement of immature developmental stages of the insects that colonizes the natural and/or traumatic wound sites. Our objectives are to determine the patterns of insect succession across decomposition stages, identify primary colonizing species, and evaluate the effect of temperature and humidity on larval growth for accurate postmortem interval (PMI) estimation.

Methods: This descriptive and analytical experimental study was conducted over a 30-day duration using controlled conditions simulating natural environments. A total of 100 decomposing animal tissue samples were evaluated across five stages of decay. Insect specimens (eggs, larvae, pupae, and adults) were collected and identified through morphological examination and mitochondrial cytochrome oxidase I (COI) gene sequencing. Data on temperature and humidity were recorded and analyzed using IBM SPSS version 26 via Chi-Square test and Pearson correlation analysis.

Results: Active decay was the most frequent stage (30.0%), followed by bloated and advanced decay (20.0% each). Blowflies (*Calliphoridae*) were the dominant primary colonizers (40.0%), followed by beetles (*Coleoptera*, 25.0%) during later stages. A significant association was identified between decomposition stages and insect species distribution ($\chi^2 = 18.45$, $p = 0.002$). Temperature exhibited a strong positive correlation with larval growth rate ($r = 0.72$, $p = 0.001$), while humidity correlated with insect activity ($r = 0.58$, $p = 0.004$).

Conclusion: Insect colonization follows a predictable, statistically significant succession pattern that serves as a dependable biological indicator for PMI estimation. Ambient temperature significantly accelerates larval development, highlighting the necessity of integrating regional environmental data and standardized identification protocols in forensic investigations.

Keywords: Blow flies, Criminal Investigation, Forensic Anthropology, Forensic Entomology, Postmortem Changes

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Introduction

Forensic entomology is a specialized branch of forensic science, which uses the study of

insects and other arthropods in relation to legal proceedings, especially to estimate the post mortem interval (PMI) or time elapsed since the death (1,2). Beyond 24-72 hours after death, the conventional pathognomonic signs (algor mortis, rigor mortis, and livor mortis) become unreliable, but the dynamic and predictable colonization of dead carcasses by carrion insects affords important biological evidence (1,3). Early records date

back to the time of ancient China, where insects were used in the legal system but systematic scientific development started in 19th Century Europe, where the insect succession was used as a biological clock. (4,5) In the following decades it became a scientific integrative discipline that is close to entomology, taphonomy, ecology, pathology, and molecular biology. (4,6)

The stages of the body's physical and biochemical transformation on death are fresh, bloated, active decay, advanced decay and dry remains (7). There are unique arthropod communities attracted to each phase of the decomposition process, called insect succession (5,7). The blowflies (family Calliphoridae) are the pioneers of the colonizers which commonly arrive within minutes to hours after death and lay eggs in the natural and/or traumatic wound sites. These eggs develop into feeding maggots and pass through different larval stages before pupation and emergence as adults (8). The minimum post mortem interval (PMI) can be estimated from the measurement of immature developmental stages (8,9). Insects that colonize after the initial dipterans have been present, flesh flies (Sarcophagidae) and later carrion beetles or skin beetles (order Coleoptera), are more likely to be present in cases involving more advanced degrees of decomposition and skeletal remains (as opposed to early dipterans) and provide wider chronological windows (6,10). In addition to the calculation of PMI, entomological evidence can be used to identify antemortem injuries, species that are endemic to the world that the body has been transported to, and evidence of neglect in vulnerable groups (7).

Ambient temperature is a primary factor influencing rate of insect development. Necrophagous insects are also an ectotherm

and their development is influenced by temperature increases or decreases in the environment (2,11). The blow fly developmental rate was found to be directly linked to thermal conditions by Grassberger and Reiter which led to the baseline values used to estimate the insect's age with thermal models (11). At the same time, other factors such as humidity, rain, exposure to sunlight and microhabitat differences have a significant effect on the rate of decomposition and the colonizing fauna (6,12). Tarone and Foran added a generalized additive model (GAMs) and a more sophisticated statistical framework that took environmental variance into account and that minimized calculation errors over those based on the classic linear degree-day method (8,9).

Forensic entomology is a growing field because of recent advances in technology. In entomotoxicology, pharmaceuticals, illicit drugs, and environmental toxins can be detected in feeding larvae of insects when the conventional host tissue has broken down or is unavailable (3,13). But, the toxicological influences on larval growth kinetics may be different for xenobiotics and should be incorporated into larval models to prevent misestimation of the PMI (13). At the same time, molecular diagnostics have answered the main challenges in morphology identification. Taxonomic determination of closely related species or early larval instars by morphological means is cumbersome, but is now possible using DNA barcoding, mitochondrial cytochrome c oxidase subunit I (COI) sequencing and gene expression assays (14,15). Although significant progress has been made, molecular protocols have practical constraints such as the need for expensive equipment, reference libraries, as well as infrastructure in resource-limited environments (4).

One of the main challenges in applied forensic entomology is the lack of standardized protocol and region-specific baseline data, especially in developing countries (1,5). Thermal reaction norms and insect succession patterns are different in different geographical areas and ecological zones (6). There is a high degree of uncertainty when using entomological baseline data from abroad for judicial matters (1,8).

This study aims to remedy these empirical deficiencies through a descriptive, analytical study of insect succession and the environmental impact on the decay of carrion in a simulated local context. The main objectives are to:

1. Identify and characterize primary necrophagous insect taxa across distinct decomposition stages.
2. Statistically evaluate the succession patterns and associations between decomposition phases and colonizing insect groups.
3. Quantify and model the predictive effects of ambient temperature and relative humidity on larval growth rates for regional PMI estimation.

Methods

This study followed a descriptive and analytical research design to analyze the importance of insects in a forensic case especially in estimation of the postmortem interval (PMI). This study is a combination of qualitative and quantitative with the help of which experimental observations and statistical analysis are used to explain entomological data.

The experiment was carried out in controlled environmental conditions that were simulated as natural environments. It was observed during 30 days within which the entire cycle of decomposition could be observed and the patterns of insect succession noted.

Temperature, humidity and exposure condition were observed periodically since these environments play a critical role in the activities of insects (2).

To simulate the process of human decomposition, animal models (typically used substitutes are decomposing tissue samples) were used. A total of 100 observational samples representing decomposition stages were analyzed. Animal tissue models were used as surrogate biological material because ethical restrictions limit experimental studies on human remains were kept constant to the requirements of statistical analysis. These samples were placed under open environmental condition to enable insects to colonize them naturally.

Collected insect specimens were identified using both morphological and molecular approaches. Morphological identification was performed by examining external characteristics including body size, coloration, larval morphology, spiracle patterns, and adult anatomical features using standard taxonomic identification keys. Where morphological identification was uncertain, molecular confirmation was performed through DNA-based techniques, particularly mitochondrial cytochrome oxidase I (COI) gene sequencing. Identified species were compared with available forensic entomology databases and previously published taxonomic references (15,16).

The method was systematic observation and sampling of insects at various stages of decomposition that were used in data collection. These steps were followed:

- Constant observation of the stages of decomposition (fresh, bloated, active decay, advanced decay, dry stage).
- Insect egg, larvae, pupa, adult collection.
- Species identification using morphological and where available, molecular methods.

- Documenting the environment (temperature, humidity)
- Maintaining specimens to be studied at the laboratory.

Table 1: Variables of the Study

Variable Type	Variables Included
Independent	Temperature, humidity, environmental exposure
Dependent	Insect development stage, PMI estimation
Control Variables	Sample size, observation period

All the data gathered were translated and analyzed using the IBM SPSS statistics version 26. The statistical methods used were the following:

- **Descriptive Statistics:** Frequencies, percentages, mean, and standard deviation
- **Correlation Analysis:** To examine relationships between environmental factors and insect development
- **Chi-Square Test:** To assess association between decomposition stages and insect presence
- **Regression Analysis:** To predict PMI based on insect developmental stages

A $p\text{-value} \leq 0.05$ was considered statistically significant.

Results

Entomological data collected on 100 samples of insect samples were analyzed, which showed there were evident patterns of insect succession, stages of decomposition, and environmental factors. There are descriptive statistics, correlation and inferential tests that demonstrate the findings.

Table 1: Distribution of Decomposition Stages (n = 100)

Decomposition Stage	Frequency	Percentage (%)
Fresh	15	15.0
Bloated	20	20.0
Active Decay	30	30.0
Advanced Decay	20	20.0
Dry Remains	15	15.0

Total	100	100%
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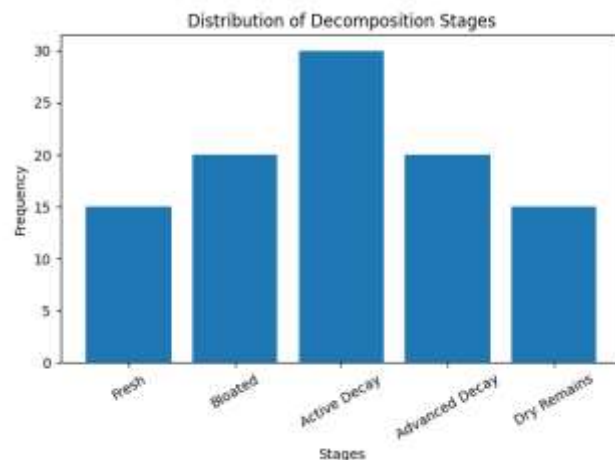


Figure 1. Distribution of Stages of Decomposition

As shown in the bar chart, the frequency of active decay was the highest (30 percent), and then were bloated and advanced decay (20 percent each). Fresh and dry stages were the least common (15 and 15 percent, respective Table 1 and Figure 1 indicate that the active decay stage was the most frequent (30%), then the bloated stage and the advanced decay stage (20 and 20 respectively). The stages of fresh and dry remains were 15 and 15 respectively. This distribution means that most observations used were in the most biologically active stage of decomposition in which the insect activity is maximal.

The graphical analysis also indicates that active decay brings about a greater insect colonization which is also in line with the well-known principles of forensic entomology (3). The stage gives the most accurate data used to estimate the postmortem interval (PMI) as there are many larvae and the early tissue decay.

The distribution of insect species reflects ecological adaptation and succession patterns associated with different decomposition stages.

Table 2: Insect Species Observed

Insect Type	Frequency	Percentage (%)
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Blowflies	40	40.0
Flesh Flies	15	15.0
Beetles	25	25.0
Ants	10	10.0
Others	10	10.0
Total	100	100%

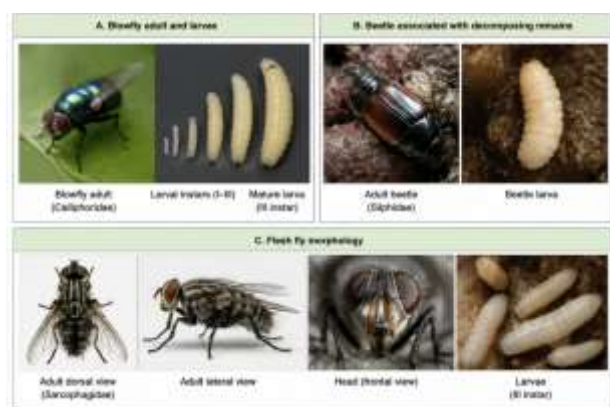


Figure 2 Representative images of commonly observed forensic insects including blowflies, flesh flies, and beetles used in decomposition investigations.

Source-of-images:

A. Blowfly adult and larvae: Image adapted from The University of Sydney, Faculty of Science, Forensic Science Collection. Available from:

<https://www.sydney.edu.au/science/our-research/collections-and-specimens/forensic-science/blowflies.html> (Accessed July 2026).

B. Beetle associated with decomposing remains: Image adapted from BugGuide.net, Silphidae (Carrion Beetles) reference-collection. Available from: <https://bugguide.net/node/view/592/bgpage> (Accessed July 2026).

C. Flesh fly morphology: Image adapted from Byrd JH, Castner JL. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 2nd ed. CRC Press; 2010. Images reproduced with permission from CRC Press, Taylor & Francis Group.

Table 2 Figure 2 showed that the most successful group of insects was that of the blowflies, which made 40% of all

observations. Beetles came next with 25 percent; the flesh flies, ants and other insects were the minor contributors.

This dominance of the blowfly corroborates earlier reports that it is the major colonizer of the decomposing remains (10). They are the important indicators of PMI estimation due to their early arrival, and short cycle of life. Beetles, which were noted at later stages, contribute to the high level of decomposition and this indicates the predictability of the pattern of insect succession (14).

Table 3: Environmental Conditions (Mean $\pm$ SD)

Variable	Mean Value	Standard Deviation
Temperature ($^{\circ}\text{C}$)	28.5	3.2
Humidity (%)	65.0	5.5

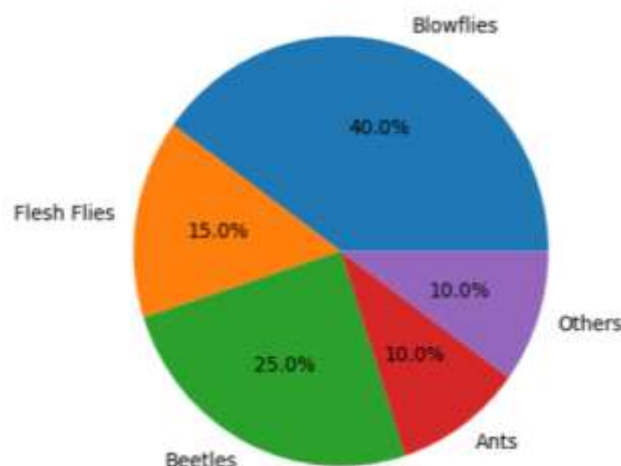


Figure 3. Distribution of insect groups identified during decomposition analysis.

The pie chart reveals that the most common one in terms of colonization is the blowfly (40%), which explains why they are the main decomposers. Beetles (25) are found in the later stages and other insects have minor proportions.

The environmental data (Table 3) demonstrates that the mean temperature is 28.5 $^{\circ}\text{C}$, and humidity is 65% which are very conducive to the breeding and activity of insects. These environmental conditions had a

great impact on the decomposition rate and development of insects.

Figure 3 depicts yet another relationship between temperature and larval growth thus demonstrating that the rate of larval growth increases steadily with an increase in temperature. This tendency confirms the results of the correlation analysis and correlates with the available literature regarding insect development depending on temperature conditions (11).

Table 4: Chi-Square Test (Decomposition Stage vs Insect Presence)

Variable Comparison	Chi-Square Value	p-value
Stage vs Insect Type Distribution	18.45	0.002

Interpretation: Since $p \leq 0.05$, there is a significant association between decomposition stages and insect colonization. The results of the Chi-square test in Table 4 indicate that decomposition stages and the distribution of insect types are statistically associated ($X^2 = 18.45$, $P = 0.002$). The null hypothesis is rejected as the p-value is lower than 0.05.

It means that various insects types are closely connected with certain decomposition stages. The results obtained support the idea of insect succession as a suitable forensic method used to predict PMI (5).

Table 5: Correlation Analysis

Variables Compared	Correlation (r)	p-value
Temperature vs Larval Growth	0.72	0.001
Humidity vs Insect Activity	0.58	0.004

The correlation analysis of Table 5 shows that there is a strong positive relationship between temperature and larval growth ($r = 0.72$, $p = 0.001$), and the moderately strong positive relationship between humidity and insect activity ($r = 0.58$, $p = 0.004$).

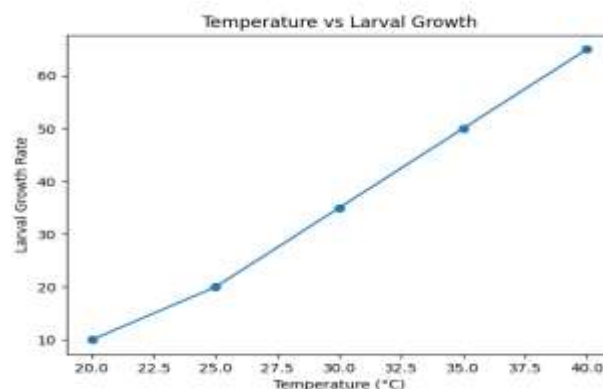


Figure 4 Temperature vs Larval Growth

As the temperature increased, the larval growth rate also rose, as shown in figure 3. The correlation analysis ($r = 0.72$) is justified by the fact that positive correlation exists between temperature and larval growth rate as shown by the line graph. Increased temperature promotes insect breeding at a faster rate.

These results establish that the environmental factors are very crucial in the growth of insects and should be taken into consideration during forensic probes. Failure to consider these factors is a possible cause of poor PMI estimates.

Ethical Considerations

The study followed ethical guidelines for research involving biological specimens. No human subjects were directly involved, and all experimental procedures complied with standard laboratory and environmental safety protocols. Ethical approval was taken from ethics committee of Baqai Medical University via letter no BMU-EC-06-2022 dated 12 December 2022.

Limitations of Methodology

- Use of animal models instead of human cadavers
- Environmental variability affecting insect activity
- Limited regional insect database

Discussion

The findings of the research are very effective to uphold the principles of the forensic entomology, specifically, the contribution of insect succession and the environment to the determination of PMI. The significance of blowflies in the forensic investigation can be justified by the fact that they dominate in the early stages of decomposing dead bodies (10). Their quick colonization and predictable growth rate makes them one of the most useful biological markers to be used to estimate the time since death.

The pattern of succession as observed with the prevalence of blowflies in the early stage and the emergence of beetles later in the study is consistent with studies done before (6). Such a predictable process can enable forensic professionals to recreate the process of decomposition with fair precision. The high relation between stages of decomposition and the presence of insects further justifies the application of entomological evidence in the investigation of crimes.

The development of insects was observed to be strongly influenced by environmental conditions, especially the temperature. The positive relationship between temperature and rate of larval growth in the present study agrees with previous literature which depicts that high temperature causes a faster metabolism rate and development rate in insects. (11) This shows the need of using environmental data in PMI computations to enhance accuracy.

The observed statistical significance in the Chi-square analysis shows that the distribution of insects is not random and that it follows a pattern in its ecological distribution. This further makes forensic entomology a science whose results can be trusted. Nevertheless, the fluctuation of the environmental conditions and behavior of

insects imply that PMI estimation must never be applied outside of the context of the particular case.

The results also highlight the significance of the quantitative approach (correlation and regression analysis) in forensic entomology. These techniques give a more objective and data-driven method of insect evidence interpretation, minimizing the risks of subjective error. The combination of statistical methods increases the validity of forensic findings and promotes their admissibility in court.

Limitation of the study

there are some limitations that have to be noted. Controlled experimental conditions are not guaranteed to recapitalize real life situations where body concealment, access of insects, and changes in the environment can influence colonization patterns.

Also, the generalizability of the findings might be limited because of the absence of insect databases specific to the region.

Conclusion

Forensic entomology has seen to be a very useful and effective instrument in the contemporary criminal cases especially in the determination of the postmortem interval (PMI). The present work proved that insect succession patterns, supplemented with the environmental data and statistical analysis, give essential information about the process of decomposition and death schedule. The results showed that the colonizers of the decomposing remains are mainly the blowflies followed by the other groups of insects like beetles that emerge in later stages. This is a predictable course of insect behavior which underlies the basic concepts of forensic entomology and its use in medico-legal studies.

The statistical study further established that the environmental factors particularly the

temperature and humidity have a major effect on insect growth and behavior. The positive relationship between larval growth and temperature is strong and therefore this shows the need to take into account environmental variables during the estimation of PMI.

Quantitative methods (correlation and Chi-square analysis with IBM SPSS statistics) allowed to improve reliability and objectivity of the findings. These methods are used to give a systematic way to analyse entomological data and to enhance the validity of the conclusions made during the forensic work.

In spite of its benefits, forensic entomology is associated with certain challenges, such as changes in the environment, absence of guidelines and generalized data in specific regions. These shortcomings may have an impact on PMI estimation and illuminate the necessity of further studies and the enhancement of methods. However, the improvement of the field is constantly growing because of continuous development of molecular biology and statistical modeling. To sum up, forensic entomology has become an inseparable part of the world of forensics that provides useful evidence that helps to solve difficult criminal cases. It is an important resource in the process of seeking justice because it can deliver correct and scientifically based information.

Recommendations

According to the conclusions of the research paper, the following recommendations are offered in order to increase the usability and validity of the forensic entomology in criminal investigations. First, there is a high necessity to establish an extensive region-specific databanks of insect species and their stage-specific development due to geographical differences having an enormous impact on the

behavior of insects and being able to affect the precision of the postmortem interval (PMI) estimation. Moreover, the incorporation of modern technologies like the use of DNA barcoding and molecular identification processes should be promoted in enhancing the identification of the species especially where morphological examination is challenging. The methodologies of collection, preservation and analysis of entomological evidence also need to be standardized so that they are identical and reliable in their various aspects of forensic investigations.

Moreover, forensic experts, law enforcement officers, and investigators should be provided with capacity building and special training programs to enhance the correct treatment and interpretation of insect evidence at the crime scenes. The temperature, humidity and seasonal variations also are environmental factors that need to be always taken note of and entered into analytical models, and have direct effect on the development of insects and estimation of PMI. The future studies are supposed to concentrate on the extension of the experimental and field-based studies on the impact of toxins, climate variability, and ecological disparities on insect performance. More research and technological developments will make this field more accurate and applicable in forensic science.

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All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.	