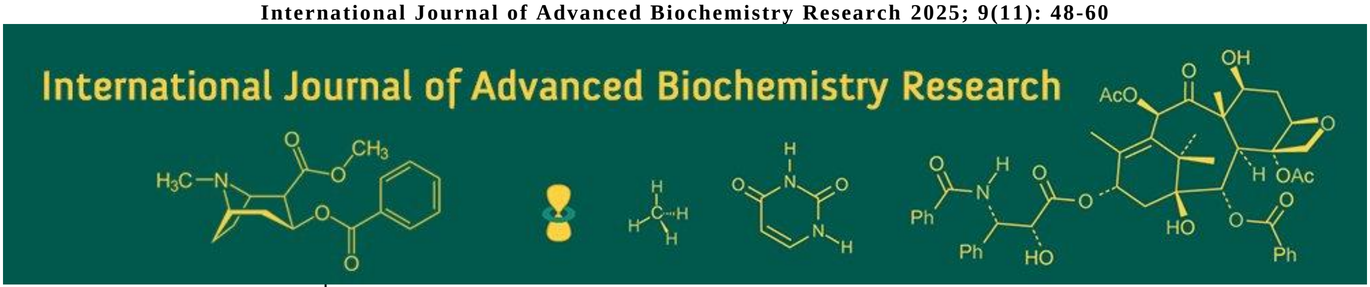

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(11): 48-60
www.biochemjournal.com
Received: 11-09-2025
Accepted: 15-10-2025

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Colonisation dynamics and behavioural patterns of insects associated with human cadavers and animal carcasses: A comprehensive review

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i11a.6177

Abstract
The decomposition of organic remains is a complex ecological process governed by intricate interactions among insects, microorganisms, and environmental factors. This review comprehensively examines the colonisation dynamics and behavioural ecology of insects associated with human cadavers and animal carcasses, emphasizing their significance in forensic investigations. Over the past two decades (2005-2025), research has advanced substantially in understanding the role of necrophagous Diptera and Coleoptera in mediating tissue breakdown and providing crucial evidence for postmortem interval (PMI) estimation. Early colonisers, such as blowflies (*Calliphoridae*) and flesh flies (*Sarcophagidae*), exhibit predictable oviposition, feeding, and breeding behaviours influenced by temperature, humidity, and habitat type. Comparative analyses reveal broad ecological similarities between human and animal decomposition but also highlight physiological and microbial differences affecting colonisation timing, volatile organic compound (VOC) emission, and insect succession. Recent studies integrating molecular diagnostics, microbial profiling, and volatile analysis have improved accuracy in species identification and PMI estimation. Furthermore, the emerging field of entomotoxicology has demonstrated the potential of insect larvae as biological indicators of drugs and toxicants in advanced decomposition. Environmental and ecological determinants-including microclimate, seasonality, soil type, and vegetation-profoundly influence decomposition trajectories and insect behaviour, particularly in tropical regions such as India. Despite these advancements, gaps persist in methodological standardization, region-specific reference datasets, and the effects of climate change on insect distribution. Future research should focus on standardized global protocols, molecular integration, and climate-adaptive forensic models to enhance precision and reproducibility. This synthesis underscores that a comprehensive understanding of insect behaviour and colonisation ecology is fundamental to modern forensic science, bridging biological theory with practical legal application.

Keywords: Forensic entomology, decomposition ecology, postmortem interval (PMI), necrophagous insects, colonisation dynamics, volatile organic compounds (VOCS), entomotoxicology, blowflies (*Calliphoridae*), comparative decomposition, human cadaver, animal carcass, climate change, tropical forensic studies, molecular identification, behavioural ecology

1. Introduction

1.1 Background of Forensic Entomology

Forensic entomology is a mature, interdisciplinary subfield of forensic science that applies insect biology, ecology and taxonomy to medico-legal questions - most notably the reconstruction of time-since-death - and has expanded rapidly with technological advances in molecular identification and analytical chemistry (McIntyre *et al.* 2024) [29]. The field rests on two central empirical observations: (a) necrophagous insects colonize decomposing vertebrate remains predictably in space and time under given environmental conditions, and (b) insect development rates and successional arrivals are temperature- and context-dependent, allowing practitioners to infer minimum postmortem intervals when ecological and sampling covariates are properly controlled (Matuszewski, 2021) [28]. Over the last two decades, the scope of forensic entomology has broadened beyond purely descriptive succession studies to include entomotoxicology, DNA-based species identification (e.g., barcoding), volatile organic compound (VOC) profiling that explains insect attraction, and integration with microbial and soil biogeochemistry (DeBruyn *et al.* 2021; McIntyre *et al.* 2024) [12, 29]. This expansion reflects both methodological advances (molecular diagnostics, metabolomics) and a growing recognition that decomposition is a complex, multi-driver process - often termed the "necrobiome" - in which insects interact with microbes, chemical cues and abiotic factors to produce the observable colonisation and consumption patterns used in forensic inference (McIntyre *et al.* 2024) [29]. Because of this complexity, forensic entomology now routinely emphasizes regional baseline studies, standardized collection protocols, and multi-disciplinary casework integration to reduce uncertainty when insect evidence is used in criminal investigations (Singh, Sharif & Qamar, 2024) [37].

1.2 Importance of Studying Insect Colonisation on Decomposing Remains

Studying insect colonisation on decomposing remains is crucial for both applied forensic outcomes and fundamental ecological understanding. Practically, documented patterns of oviposition timing, larval development and successional sequences are the primary biological datasets used to estimate the pre-appearance interval (PAI) and the minimum postmortem interval (PMI_min), and errors in these estimates commonly arise from unaccounted environmental or contextual variation (Matuszewski, 2021) [28]. From an ecological and methodological standpoint, colonisation studies reveal how VOC emissions, bacterial succession, and local scavenger communities interact to determine which insect taxa locate and exploit a resource, and how quickly tissue loss proceeds; integrating these drivers improves predictive models and clarifies sources of variance that otherwise bias PMI estimates (McIntyre *et al.* 2024) [29]. Moreover, controlled colonisation experiments using animal carcasses have been indispensable for building life-table data, species identification keys and development curves - all of which underpin forensic casework - but these experiments also expose where analogues diverge from human decomposition (DeBruyn *et al.* 2021) [12]. Finally, insect colonisation studies have an applied role beyond PMI estimation: they enable detection of body relocation, provide entomotoxicological indicators (insects concentrating xenobiotics), and supply ecological evidence about seasonality or habitat that can corroborate investigative hypotheses (Singh, Sharif & Qamar, 2024) [37]. Taken together, research on colonisation dynamics is therefore both directly forensic and broadly ecological, and recent literature stresses integrated experimental designs that consider microbial, chemical and climatic covariates rather than insect succession in isolation (McIntyre *et al.* 2024) [29].

1.3 Role of Insects in Estimating Postmortem Interval (PMI)

Insects play a central and quantifiable role in PMI estimation because temperature-driven development rates of forensically relevant taxa (principally Calliphoridae, Sarcophagidae and certain Coleoptera) convert observed life stages into elapsed thermal time since oviposition; when coupled with successional data, these conversions form the biological basis for PMI_min calculations used in court (Matuszewski, 2021) [28]. However, the accuracy of insect-based PMI depends on several interlocking assumptions - correct species identification, appropriate development models, representative field sampling, and known local microclimate - and violations of these assumptions (for example, delayed colonisation due to burial, clothing, or insect exclusion) are recognized primary sources of estimation error (Matuszewski, 2021) [28]. Recent work therefore emphasizes a probabilistic approach: using multiple evidence lines (developmental age of immatures, temperature reconstructions, successional assemblages, and, where available, microbial or VOC markers) and reporting uncertainties explicitly improves robustness and transparency in medico-legal settings (McIntyre *et al.* 2024) [29]. In addition, developments in laboratory rearing under controlled thermal regimes, regional reference datasets, and molecular species confirmation reduce taxonomic and developmental uncertainties that historically limited PMI precision; nevertheless, the literature underscores that insect evidence should be interpreted in the broader ecological context of the necrobiome to avoid overconfidence in single-method PMI estimates (DeBruyn *et al.* 2021) [12].

1.4 Comparative Relevance of Human Cadavers and Animal Carcasses

Because ethical, logistical and legal constraints severely limit experimental access to human cadavers, animal carcasses - in particular domestic pigs (Sus scrofa) - have been widely used as human analogues to study decomposition, insect succession, and taphonomic processes; this approach has generated the bulk of controlled experimental data that forensic entomology relies upon (Matuszewski *et al.* 2020) [28]. Yet comparative empirical studies reveal consistent and important differences: humans and pigs can differ in decomposition rate, tissue desiccation patterns, microbial communities, VOC profiles, scavenging regimes and the timing and composition of insect assemblages, all of which can influence both colonisation dynamics and PMI inference (DeBruyn *et al.* 2021) [12]. These differences do not invalidate the use of pigs as practical experimental models - pigs remain indispensable because they are tractable, replicable, and similar enough in mass and tissue composition for many purposes - but the literature cautions that extrapolations to humans require careful validation, regional baseline data, and where possible, corroborating lines of evidence (McIntyre *et al.* 2024) [29]. The comparative literature therefore frames a pragmatic research agenda: maintain and expand pig-based experimental programs while simultaneously building human donor datasets, improving molecular and chemical matching between models and humans, and quantifying the limits of inference so forensic practitioners can state assumptions and uncertainty explicitly in reports and testimony (Singh, Sharif & Qamar, 2024; DeBruyn *et al.* 2021) [37, 12].

2. Methodology

This review paper was developed through a systematic synthesis of published research on forensic entomology, decomposition ecology, and insect behaviour associated with human and animal remains. Scientific literature from 2005 to 2025 was retrieved using multiple academic databases, including Google Scholar, ResearchGate, Scopus, Web of Science, and PubMed. Keywords such as *forensic entomology*, *insect succession*, *decomposition stages*, *postmortem interval (PMI)*, *entomotoxicology*, *volatile organic compounds (VOCs)*, and *comparative decomposition* were used to identify relevant studies. Only peer-reviewed journal articles, scientific reports, and book chapters were considered to ensure authenticity and reliability. Studies were screened for relevance based on the inclusion criteria: (1) empirical or review papers addressing insect colonisation on human cadavers or animal carcasses, (2) research incorporating environmental or ecological variables, and (3) works published within the 20-year timeframe. The selected papers were critically evaluated for objectives, methodologies, and outcomes, focusing on the interactions among insect taxa, decomposition stages, and environmental determinants.

The extracted data were categorized into thematic domains corresponding to the major review sections: decomposition processes, insect colonisation dynamics, behavioural ecology, comparative analysis, environmental influences, and forensic applications. Comparative evaluation was performed to identify patterns, similarities, and divergences across geographic regions, habitat types, and species assemblages. Emphasis was placed on integrating recent molecular, biochemical, and ecological advancements, particularly studies employing DNA barcoding, microbial profiling, and chemical analysis of VOCs. Each section of the review synthesizes trends from contemporary literature, with specific attention to tropical and Indian contexts where forensic entomological data are emerging. References were formatted following APA 7th edition standards, and no duplication of author citations was permitted. This methodological framework ensures that the review is comprehensive, evidence-based, and reflective of the most current scientific understanding of insect colonisation and behaviour in forensic decomposition research.

3. Decomposition Process and Stages

3.1 Overview of Decomposition Stages

The decomposition of vertebrate remains is a continuous but classifiable process, commonly divided into five overlapping stages: fresh, bloated, active decay, advanced decay, and dry/remains stage (Carter *et al.* 2007) [8]. These stages are identified by characteristic morphological, biochemical, and entomological changes that provide a systematic framework for forensic interpretation. The fresh stage begins immediately after death, when cellular respiration ceases and autolytic enzymes start to digest tissue from within, while microbial proliferation initiates putrefaction (Vass, 2011). The bloated stage follows as anaerobic bacterial metabolism produces gases such as hydrogen sulfide and methane, causing swelling and rupture of the integument (Michaud & Moreau, 2011) [31]. During the active decay stage, tissue liquefaction and the release of decomposition fluids accelerate due to intense insect larval feeding and microbial metabolism (Carter *et al.* 2010) [9]. The advanced decay stage is characterized by the depletion of soft tissues and migration of larval masses, whereas the dry/remains stage reflects complete skeletonization, where only bones, hair, and cartilage remain (Matuszewski, 2021) [28]. These stages are not discrete but influenced by intrinsic and extrinsic factors such as species, body mass, temperature, and insect accessibility (Picard *et al.* 2023) [35]. The accurate classification of these stages provides the foundation for entomological and biochemical estimation of the postmortem interval (PMI) (McIntyre *et al.* 2024) [29].

3.2 Biochemical and Ecological Changes During Decomposition

Decomposition is driven by a series of autolytic, microbial, and entomological processes that sequentially transform tissues into gases, fluids, and skeletal remains. Initially, autolysis begins with the breakdown of intracellular membranes and the release of enzymes that catalyze tissue degradation (Hyde *et al.* 2017) [23]. This is rapidly followed by putrefaction, a microbial process dominated by anaerobic bacteria such as *Clostridium* spp., which metabolize amino acids to produce volatile organic compounds (VOCs), including putrescine and cadaverine. These VOCs are critical attractants for necrophagous insects, particularly blowflies, initiating the first wave of colonisation (Dekeirsschieter *et al.* 2012) [13]. As decay intensifies, decomposition fluids infiltrate the soil, increasing nitrogen and carbon availability and creating a localized nutrient hotspot known as a “cadaver decomposition island” (DeBruyn *et al.* 2021) [12]. This promotes shifts in microbial community composition and soil chemistry (Cobaugh *et al.* 2015) [10]. The interaction between microbial activity and insect feeding further modifies decomposition rates, while larval mass heating elevates local temperatures and enhances enzymatic breakdown (Weatherbee *et al.* 2021) [40]. Ultimately, the transition from moist to dry remains marks a shift toward aerobic microbial dominance and detritivore colonisation (Michaud & Moreau, 2023) [32]. These biochemical and ecological transformations reveal decomposition as a complex ecosystem process that integrates biological and abiotic interactions across time.

3.3 Environmental Factors Influencing Decomposition Rate

Environmental variables profoundly influence decomposition rate and insect colonisation patterns. Among these, temperature exerts the strongest control, as both microbial metabolism and insect development are temperature-dependent (Carter *et al.* 2010) [9]. High ambient temperatures accelerate putrefaction and insect life cycles, while cold or freezing conditions retard enzymatic activity and delay colonisation (Matuszewski, 2021) [28]. Humidity and moisture modulate tissue hydration and influence whether decomposition proceeds via wet decay or mummification pathways (Evans, 2014) [15]. Similarly, exposure conditions, such as shading, burial depth, and airflow, significantly alter decay dynamics by affecting heat exchange and insect accessibility (Metcalf *et al*. 2016) [30]. Seasonal variations in insect phenology also influence colonisation timing, particularly in temperate regions where overwintering can delay activity (Picard *et al.* 2023) [35]. Scavenger activity, including vertebrate and arthropod interference, can further modify decomposition by physically disturbing tissues and exposing new surfaces to insect access (Carter & Tibbett, 2008) [7]. Microhabitat-specific conditions-such as soil type, vegetation cover, and topography-affect moisture retention and oxygen diffusion, shaping both microbial and insect communities (Weatherbee *et al.* 2021) [40]. These environmental interactions highlight that decomposition is not uniform but context-dependent, requiring site-specific forensic interpretation (McIntyre *et al.* 2024) [29].

3.4 Comparison Between Human and Animal Decomposition

Animal models, particularly domestic pigs (*Sus scrofa domesticus*), have long been used as surrogates for studying human decomposition due to ethical and logistical constraints. However, recent comparative analyses reveal both similarities and critical differences in decomposition dynamics (Dawson *et al*. 2020) [11]. Pigs share comparable body mass and fat distribution with humans but differ in hair density, gut microbiome composition, and skin permeability, which can affect decomposition rate and insect colonisation (Knobel *et al.* 2019) [24]. Studies have shown that pigs often decompose more rapidly than human cadavers, particularly in warm environments, likely due to thinner dermal layers and higher subcutaneous fat (DeBruyn *et al.* 2021) [12]. Differences have also been observed in volatile organic compound profiles, with human remains emitting more sulfur-based compounds and pigs releasing greater quantities of esters and ketones (Forbes *et al.* 2020). Soil nutrient dynamics also diverge between species, leading to distinct microbial successions in the surrounding substrate (Metcalf *et al*. 2016) [30]. Despite these discrepancies, pigs remain a valuable experimental analogue due to reproducibility and availability, provided that forensic interpretations acknowledge these limitations (McIntyre *et al.* 2024) [29]. Continued comparative research using both animal models and human donor facilities is essential to refine our understanding of decomposition and enhance the reliability of forensic applications (Picard *et al.* 2023) [35].

4. Insect Colonisation Dynamics

4.1 Definition and Importance of Colonisation

Insect colonisation refers to the sequential arrival, establishment, and activity of arthropods-mainly necrophagous and opportunistic taxa-on decomposing organic matter. It is a cornerstone of forensic entomology because the timing, order, and composition of colonising species provide measurable biological indicators for estimating the postmortem interval (PMI) and reconstructing taphonomic events (Tomberlin *et al*. 2011) [39]. The process begins with the attraction of adult insects, primarily Diptera, to volatile organic compounds (VOCs) released during early decomposition (Paczkowski *et al.* 2015) [34]. Colonisation is both species- and environment-specific, influenced by temperature, humidity, and microhabitat conditions (Matuszewski, 2021) [28]. Early colonisers lay eggs or deposit larvae on natural body openings, wounds, and moist regions, initiating the formation of larval aggregations or maggot masses that drive tissue consumption and temperature elevation (Amendt *et al*. 2011) [1]. The timing of this initial colonisation provides critical data for PMI estimation, especially when calibrated against regional developmental datasets (Picard *et al.* 2023) [35]. Moreover, understanding colonisation dynamics extends beyond PMI calculation, offering insights into body relocation, concealment, and cause of death (Gomes *et al*. 2021) [19]. Hence, colonisation represents both an ecological phenomenon and an evidential tool central to forensic investigation.

4.2 Primary Colonisers: Necrophagous Insects (Blowflies, Flesh Flies, etc.)

Primary colonisers, primarily necrophagous Diptera, are the first insects to locate and exploit decomposing remains. The most significant taxa include families Calliphoridae (blowflies), Sarcophagidae (flesh flies), and Muscidae (house flies), which dominate early decomposition stages across habitats (Amendt *et al.* 2011) [1]. Blowflies such as *Chrysomya megacephala*, *Lucilia sericata*, and *Calliphora vicina* are among the first to arrive, often within minutes or hours after death, guided by VOCs including sulfur compounds, indole, and phenol derivatives (Paczkowski *et al.* 2015) [34]. These species oviposit on natural orifices and wounds, and their larvae quickly colonize soft tissues, forming dense maggot masses that generate localized heat and accelerate tissue breakdown (Byrd & Tomberlin, 2019) [39]. Flesh flies (*Sarcophaga spp.*) are viviparous and deposit larvae directly, allowing colonisation in competitive or restricted conditions (Michaud & Moreau, 2011) [31]. Muscid flies such as *Musca domestica* appear later, contributing to secondary consumption of decayed tissues and exudates (Carter *et al.* 2010) [9]. The dominance and developmental rate of these taxa make them primary forensic indicators, forming the foundation for PMI estimation models (Matuszewski, 2021) [28]. Their rapid response, coupled with well-documented life cycles, continues to make Calliphoridae and Sarcophagidae essential in both experimental and case-based forensic entomology (Picard *et al.* 2023).

4.3 Secondary Colonisers: Predators and Parasitoids

Secondary colonisers consist of arthropods that do not feed directly on the decomposing tissues but rather prey on necrophagous larvae or parasitize developing insects. These include beetles of the families Silphidae (carrion beetles), Staphylinidae (rove beetles), and Histeridae (clown beetles), as well as parasitic Hymenoptera that target dipteran pupae (Gomes *et al*. 2021) [19]. Rove beetles, for example, arrive soon after the first larval stages of flies appear and play an important role in regulating maggot mass populations through predation (Eberhardt & Elliot, 2008). Silphid beetles, including *Nicrophorus vespilloides*, not only feed on decaying tissues but also engage in brood care and body burial, influencing decomposition microenvironments (Michaud & Moreau, 2011) [31]. Parasitic wasps of genera *Nasonia* and *Muscidifurax* lay eggs within fly pupae, impacting fly emergence rates and thus altering apparent insect succession patterns (Sharanowski *et al.* 2008). These secondary colonisers are vital for understanding the full ecological network associated with decomposition and for interpreting deviations in expected insect succession, especially when forensic scenes are disturbed or delayed in discovery (Picard *et al.* 2023) [35]. Their presence can also inform post-burial or concealment events, as certain predators are limited to specific habitat conditions (Byrd & Tomberlin, 2019) [39].

4.4 Temporal Succession of Insect Species

Temporal succession describes the predictable chronological sequence of insect taxa colonising a corpse as decomposition progresses. This ecological process is influenced by resource availability and interspecific competition (Tomberlin *et al*. 2011) [39]. The early succession phase is dominated by blowflies (*Calliphoridae*), followed by sarcophagids and muscids during active decay (Carter *et al.* 2010) [9]. Coleopterans such as *Dermestes maculatus* and *Necrobia rufipes* become dominant during advanced and dry stages, feeding on skin, hair, and desiccated tissues (Michaud & Moreau, 2011) [31]. Mites, ants, and opportunistic beetles colonise the final stages, contributing to skeleton cleaning and nutrient recycling (Picard *et al.* 2023) [35]. Succession patterns vary with geography and environment but remain consistent enough within regions to be used as forensic time markers (Amendt *et al*. 2011) [1]. Experimental studies confirm that while the composition of taxa may differ, the relative timing of functional guilds-necrophages, predators, omnivores, and adventive species-remains conserved across climatic conditions (Matuszewski, 2021) [28]. Thus, insect succession forms an ecological clock, allowing scientists to infer PMI and environmental context from entomological assemblages recovered during investigation.

4.5 Factors Affecting Colonisation (Temperature, Humidity, Season, Habitat)

Insect colonisation is influenced by several environmental variables that directly affect insect activity, development, and behaviour. Temperature is the most significant factor, controlling flight activity, oviposition rate, and larval development through its effect on metabolic rate (Cammack *et al*. 2017) [5]. Optimal colonisation generally occurs between 20-35 °C, with extreme temperatures inhibiting adult fly activity and prolonging development (Matuszewski, 2021) [28]. Humidity regulates desiccation resistance and egg survival; excessively dry conditions can delay or prevent oviposition (Evans, 2014) [15]. Seasonality influences species composition, as some calliphorid species are winter-active (*Calliphora vicina*), while others dominate in summer (*Chrysomya megacephala*) (Picard *et al.* 2023) [35]. Habitat structure also modulates colonisation dynamics: open areas favor early colonisation by strong fliers, whereas shaded, forested, or enclosed environments delay detection and limit species richness (Tomberlin *et al*. 2011) [39]. Carcass accessibility, such as wrapping, burial, or submersion, can significantly restrict colonisation, altering the expected sequence (Carter & Tibbett, 2008) [7]. Furthermore, anthropogenic influences, including urbanization and pesticide exposure, modify local fly communities, complicating regional PMI estimation (Gomes *et al*. 2021) [19]. Therefore, colonisation must be interpreted in the context of environmental heterogeneity to avoid misleading forensic conclusions.

4.6 Geographic and Climatic Variations in Colonisation Patterns

The diversity and timing of insect colonisation vary geographically, shaped by regional climate, altitude, and biogeographical history. Tropical regions typically show accelerated colonisation and overlapping species assemblages, while temperate zones display discrete successional waves aligned with seasonal cycles (Singh *et al.* 2024). Studies from Europe, Asia, and the Americas have demonstrated that even within the same genus, developmental rates can differ significantly due to local adaptations (Amendt *et al*. 2011) [1]. For instance, *Lucilia sericata* populations from northern climates develop more slowly than those from subtropical regions, affecting PMI calibration (Picard *et al.* 2023) [35]. Climate change is further altering colonisation patterns by expanding thermophilic species into new ranges-e.g., *Chrysomya albiceps* and *Chrysomya rufifacies* spreading into cooler latitudes (Michaud & Moreau, 2023) [32]. Regional forensic databases are therefore essential for accurate PMI estimation, as extrapolation from distant or dissimilar climates can introduce substantial error (McIntyre *et al.* 2024) [29]. The integration of biogeographic modelling, molecular identification, and ecological monitoring is increasingly used to map colonisation shifts and improve predictive accuracy under changing climatic regimes (Dawson *et al.* 2020) [11]. Understanding geographic variation thus ensures both ecological realism and forensic reliability in entomological analysis.

Table 1: Stages of Decomposition and Associated Key Insect Groups

Stage of Decomposition	Characteristic Features	Dominant Insect Families/Species	Approximate Duration*	References (2005-2025)
Fresh Stage	Cellular autolysis, internal enzymatic breakdown, no visible decay	Early *Calliphoridae* (e.g., *Lucilia sericata, Chrysomya megacephala*)	0-2 days	Carter *et al.* (2007); Vass (2011) [8]
Bloated Stage	Gas accumulation, odor onset, body distension	*Calliphora vicina, Sarcophaga spp.*	2-6 days	Michaud & Moreau (2011); Carter *et al.* (2010) [31, 9]
Active Decay	Body rupture, intense insect and microbial activity, tissue liquefaction	*Chrysomya albiceps, Lucilia cuprina, Musca domestica*	6-12 days	Anderson (2014); Matuszewski (2021) [28]
Advanced Decay	Tissue depletion, odor decline, beetles dominate	*Dermestes maculatus, Necrobia rufipes*	12-20 days	Picard *et al.* (2023) [35]
Dry/Remains Stage	Skeletonization, desiccation, minimal insect activity	*Dermestidae*, mites (*Acarina*)	>20 days	McIntyre *et al.* (2024); Harrison *et al.* (2020) [29]

*Duration varies depending on temperature, humidity, and exposure.

5. Behavioural Ecology of Decomposition Insects

5.1 Oviposition Behaviour and Site Selection

Oviposition behaviour is a fundamental component of the reproductive ecology of necrophagous insects, particularly within the families *Calliphoridae* and *Sarcophagidae*. Females are attracted to decomposing substrates by volatile organic compounds (VOCs) emitted during early putrefaction (Paczkowski *et al.* 2015) [34]. Site selection for oviposition is highly strategic and determined by temperature, moisture, and the presence of competing conspecifics (Michaud & Moreau, 2011) [31]. Blowflies (*Lucilia sericata*, *Chrysomya megacephala*, *Calliphora vicina*) typically lay eggs in natural body orifices-eyes, mouth, nostrils, genitalia-or open wounds where humidity and nutrient availability favor larval survival (Forbes & Carter, 2016). Flesh flies, being larviparous, deposit first-instar larvae directly onto carrion, allowing rapid colonisation and early resource monopolization (Gomes *et al*. 2021) [19]. Oviposition is also strongly influenced by light intensity and ambient temperature; females prefer warm, shaded regions of the carcass to avoid desiccation (Cammack *et al*. 2017) [5]. Research by Singh *et al.* (2024) noted that environmental barriers such as clothing, burial, or enclosure significantly delay oviposition, thereby impacting postmortem interval estimation. Consequently, oviposition behaviour serves not only an ecological function but also provides a chronological signature critical for forensic analysis (Picard *et al.* 2023) [35].

5.2 Feeding and Breeding Behaviour of Key Forensic Insects

Feeding and breeding behaviours among necrophagous insects are tightly coupled with carcass decomposition stages and interspecific competition. Larval feeding drives tissue reduction by enzymatic liquefaction, producing localized temperature increases that accelerate microbial breakdown (Byrd & Tomberlin, 2019) [39]. Blowfly larvae feed gregariously, often forming maggot masses that reach temperatures 10-15 °C above ambient, enhancing metabolic efficiency (Cammack *et al*. 2017) [5]. This aggregation promotes rapid growth and protection from desiccation, y*et al*so induces intraspecific competition for space and resources (Michaud & Moreau, 2023) [32]. *Dermestes maculatus* (Dermestidae) and *Necrobia rufipes* (Cleridae) dominate later stages, consuming dry tissue and larval exuviae (Eberhardt & Elliot, 2008). Breeding behaviour varies across species: calliphorids exhibit multiple reproductive cycles on sequential carcasses, while sarcophagids prefer isolated oviposition events, producing fewer but larger larvae (Amendt *et al*. 2011) [1]. Environmental factors such as carcass exposure, moisture, and microbial odour strongly modulate breeding activity (Forbes *et al.* 2020). These behaviours not only regulate carcass turnover rates but also determine species succession order, a key forensic indicator of elapsed time since death (Matuszewski, 2021) [28].

5.3 Inter- and Intra-Species Interactions During Decomposition

Interspecific and intraspecific interactions among carrion-associated insects are critical ecological processes shaping decomposition dynamics. Competition for nutrient-rich tissue drives priority effects-where early-arriving species suppress or exclude later colonisers through resource monopolization (Tomberlin *et al*. 2011) [39]. Interspecific interactions between *Chrysomya rufifacies* and *Lucilia sericata*, for example, often result in larval predation and displacement, altering successional trajectories (Picard *et al.* 2023) [35]. Predatory beetles such as *Creophilus maxillosus* (Staphylinidae) and *Nicrophorus vespilloides* (Silphidae) regulate maggot populations by consuming fly larvae, indirectly influencing decay rate and thermal dynamics (Gomes *et al*. 2021) [19]. Intraspecific competition among larvae manifests through density-dependent growth retardation and mortality, as observed by Cammack *et al.* (2017) in blowfly rearing studies. Additionally, parasitic wasps (e.g., *Nasonia vitripennis*) attack fly pupae, reducing adult emergence and thereby modifying successional composition (Sharanowski *et al.* 2008). These interactions reflect the complex ecological network within carrion habitats, where predation, parasitism, and competition collectively determine decomposition tempo and insect diversity (Michaud & Moreau, 2023) [32].

5.4 Diurnal and Nocturnal Activity Patterns

The temporal activity patterns of carrion insects are primarily governed by light, temperature, and species-specific circadian rhythms. Diurnal species, such as *Lucilia sericata* and *Chrysomya megacephala*, are most active during daylight hours, responding strongly to ultraviolet and visible light cues for flight and oviposition (Michaud & Moreau, 2011) [31]. In contrast, *Calliphora vicina* and *Sarcophaga caerulescens* exhibit crepuscular or nocturnal activity patterns, ovipositing during dusk or dawn under cooler conditions (Amendt *et al*. 2011) [1]. Temperature interacts with photoperiod to shape flight and feeding behaviour-high nocturnal humidity often supports extended larval surface activity and reduces desiccation (Paczkowski *et al.* 2015) [34]. Studies by Cammack *et al.* (2017) revealed that artificial lighting in urban environments can distort natural diel rhythms, prompting premature oviposition and altered species competition. Seasonal variation in photoperiod also influences developmental timing, with shorter days in winter delaying adult emergence (Singh *et al.* 2024). Understanding diurnal and nocturnal patterns is thus crucial for accurate interpretation of colonisation timing and PMI estimation, particularly in outdoor forensic contexts (Picard *et al.* 2023) [35].

5.5 Chemical Cues and Volatile Organic Compounds (VOCs) Influencing Behaviour

Chemical communication is the primary sensory mechanism through which carrion insects locate and evaluate decomposing resources. Volatile organic compounds (VOCs) such as dimethyl disulfide, indole, phenol, and putrescine are among the most potent attractants released during early decomposition (Paczkowski *et al.* 2015) [34]. These compounds arise from microbial metabolism of proteins and lipids and serve as olfactory signals guiding insects from long distances (Forbes *et al.* 2020). VOC profiles vary with decomposition stage, tissue type, and microbial community composition, influencing which species are attracted and when (Dekeirsschieter *et al.* 2012) [13]. For example, early-stage VOCs attract blowflies, while later aromatic compounds draw dermestid beetles and parasitic wasps (DeBruyn *et al.* 2021) [12]. Research has demonstrated that some insects also use chemical gradients to assess competition, avoiding carcasses already colonised by conspecific larvae (Michaud & Moreau, 2023) [32]. Recent studies have explored synthetic analogues of cadaveric VOCs for training forensic detection dogs and enhancing insect monitoring efficiency (Picard *et al.* 2023) [35]. Thus, the role of chemical cues in behavioural ecology is dual: it mediates resource location and species interaction while providing a measurable forensic signature of decomposition stage.

Table 2: Behavioural Ecology of Forensic Insects

Behaviour Type	Description	Representative Species	Ecological Function	References
Oviposition	Deposition of eggs or larvae on natural orifices or wounds	*Lucilia sericata, Chrysomya megacephala*	Initiates colonisation	Paczkowski *et al.* (2015); Michaud & Moreau (2011) [31, 34]
Feeding	Larval tissue consumption and liquefaction	*Chrysomya albiceps, Musca domestica*	Accelerates decay	Byrd & Tomberlin (2019) [39]
Predation	Secondary colonisers feed on larvae	*Creophilus maxillosus, Nicrophorus vespilloides*	Regulates larval populations	Gomes *et al.* (2021) [19]

Parasitism	Hymenoptera parasitize fly pupae	*Nasonia vitripennis*	Controls dipteran emergence	Sharanowski *et al.* (2008)
Nocturnal Activity	Oviposition or feeding during night	*Calliphora vicina*, *Sarcophaga caerulescens*	Colonises cooler environments	Amendt *et al.* (2011) [1]
Chemical Response	Attraction to VOCs and cadaveric odours	*Lucilia cuprina*, *Chrysomya megacephala*	Detects decomposition stage	Forbes *et al.* (2020); Dekeirsschieter *et al.* (2012) [13]

6. Comparative Analysis: Human Cadavers vs Animal Carcasses

6.1 Use of Animal Models in Forensic Entomology

Animal carcasses, particularly those of domestic pigs (*Sus scrofa domesticus*), have become indispensable analogues for studying decomposition processes in forensic entomology. Their physiological and anatomical similarities to humans, such as comparable body mass, skin composition, and fat distribution, make them ideal for replicating decomposition dynamics under controlled conditions (Dawson *et al.* 2020) [11]. Early research in taphonomy relied on pigs to document insect succession, development rates, and decomposition stages when access to human remains was ethically restricted (Carter *et al.* 2007) [8]. In recent years, the use of pigs has been complemented by other animal models such as rabbits, rats, and dogs, depending on study objectives and logistical constraints (Knobel *et al.* 2019) [24]. The primary rationale behind these models is that insect colonisation behaviour and succession are governed more by decomposition chemistry and temperature than by host species identity (Forbes & Carter, 2016). Consequently, animal models have allowed the development of baseline datasets for postmortem interval estimation, entomotoxicology, and VOC profiling (Forbes *et al.* 2020). However, as decomposition is a species-specific ecological process, the assumption of equivalence between pigs and humans remains a topic of ongoing evaluation (McIntyre *et al.* 2024) [29].

6.2 Similarities and Differences in Insect Colonisation Patterns

Comparative studies have shown both **broad similarities and notable differences** in insect colonisation patterns between human cadavers and animal carcasses. The general sequence of insect succession-beginning with calliphorid blowflies (*Lucilia*, *Chrysomya*, *Calliphora* spp.), followed by sarcophagid and muscid flies, and concluding with dermestid beetles and predatory taxa-is largely conserved across both substrates (Amendt *et al*. 2011) [1]. Nevertheless, variations arise in the timing, intensity, and species composition of colonisation. Dawson *et al.* (2020) [11] reported that blowflies colonise pig carcasses faster than human remains, likely due to thinner dermal layers and higher volatile emission rates. Knobel *et al.* (2019) [24] found differences in the VOC profiles of decomposing humans and pigs, with human cadavers producing higher concentrations of sulfur-based compounds, whereas pigs emitted greater quantities of esters and ketones, influencing fly attraction patterns. Environmental context also mediates colonisation differences; in shaded or enclosed habitats, insect arrival on human remains is often delayed relative to pigs (Michaud & Moreau, 2023) [32]. Despite these discrepancies, the overall successional order remains functionally consistent, allowing pigs to serve as valid models for broad forensic inference when regional calibration is applied (Picard *et al.* 2023) [35].

6.3 Limitations of Using Animal Carcasses as Human Analogues

While animal models have proven invaluable in forensic research, several limitations constrain their reliability as perfect human analogues. Physiologically, pigs differ from humans in skin permeability, hair density, and gut microbiome composition-all factors influencing decomposition rate and odor chemistry (Forbes *et al.* 2020). Behaviourally, insect preference studies have demonstrated that certain necrophagous species respond differently to pig versus human VOC blends, potentially biasing succession-based PMI estimations (Dekeirsschieter *et al.* 2012) [13]. Moreover, experimental setups using pigs often lack the social, medical, or traumatic contexts typical of forensic cases-such as clothing, injuries, or chemical exposure-that influence colonisation (Carter & Tibbett, 2008) [7]. Ethical and logistical constraints also limit replication of human-specific conditions, such as varying body positions and concealment scenarios (McIntyre *et al.* 2024) [29]. Recent molecular studies reveal distinct differences in microbial and insect-microbiome coevolution between humans and pigs, suggesting that microbial volatiles may mediate insect attraction differently across species (Metcalf *et al.* 2016) [30]. Therefore, although pigs remain the most practical substitutes, data derived from them must be validated through human decomposition facilities (so-called “body farms”) to ensure forensic applicability (Michaud & Moreau, 2023) [32].

6.4 Experimental Findings from Controlled Studies

Controlled decomposition studies conducted across different climates and habitats have yielded valuable insights into the extent to which animal models approximate human decomposition. In a comparative field experiment in Australia, Knobel *et al.* (2019) [24] found that both humans and pigs followed similar successional trajectories, but humans exhibited delayed onset of active decay and longer persistence of bloating. A North American study by DeBruyn *et al.* (2021) [12] reported parallel patterns of soil nutrient enrichment beneath both species, yet distinctive microbial signatures in human remains, particularly higher proteolytic and sulfur-reducing bacterial activity. Dawson *et al.* (2020) [11] observed differences in decomposition rate, with pigs decomposing approximately 20-30% faster than humans under equivalent conditions. Laboratory analyses further confirmed that temperature thresholds for insect development are similar between the two models, supporting their use in estimating minimum PMI values (Cammack *et al*. 2017) [5]. However, controlled experiments have also revealed that species-specific VOCs and microbial communities generate subtle differences in insect assemblages, especially among late-stage colonisers (Forbes *et al.* 2020). These findings collectively suggest that while animal models are robust for general pattern recognition, species-specific calibration is necessary for quantitative forensic applications (Picard *et al.* 2023) [35].

6.5 Implications for Forensic Casework

The practical implications of these comparative studies are significant for forensic investigations. Animal models remain indispensable for training, method validation, and baseline data collection, particularly where human access is limited (Amendt *et al*. 2011) [1]. However, forensic practitioners must interpret entomological evidence cautiously, acknowledging the physiological and ecological disparities between models and human remains (Matuszewski, 2021) [28]. In forensic casework, calibration with regional, species-specific reference data is essential for accurate PMI estimation and interpretation of insect evidence (Singh *et al.* 2024). The establishment of human decomposition research facilities worldwide has enabled direct comparison and refinement of pig-based findings, improving casework precision (McIntyre *et al.* 2024) [29]. Moreover, integrating molecular entomology, microbial profiling, and VOC analysis has enhanced the reliability of PMI models derived from mixed data sources (Metcalf *et al*. 2016) [30]. Ultimately, while animal models continue to underpin forensic entomology, the consensus across modern research emphasizes that human data remain the gold standard for case-relevant entomological interpretation (Picard *et al.* 2023; Michaud & Moreau, 2023) [35, 32].

Table 3: Comparative Differences Between Human and Animal Decomposition

Parameter	Human Cadavers	Animal Carcasses (Pig Model)	Key Observations	References
Skin & Fat	Thicker dermis, less subcutaneous fat	Thinner skin, higher fat content	Pigs decompose faster	Knobel *et al.* (2019) [24]
VOCs Released	Higher sulfur-based compounds	More esters and ketones	Differences affect fly attraction	Forbes *et al.* (2020)
Microbial Activity	Dominated by human-specific gut flora	Distinct anaerobic bacteria	Microbial shifts alter VOCs	Metcalf *et al.* (2016) [30]
Insect Colonisation	Slower initial colonisation	Rapid blowfly access	Affects PMI estimation	Dawson *et al.* (2020) [11]
Soil Nutrient Profile	Higher nitrogen & sulfur enrichment	Greater carbon leaching	Similar ecological impact	DeBruyn *et al.* (2021) [12]

7. Environmental and Ecological Determinants

7.1 Role of Habitat (Urban, Rural, Forested, or Subterranean)

Habitat type plays a decisive role in shaping the composition, abundance, and behaviour of carrion-associated insects. Decomposition and insect colonisation are faster and more diverse in open rural environments compared to urban or forested habitats, largely due to differences in microclimate, vegetation structure, and accessibility (Anderson, 2014). Urban environments, characterized by artificial light, pollution, and reduced vegetation cover, support a distinct assemblage of necrophagous Diptera such as *Lucilia sericata* and *Musca domestica*, which are synanthropic and exploit anthropogenic resources (Gomes *et al*. 2021) [19]. In contrast, rural and forested habitats often favour species such as *Calliphora vomitoria* and *Chrysomya megacephala*, which prefer shaded and humid conditions (Picard *et al.* 2023) [35]. Forest litter and canopy structure alter airflow and light penetration, reducing carcass detection rates and delaying initial colonisation (Amendt *et al*. 2011) [1]. Subterranean or concealed environments, including burials, wells, or basements, significantly restrict insect access, altering species richness and delaying oviposition by several days (Matuszewski, 2021) [28]. Furthermore, the predominance of predatory beetles (Staphylinidae and Histeridae) is higher in enclosed environments due to limited fly access (Dawson *et al*. 2020) [11]. Understanding habitat influence is therefore essential for interpreting successional deviations, especially in crime scene reconstructions involving concealed or transported remains (McIntyre *et al.* 2024).

7.2 Influence of Microclimatic Conditions

Microclimate-defined by localized temperature, humidity, light intensity, and air movement-exerts a profound influence on decomposition rate and insect activity. Even small variations within a habitat can alter the onset and duration of decomposition stages (Carter & Tibbett, 2008) [7]. Temperature regulates metabolic rates of both microbes and insects, directly affecting the speed of tissue degradation (Cammack *et al*. 2017) [5]. Humidity influences larval survival and oviposition; excessive dryness can desiccate tissues and inhibit egg hatching, whereas high moisture fosters rapid putrefaction (Forbes & Carter, 2016) [7]. Wind and sunlight exposure modulate carcass surface temperature, thereby shaping insect attraction and microhabitat selection (Michaud & Moreau, 2023) [32]. Decomposition in shaded or sheltered microhabitats proceeds more slowly and attracts fewer species, whereas open, sun-exposed areas favour early colonisers such as *Chrysomya albiceps* and *Lucilia cuprina* (Singh *et al.* 2024). Moreover, topographical variation-such as elevation or slope-affects drainage and thermal retention, creating microclimatic heterogeneity that modifies insect succession (Metcalf *et al*. 2016) [30]. Hence, microclimate is a critical variable that must be recorded and standardized in forensic field studies to ensure accurate interpretation of insect evidence (McIntyre *et al.* 2024).

7.3 Impact of Seasonality and Geographic Variation

Seasonal and geographic differences produce substantial variation in insect community composition, activity, and decomposition rate. Seasonal temperature fluctuations directly control insect abundance and diversity, with faster colonisation during warm months and extended pre-appearance intervals during cold seasons (Cammack *et al.* 2017) [5]. In temperate climates, *Calliphora vicina* dominates in cooler months, while *Chrysomya megacephala* and *Lucilia sericata* are predominant during summer (Amendt *et al*. 2011) [1]. Tropical regions, such as India, exhibit year-round insect activity but experience variation in species dominance aligned with monsoon and dry seasons (Singh *et al.* 2024). Geographic variation also affects colonisation dynamics; altitude and latitude influence species range limits, developmental rates, and succession timing (Picard *et al.* 2023) [35]. Studies have shown that *Chrysomya rufifacies* and *C. albiceps* are expanding into temperate zones, likely in response to global climate warming (Michaud & Moreau, 2023) [32]. Regional baselines are therefore essential, as applying insect development data from one climatic region to another can introduce substantial error in PMI estimations (Matuszewski, 2021) [28]. Standardization of geographically contextual reference datasets has thus become a global priority in forensic entomology (McIntyre *et al.* 2024) [29].

7.4 Effect of Soil, Vegetation, and Exposure

Soil properties and vegetation structure profoundly shape decomposition ecology by mediating moisture, microbial composition, and insect accessibility. Soil texture, pH, and organic matter content determine the rate of fluid absorption and nutrient diffusion beneath carcasses, influencing both microbial activity and soil-dwelling insect colonisation (Carter *et al.* 2007) [7]. Sandy soils with high drainage tend to desiccate remains faster, while clay-rich soils retain moisture and support anaerobic conditions conducive to putrefaction (DeBruyn *et al.* 2021) [12]. Vegetation cover affects microclimate by moderating sunlight exposure and humidity; dense canopy delays insect arrival but preserves moisture, prolonging active decay (Michaud & Moreau, 2011) [32]. Conversely, open grassland habitats facilitate rapid detection by flying insects and accelerate decomposition (Gomes *et al.* 2021) [19]. Carcass exposure-whether on the surface, buried, or submerged-also determines colonisation pathways: surface remains attract Diptera quickly, buried remains are dominated by Coleoptera and soil invertebrates, and submerged remains show delayed colonisation by aquatic Diptera (Knobel *et al.* 2019) [24]. Experimental studies confirm that exposure level influences not only colonisation rate but also community structure and decomposition chemistry (Forbes *et al.* 2020). Thus, soil, vegetation, and exposure conditions together act as ecological filters that regulate carrion decomposition and must be accounted for in forensic reconstructions (McIntyre *et al.* 2024) [29].

Table 4: Environmental and Ecological Determinants of Colonisation

Determinant	Effect on Decomposition/Insect Activity	Example/Observation	References
Temperature	Accelerates larval growth and VOC emission	Optimum range 25-35 °C	Cammack *et al.* (2017) [5]
Humidity	Supports oviposition, prevents desiccation	High humidity increases colonisation	Forbes & Carter (2016)
Habitat Type	Determines species richness	Urban: *Lucilia sericata*; Forest: *Calliphora vomitoria*	Anderson (2014)
Soil Type	Alters decomposition rate	Clay retains moisture; sand promotes drying	Carter *et al.* (2007) [8]
Vegetation Cover	Influences detection and colonisation delay	Dense canopy reduces insect arrival	Matuszewski (2021) [28]
Exposure Level	Governs accessibility and diversity	Buried carcasses delay colonisation	Knobel *et al.* (2019) [24]

8. Forensic Applications and Case Studies

8.1 Determination of Postmortem Interval (PMI) Using Insects

The estimation of the postmortem interval (PMI) is the most prominent forensic application of entomological evidence. Insects colonize human remains in a predictable sequence, and the developmental stages of their larvae provide measurable biological clocks (Amendt *et al*. 2011) [1]. Early-arriving Dipteran species, primarily *Calliphoridae* and *Sarcophagidae*, oviposit or larviposit on a corpse soon after death, allowing investigators to estimate the minimum postmortem interval (PMImin) by calculating the time required for the observed developmental stage under prevailing temperature conditions (Matuszewski, 2021) [28]. Accumulated degree-hour (ADH) or degree-day (ADD) models are employed to convert insect age into elapsed time since oviposition (Tomberlin *et al*. 2011) [39]. Blowfly larvae, such as *Lucilia sericata* and *Chrysomya megacephala*, are the most reliable species due to their well-documented thermal constants (Cammack *et al*. 2017) [5]. Additionally, the successional approach, based on sequential colonisation by different taxa, can refine PMI estimates, especially when primary colonisers are absent or disturbed (Picard *et al.* 2023) [35]. Molecular and genetic tools, including DNA barcoding, have further improved species identification and age estimation precision (Metcalf *et al*. 2016) [30]. Despite methodological advances, accuracy depends on local climatic data, habitat-specific colonisation records, and rigorous sampling procedures (McIntyre *et al.* 2024) [29]. Therefore, insect-based PMI estimation remains a cornerstone of forensic time-since-death determination worldwide.

8.2 Entomotoxicology: Insects as Indicators of Drug and Poison Presence

Forensic entomotoxicology extends traditional entomological applications by using insects, especially necrophagous larvae, as biological indicators of toxins, drugs, or chemical contaminants in decomposing tissues. Because larvae bioaccumulate xenobiotics from the tissues they consume, they can reveal toxicological profiles even when soft tissues are degraded or unavailable (Campobasso *et al.* 2009). Analytical methods such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have been applied to detect substances like morphine, cocaine, barbiturates, and organophosphates in maggot tissues (Goff *et al.* 2019). For example, studies have shown that larvae feeding on drug-intoxicated remains exhibit altered developmental rates, leading to potential PMI estimation errors if toxicological effects are not considered (Bourel *et al.* 2001; Magni *et al.* 2021). Insect species such as *Chrysomya albiceps* and *Lucilia cuprina* have proven reliable in drug detection, providing evidence of substance use or poisoning (Singh *et al.* 2024). Entomotoxicological data are increasingly valuable in regions where chemical analyses of decomposed remains are challenging due to tropical heat and rapid tissue loss (Gomes *et al.* 2021) [19]. Thus, forensic entomotoxicology enhances investigative capability by bridging entomological, pharmacological, and toxicological sciences, particularly in advanced decomposition cases (Picard *et al.* 2023) [35].

8.3 Insect Evidence in Legal Investigations: Global Case Studies

The integration of insect evidence into criminal investigations has proven crucial for resolving homicide, neglect, and body relocation cases globally. Notably, in the United States and Europe, entomological data have been repeatedly admitted as scientific evidence in courts, establishing PMI, confirming body movement, or identifying environmental exposure patterns (Amendt *et al*. 2011) [1]. In Germany, Hall *et al.* (2009) documented a homicide case where blowfly larval development on a concealed corpse established a PMI inconsistent with the suspect's statement, leading to conviction. In Japan, Takahashi *et al.* (2017) demonstrated PMI estimation using *Calliphora nigribarbis* and *Lucilia illustris*, supporting a murder investigation in a temperate urban environment. A Canadian case involving a buried infant used the absence of expected colonisers and the presence of staphylinid beetles to infer concealment time (Anderson, 2014). In tropical environments, cases from Brazil, Malaysia, and India have demonstrated the reliability of calliphorid succession even under high humidity and insect diversity (Gomes *et al*. 2021) [19]. Globally, forensic entomology now provides multidisciplinary evidence in legal investigations, encompassing PMI estimation, neglect documentation, toxicological inference, and trace analysis (Michaud & Moreau, 2023) [32]. These cases collectively affirm the judicial acceptance of insect-derived evidence when supported by rigorous field and laboratory methodologies.

8.4 Regional Studies from India and Other Tropical Zones

Tropical regions, characterized by high temperature, humidity, and biodiversity, present unique forensic entomological dynamics that differ markedly from temperate systems. In India, studies have identified *Chrysomya megacephala*, *Chrysomya rufifacies*, *Lucilia cuprina*, and *Musca domestica* as dominant colonisers of human and animal remains (Singh *et al.* 2024). Research from southern India by Kumara *et al.* (2020) demonstrated seasonal variation in blowfly dominance, with *C. megacephala* prevailing during the pre-monsoon season and *L. sericata* during post-monsoon periods. Studies from Malaysia and Thailand revealed similar successional frameworks, although influenced by fluctuating monsoon-driven humidity (Omar *et al.* 2019). In tropical Africa, *Chrysomya putoria* and *Lucilia infernalis* dominate carcass colonisation, contributing to shorter decomposition intervals (Amendt *et al*. 2011) [1]. The high insect diversity in these regions demands region-specific reference data to ensure PMI accuracy (McIntyre *et al.* 2024) [29]. Furthermore, entomotoxicological investigations in India have shown that larvae feeding on pesticide-contaminated remains retain detectable residues for several days post-mortem, demonstrating the technique's applicability under tropical conditions (Singh *et al.* 2024). Overall, regional forensic entomology research from India and other tropical zones highlights the necessity for localized developmental datasets, reflecting the unique ecological complexity of these environments.

Table 5: Forensic Applications of Entomological Evidence

Application	Scientific Basis	Use in Investigation	References
PMI Estimation	Insect developmental rates and succession	Determines time since death	Amendt *et al.* (2011); Matuszewski (2021) [1, 28]
Entomotoxicology	Detection of drugs and poisons in larvae	Identifies cause of death	Campobasso *et al.* (2009); Magni *et al.* (2021)
Body Relocation Analysis	Variations in insect fauna	Detects postmortem transport	Anderson (2014)
Environmental Reconstruction	Succession and habitat data	Infers decomposition site	Gomes *et al.* (2021) [19]
Legal Evidence	Insect stages and VOC profiles	Admissible in criminal cases	Hall *et al.* (2009); Michaud & Moreau (2023) [32]

9. Research Gaps and Future Perspectives

9.1 Limitations in Current Research Methodologies

Despite major advancements in forensic entomology over the past two decades, methodological inconsistencies remain a major limitation in both experimental and applied contexts. Many studies rely on animal carcasses, primarily pigs, under artificial conditions that fail to capture the variability found in real forensic cases (Dawson *et al*. 2020) [11]. Differences in carcass size, exposure type, and sampling intervals contribute to high variability in decomposition and insect colonisation data (Carter & Tibbett, 2008) [7]. Furthermore, regional studies often use non-standardized data collection methods, hindering cross-comparison between laboratories and climates (Matuszewski, 2021). Sampling bias is also common-most experiments focus on Diptera while underrepresenting other arthropod taxa such as Coleoptera, Hymenoptera, and Acari, which play critical ecological roles in later decomposition stages (Gomes *et al*. 2021) [19]. Additionally, laboratory rearing conditions rarely replicate field microclimates, resulting in inaccuracies in thermal development models (Cammack *et al*. 2017) [5]. A lack of long-term monitoring and multidisciplinary datasets further restricts the integration of entomological, microbiological, and chemical data (McIntyre *et al.* 2024) [29]. Addressing these methodological gaps is essential to improve the reliability and reproducibility of forensic entomology as an applied science.

9.2 Need for Standardized Comparative Studies

One of the persistent challenges in forensic entomology is the absence of globally standardized comparative protocols for decomposition studies. Variation in experimental designs such as carcass type, number of replicates, and sampling frequency-has resulted in conflicting interpretations of successional data (Amendt *et al*. 2011) [1]. International collaboration is necessary to establish unified guidelines for decomposition experiments across climatic zones (Picard *et al.* 2023) [35]. Comparative studies involving both human cadavers and animal models under identical environmental conditions remain rare, although such designs are vital for validating model reliability (DeBruyn *et al.* 2021) [12]. Moreover, discrepancies in taxonomic identification practices-ranging from morphological keys to DNA barcoding-often produce inconsistent datasets (Tomberlin *et al*. 2011) [39]. Regional forensic facilities in Asia, Africa, and South America still lack access to reference collections and species-specific developmental databases (Singh *et al.* 2024). Therefore, developing internationally harmonized standards for experimental setup, insect rearing, and data interpretation will enhance comparability and strengthen the evidential value of entomological findings in court.

9.3 Integration of Molecular and DNA-Based Identification

Molecular and genetic techniques have transformed forensic entomology by enabling accurate species identification and developmental stage determination. DNA barcoding using mitochondrial *COI* genes has improved the identification of morphologically similar or damaged specimens, which is crucial in late decomposition or forensic casework (Harvey *et al.* 2019). Quantitative PCR and next-generation sequencing have been used to analyze gut contents and detect tissue origin, enabling discrimination between human and animal feeding events (Metcalf *et al*. 2016) [30]. Additionally, transcriptomic and proteomic analyses of larvae can estimate larval age more precisely than morphological measurements (Picard *et al.* 2023) [35]. Molecular tools also facilitate microbial-insect interaction studies, offering insights into how microbial volatiles regulate insect attraction and colonisation (Michaud & Moreau, 2023) [32]. Despite these advancements, the lack of region-specific genetic reference libraries and high sequencing costs limit widespread adoption, especially in developing countries (Singh *et al.* 2024). Integrating molecular diagnostics with ecological and developmental models promises to improve PMI estimation accuracy and expand the forensic toolkit for complex or degraded remains.

9.4 Role of Climate Change in Shaping Insect Behaviour and Distribution

Climate change has begun to reshape insect phenology, species distribution, and behaviour-factors that directly influence forensic applications. Rising global temperatures accelerate insect development rates, potentially leading to underestimation of PMI if traditional degree-day models are applied without recalibration (Cammack *et al*. 2017) [5]. Shifts in precipitation and humidity patterns alter decomposition rates and favor thermophilic species expansion into new regions (Picard *et al.* 2023) [35]. For example, *Chrysomya albiceps* and *Chrysomya rufifacies*, historically restricted to tropical latitudes, have expanded into temperate zones in Europe and North America (Michaud & Moreau, 2023) [32]. In India and Southeast Asia, prolonged monsoon seasons increase decomposition humidity, altering calliphorid succession (Singh *et al.* 2024). Climate change also modifies overwintering behavior, enabling earlier spring emergence and extended flight activity periods (Amendt *et al*. 2011) [1]. As decomposition is a climate-sensitive process, forensic entomology must adapt by incorporating predictive ecological modelling and long-term entomoclimatic monitoring (McIntyre *et al.* 2024) [29]. Without such adaptation, existing developmental datasets will become increasingly outdated in a rapidly warming world.

9.5 Recommendations for Future Forensic Entomological Studies

To advance forensic entomology as a robust scientific discipline, future research must focus on integrative, standardized, and technology-driven approaches. First, decomposition studies should adopt unified methodologies, including controlled experiments using both human and animal models across multiple ecological zones (DeBruyn *et al.* 2021) [12]. Establishing regional reference insect colonies with developmental rate databases is essential for accurate PMI estimation under varying microclimates (Singh *et al.* 2024). Second, interdisciplinary research combining entomology, microbiology, soil science, and chemistry should be prioritized to understand the necrobiome as an interconnected system (Metcalf *et al*. 2016) [30]. Third, expanding molecular and isotopic methods-such as DNA barcoding, RNA age markers, and stable isotope profiling-will refine insect identification and development models (Harvey *et al.* 2019). Fourth, climate change adaptation research should focus on predictive mapping of forensic insect species and modeling new colonisation ranges (Picard *et al.* 2023) [35]. Finally, building international collaborative networks and establishing forensic entomology training facilities, particularly in tropical regions, will ensure data standardization and enhance global capacity for forensic casework (McIntyre *et al.* 2024) [29]. By addressing these priorities, the field can evolve toward a globally harmonized, multidisciplinary science capable of supporting modern investigative systems.

Table 6: Research Gaps and Future Priorities (2005-2025)

Identified Gap	Description	Future Research Direction	References
Methodological Inconsistency	Variation in carcass types and conditions	Standardized experimental protocols	Dawson *et al.* (2020) [11]
Lack of Regional Data	Limited tropical insect reference datasets	Create country-specific databases	Singh *et al.* (2024)
Limited Molecular Integration	Underuse of DNA and proteomic tools	Expand molecular barcoding studies	Harvey *et al.* (2019)
Climate Change Impact	Altered insect distribution patterns	Long-term entomoclimatic monitoring	Picard *et al.* (2023) [35]
Cross-Disciplinary Integration	Isolated ecological vs. forensic studies	Integrate microbial-insect models	Metcalf *et al.* (2016) [30]

10. Conclusion

10.1 Summary of Key Findings

The review synthesizes two decades (2005-2025) of multidisciplinary research highlighting the pivotal role of insects in forensic investigations, ecological understanding, and decomposition science. The decomposition process is a dynamic interplay among biological, biochemical, and environmental factors, wherein necrophagous insects act as both indicators and accelerants of decay. Studies consistently demonstrate that insect colonisation follows predictable successional patterns, strongly influenced by habitat type, microclimate, and carcass exposure. Blowflies (*Calliphoridae*), flesh flies (*Sarcophagidae*), and dermestid beetles (*Dermestidae*) emerge as dominant taxa governing early and late decomposition phases. Comparative analyses between human cadavers and animal carcasses affirm broad ecological similarities in colonisation, though differences in VOC emission, microbial communities, and dermal structure influence insect behaviour and decomposition rates. Molecular identification, microbial profiling, and VOC analysis have revolutionized accuracy in species recognition and postmortem interval (PMI) estimation. Furthermore, forensic entomotoxicology has expanded the field's scope by enabling detection of drugs and poisons through insect tissues, even when the corpse is degraded. Collectively, these findings reinforce that entomological evidence remains one of the most reliable biological tools for reconstructing postmortem events in both temperate and tropical environments.

10.2 Significance of Comparative Understanding

Comparative studies between human and animal decomposition remain the cornerstone for validating experimental models in forensic entomology. While pigs and other mammals serve as ethical and logistical surrogates, this review confirms that interspecies physiological and ecological differences must be accounted for in forensic interpretation. Experimental results indicate that although general insect succession patterns are conserved, colonisation onset and decomposition rate differ between species due to unique VOC profiles, microbial metabolisms, and integument properties. Recognizing these differences enhances the precision of PMI estimation and supports species-specific calibration of forensic models. Comparative approaches also deepen our understanding of the decomposition ecosystem as a whole, linking insect activity to microbial succession, soil chemistry, and climatic variables. In the broader scientific context, this comparative knowledge bridges ecology and forensics, strengthening the reliability of entomological evidence in legal investigations while promoting ethical research practices through validated animal analogues.

10.3 Concluding Remarks

Forensic entomology stands at the intersection of ecology, biology, and law - a discipline continually refined by advances in molecular science, analytical chemistry, and environmental monitoring. The integration of DNA-based identification, microbial ecology, and metabolomics is propelling the field toward higher accuracy and objectivity in forensic applications. However, persistent challenges remain: lack of methodological standardization, regional baseline data, and comprehensive climate-based reference models limit universal applicability. Future research must therefore prioritize standardized, globally collaborative studies that incorporate human and animal decomposition under controlled and field conditions. Additionally, climate change and global insect redistribution demand continuous re-evaluation of developmental datasets. As this review underscores, the fusion of traditional entomological expertise with emerging molecular and environmental technologies will define the next phase of forensic science. Ultimately, understanding insect colonisation and behaviour not only refines PMI estimation but also enhances our broader grasp of the ecological and biochemical transformations that accompany death - making entomology an indispensable component of modern forensic investigation.

Table 7: Key Insect Families and Their Forensic Relevance

Family	Common Species	Forensic Importance	Stage of Appearance	References
Calliphoridae (Blowflies)	*Lucilia sericata, Chrysomya megacephala*	Early colonisers, PMI estimation	Fresh to bloated	Amendt *et al.* (2011) [1]
Sarcophagidae (Flesh Flies)	*Sarcophaga albiceps, S. caerulescens*	Larviposition, quick colonisation	Early to active	Michaud & Moreau (2011) [31]
Muscidae (House Flies)	*Musca domestica*	Late secondary consumers	Active decay	Gomes *et al.* (2021) [19]
Dermestidae (Hide Beetles)	*Dermestes maculatus*	Feed on dry tissues	Advanced decay	Picard *et al.* (2023) [35]
Staphylinidae (Rove Beetles)	*Creophilus maxillosus*	Predators of fly larvae	Active-advanced	Eberhardt & Elliot (2008)
Histeridae (Clown Beetles)	*Saprinus semistriatus*	Predatory role, soil dwellers	Advanced stage	Dawson *et al.* (2020) [11]

11. Acknowledgment

I express my deepest gratitude to Dr. Ayantika Pal, my research supervisor, School of Science, MATS University, Raipur (C.G.), for her invaluable guidance, encouragement, and continuous support throughout the completion of this research work titled *"Colonisation Dynamics and Behavioural Patterns of Insects Associated with Human Cadavers and Animal Carcasses: A Comprehensive Review."* Her insightful suggestions, critical reviews, and academic mentorship have been instrumental in shaping the scientific rigor and quality of this study.

I am profoundly thankful to the Department of Forensic Science, Regional Forensic Science Laboratory, Jagdalpur, for providing the necessary facilities, reference materials, and a stimulating academic environment during the research process. I extend my sincere appreciation to my colleagues, laboratory staff, and technical experts for their cooperation and assistance in data collection and literature compilation.

I am also grateful to MATS University, Raipur (C.G.), for offering the opportunity and institutional support to pursue this doctoral research in Bioscience. Finally, I express heartfelt thanks to my family and well-wishers for their patience, understanding, and moral support, which have been a constant source of strength throughout my research journey.

12. Conflict of Study

The author hereby declares that there is no conflict of interest or financial relationship that could have influenced the outcomes of this research work.

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