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Forensic Entomology: An Insect as an Evidence for Legal Investigation

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Abstract

The most prevalent species worldwide that are essential to the process of a corpse's decomposition are arthropods. Forensic entomology's investigations can benefit greatly from the entomological examination of species that are frequently found near carrion. Entomological information has been used since ancient times to help solve a variety of medico-legal cases. The most common use of forensic entomology is the calculation of the post-mortem interval by meticulous examination of the insect fauna present in a corpse that decomposing. Although not always ideal, the environment around a decomposed corpse can have an impact on how quickly it decomposes. Forensic entomologists must take these elements into account. The present article gives a thorough review of the discipline of forensic entomology, including all of its uses, affecting factors, application, drawbacks and developments over time.

Keywords: *forensic entomology, post mortem interval, corpse, decomposition*

1. Introduction

The study of insects (or even other arthropods like mites and ticks) that are used as evidence in court proceedings is known as forensic entomology but it is primarily related to death investigations (Hall & Huntington, 1990). Numerous sorts of forensic investigations can benefit from knowledge of the distribution, biology, and behaviour of insects found in areas where bodies have been found because it can reveal when, where, and how, under specific circumstances, a crime was perpetrated or a person died (Anderson *et al.*, 2002; Catts & Goff, 1992; Catts & Haskell, 1990; Greenberg & Kunich, 2002; Introna & Cacattsmpobasso, 2000). The estimation of the postmortem interval (PMI, or the amount of time since death), is the

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application that receives the most attention (Amendt *et al*., 2004; Greenberg, 1991; Nuorteva, 1977).

Blowfly larvae (maggots) or adults, for example, must be treated as observable evidence in the same way that blood stains, fingerprints, hairs, fibres, or any other biological material are (Lord & Burger, 1983). In order to properly treat them as evidence, both during the autopsy and the crime scene inspection (Catts & Haskell, 1990; Haskell *et al*., 2001). The significance, of quality assurance in medicine in general (Lo & Smego, 2004; Nuorteva, 1977) and forensic science (Budowle *et al*., 2005; Gill *et al*., 1997; Randall *et al.,* 1998; Ritz-Timme *et al.,* 2000) in particular has recently been highlighted in a number of articles. Forensic entomology deals with living organisms, which should be handled carefully, so it is important to follow a systematic, quality-assured approach while collecting, preserving, and even packaging and transporting entomological samples. This will help prevent evidence from being contaminated or destroyed and will also help ensure the chain of custody. Forensic entomology is the study of the application of insects and other arthropods in criminal investigation (Catt & Goff, 1992). Insects or arthropods are found in a decomposing vertebrate corpse or carrion (Amendt *et al*., 2004). These insect colonizers can be used to estimate the time of death i.e., time interval between death and corpse discovery, also called postmortem index (PMI), movement of the corpse, manner and cause of death and association of suspects at the death scene (Sukontason *et al.,*2007).

2. Types of forensic entomology

There are three types of forensic entomology: Urban entomology, stored product entomology and medico-legal forensic entomology. Urban forensic entomology such things as litigations and civil law action involving arthropod in dwelling or as house & garden pests. Laws suits dealing with the misuse of pesticides. Stored product forensic entomology deals with arthropod infestation or contamination of a range of commercial product like flies in ketchup, beetle and their parts in candy bars. Medico legal forensic entomology deals with the arthropod involvement in events surrounding violent crime such as suicide, abuse, murder etc (Lord & Stevenson, 1986).

3. History of forensic entomology (Benecke, 2001**)**

Year	**Scientist**	**Discovery**
1247	Sung Tzu	First to use insect evidence to solve a crime and recorded evidence of application of forensic entomology (in the book " *washing away of wrongs*")

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15th & 16th century		Some of the poets and painters had closely observed the decomposition of the human bodies and illustrate their image on the woodcuts "*dances of the death*" and some have cut ivory carving "*skeleton in the tumba*"
1668	Francesco Redi	Demonstrated the development of larvae from eggs laid by flies.
1831	Orfila	Understood that maggots play an important role in the decomposition of body.
1855	Dr. Marcel Bergeret	Used Forensic entomology to detect Post Mortem Interval in a autopsy of a mummified body.
1881	Dr. Reinhard	First systematic study in Forensic entomology
1894	Pierre Megnin	Related different stages of human decomposition to the succession of insects colonizing the body after death.
1970		Emergence of Entomotoxicology as a new branch of forensic entomology.
1996		Use of forensic entomology in routine legal investigations, specially those involving death.
2001	Jeffery Wells & Felix Sperling	Devised a method to use mitochondrial DNA to differentiate between different species of fly.

4. Insect succession

4.1 WHY INSECTS ARE USED?

Insects are coming under the largest phylum Arthropoda. They can survive successfully in all type of ecological niche and in moist seasons and environments. They are colonize on a dead body almost immediately after death. Sequences of colonizing the cadaver by the insects - based on the principle of ecological succession. Insect's rate of development and species dynamics over time can be used to accurately determine time since death (Tomberlin *et al.,* 2011).

4.2 Forensically important insects associated with the cadaver (Sardar *et al*., 2021)

Sr No.	*Category*	*Description*	*Examples*
1.	Necrophages (Megnin, 1894;	• Species that primarily feed on carrion. • Appear in early stages of decomposition	*Diptera (flies): Calliphoridae* (blowflies)

	Nainis *et al.*, 1982)	• Age determination of these insects is most commonly correlated to obtain an estimated PMI	*Sarcophagids* (flesh flies) *Coleoptera (beetles):* *Silphidae* (carrion beetles) *Dermestidae*(skin beetles)
2.	Omnivores (Early & Goff , 1986)	• Feed on both the carrion and associated fauna • May deplete necrophages, thereby retarding decomposition • At present, limited potential for the provision of forensic information is known	Various species of ants, wasps, and beetles
3.	Predators and Parasites (Goff , 1989; Smith, 1986)	• Parasitize or devour immature flies, mites, nematodes, and other insects • Some species are necrophagous in the early developmental stages that become predaceous later • May eliminate competing species • Their role in the provision of forensic information is second to the necrophages.	Hymenoptera, Uropodidae, Macrochelidae, Parasitidae, Parholaspidae
4.	Incidentals or adventive species (Goff, 1989; Megnin, 1894)	• Use the carrion as an extension of their natural habitat. • Species-specific to the habitat of the scene of crime	Spiders, Centipedes, pill bugs, and Springtails

4.3 Some important insect are used in the legal investigation in forensic entomology are following:

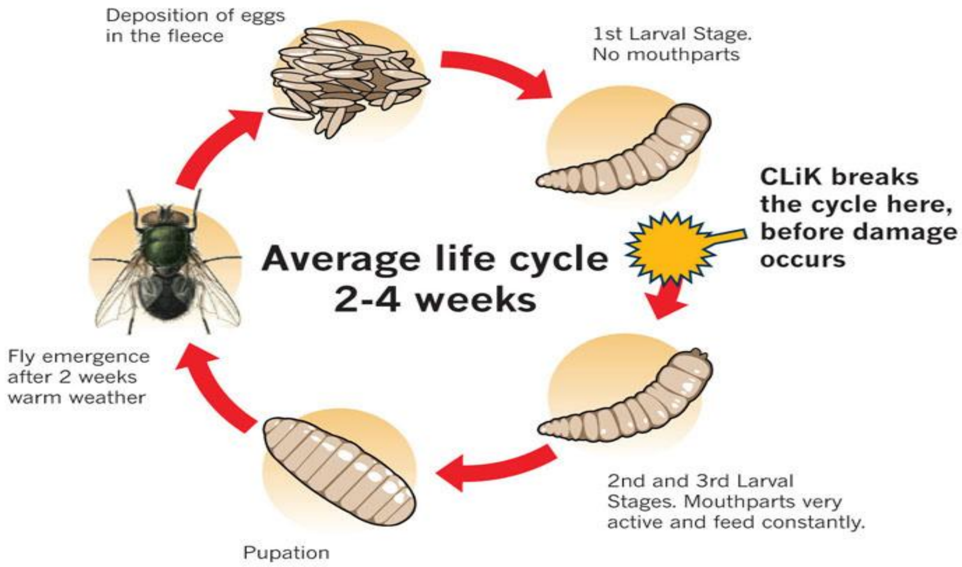
Sr no.	Families	Genera and species
Order- diptera (Krinsky, 2019)		
1	*Calliphoridae* (Blow flies)	▪ *Phaenicia sericata/ Lucilia sericata* ▪ *Phormia regina* ▪ *Calliphora vicina* ▪ *Calliphora vomitoria* ▪ *Cochliomyia macellaria*
2	*Sarcophagidae* (Flesh flies)	▪ *Sarcophaga haemorrhoidalis* ▪ *Sarcophaga bullata*
3	*Muscidae* (House flies)	▪ *Musca domestica*

		▪ *Fannia scalaris* ▪ *Hydrotaea leucostoma*
4	*Piophilidae* (Cheese skipper)	▪ *Piophilia casei*
Order- coleopteran (Krinsky, 2019)		
1	*Silphidae* (Carrion beetle)	▪ *Thanatophilus sinuatus*
2	*Histeridae* (Histerid beetle)	▪ *Margarinotus brunneus*
3	*Trogidae* (Hide beetle)	▪ *Trox* spp.
4	*Nitidulidae* (Sap beetle)	▪ *Omosita* spp.
5	*Staphilinidae* (Rove beetle)	▪ *Necrophila americana* ▪ *Nicrophorus* spp. ▪ *Oiceoptoma* spp. ▪ *Oligota pusillima*
6	*Cleridae* (Ham beetle)	▪ *Necrobia rufipes*
Order- Acarina		
1	*Parasitidae*	▪ *Poecilochirus carabi* ▪ *Poecilochirus davydovae*
2	*Histiostomatidae*	▪ *Pelzneria crenulata* ▪ *Pelzneria necrophori*
Order- Lepidoptera (Krinsky, 2019)		
1	*Pyralidae*	▪ *Aglossa* spp.
2	*Tineidae*	▪ *Tinea Pellionella* ▪ *Tineola bisselliella* ▪ *Trichophaga tapetzella*

4.3 Life cycle of blowfly

Necrophagous insects will quickly colonise a fresh body, depending on accessibility and surrounding circumstances. Flies (Diptera), especially blowflies (Calliphoridae), which can detect an odour source with incredible spatial precision and lay their eggs on a corpse within minutes to hours of death, are typically the first taxa to arive on a body. Larvae, sometimes known as "maggots," emerge from the eggs and consume the underlying tissues. In order to allow for more growth, they undergo a process known as "ecdysis" in which they shed their cuticle twice. A new larval instar (stage) develops after each ecdysis (moult). The majority of species move away from the body to find shelter once the third instar larvae have finished feeding and have entered the post-feeding stage, either in the soil or beneath things like stones or leaves (at outdoor crime scenes) or furniture (at interior crime scenes). When the metamorphosis is complete, the pupae emerge from this protective outer shell, the puparium (the hardened cuticle of the third instar larva) (Kamal, 1958).

5.



Collection of specimen (Amendt *et al*., 2007)

Group	*Equipment*	*Purpose*
1. Documentation equipment	Protocol sheets	Writing down details related to crime scene and specimens
	Camera/ Video recorder	Photographic documentation
	Scale	To be used while photographing specimen to ensure quality and identify distortion
	Labels	For identification of specimens
2. Collection equipment	Fine and coarse paintbrushes (moistened)	For collecting eggs
	Spoons	For collecting maggots
	Forceps (varying spring tensions and sizes)	For collecting insects of corresponding stages Fine – immature/fragile insects Medium – adult insects
	Shovel or trowel	For a collection of soil and leaf-litter samples, including buried larvae
	Handheld insect capture net	For catching flying insects
3. Temperature recording equipment	Thermometer	for measuring the body and ambient temperatures, as well as the larval mass temperature
	Temperature data logger	Measurement of the temperature of crime-scene for a number of days following the discovery of a dead body

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4. Storage and Transport	Tissue paper	For handling eggs and living larvae
	Vials and storage boxes of different sizes	Preservation of collect insect specimens
	Reusable ice packs in cooler bag	For storing living insect specimens
	Ethanol (70-95%)	For storage of dead specimens
	Sticker, Sealing Wax, Tapes	Material for sealing the samples
	Durable plastic or paper bags	for storage of soil samples and leaf litter specimens

5.1 If collecting living specimens: (Amendt *et al*., 2003)

For eggs:

It should be put in vials on moistened tissue paper. The vials should let air in but keep newly hatched maggots from escaping. Send the samples within 24 hours to laboratory. Kill and preserve all specimens in 70–95% ethanol if this is not required.

For larvae:

Store the samples in vials under favourable conditions. It should be lined with coarse sawdust or tissue paper to absorb fluids produced by the maggots. Living samples should be transferred within 24 h.

For pupa:

Transfer pupae for rearing within 24 h. Store each sample in vials under favourable conditions. Cool temperatures are most suitable. The lid of the vials should be punched with small holes to allow entry of air.

For adults:

Live adults should be killed by placing into a vial, which is put into a freezer (at −20°C) for 1 h; and store the dead specimens in 70–95% ethanol. If pinning possible, this should be done in addition, because it often simplifies identification. Adults which have newly emerged from puparium should be killed after allowing the wings to fully harden and the colouring to develop. Sometimes Insect remnants such as pupal skins, empty puparia or beetle faeces can also be stored after proper dehydration and then kept in 70–95% ethanol.

5.2 Where to collect entomological evidence (Amendt *et al*., 2003)

Two sites for collection of the sample

- On the corpse: Sites for sampling should include the natural orifices and eyes, traumatic wounds, at the corpse–substrate interface and under the body, in the pleats of clothes and pockets, shoes, socks, etc.
- From the carpet, bag or material in which the body might have been wrapped

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- From the plastic body bag in which the corpse or the remains have been enclosed for transport to the place of autopsy and storage.

1. Around the corpse:

- It is recommended to collect soil samples from multiple compass points up to a depth of at least 10 cm, or even more depending on the circumstances, at an outdoor scene up to 2 meters away from the corpse. If available, you should also gather some leaf litter or other soil-covering debris.
- Examine various rooms if the situation is indoors. Before pupariation (metamorphosis), larvae can leave the corpse and disperse widely, so they might be found in various rooms.

6. Stages of decomposition

There are 5 stages of decomposition

6.1 Fresh stage (1-2 DAYS):

The Fresh Stage starts as soon as someone passes away and lasts until the body starts to swell. During this stage, the body exhibits few obvious, gross decompositional changes, but greenish discoloration of the abdomen, liver, skin cracking, and tache noir may be seen. The natural body openings of the head (eyes, nose, mouth, and ears), anus and genitalia, as well as any open wounds, where insects typically enter the body. The Calliphoridae (blow flies) and Sarcophagidae (flesh flies) are typically the first insects to arrive. Arriving female flies will start to look around any potential oviposition or larviposition sites. These flies frequently enter the openings deeply and lay eggs, larvae in the first instar, or maggots. The anus and genital areas may be more or less attractive to flies depending on whether they are exposed or covered, in contrast to the head openings, which are always attractive to them. Prior to death, when blood is flowing, wounds have been found to be more appealing to flies for colonisation than wounds that were inflicted postmortem and without a blood flow. Although there may not be much visible evidence of internal feeding activity during this stage, the eggs laid in the body start to hatch (Lee Goff, 2009).

6.2 Bloated stage (2-7 DAYS):

Putrefaction, the primary aspect of decomposition, starts during the bloated stage. The gut and other areas of the body are home to anaerobic bacteria, which start to break down the tissues. Gases produced by their metabolic processes initially cause a slight inflation of the abdomen. The Bloated Stage is thought to start when this is realised. The body might start to resemble a fully inflated balloon as this continues. The internal body temperatures start to rise as a result of the putrefaction and maggot metabolic processes working together. These

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temperatures can be significantly higher than the surrounding air's temperature (50°C), at which point the body transforms into a unique habitat that is largely independent of its surroundings. During this stage of decomposition, the adult Calliphoridae are drawn to the body and large masses of maggots are seen associated with the head and other primary invasion sites. There are larger populations present internally even though these populations are visible externally. During this stage, internal pressures brought on by the production of gases cause fluid to seep from the body's natural openings, and a potent ammonia odour is noticeable (Tulis & Goff, 1987).

6.3 Decay stage (5-13 DAYS):

The beginning and ending points of the stages of decomposition are largely undefined, but the beginning of the decay stage is clearly marked by a physical occurrence. The outer layer of the skin breaks down at this point, allowing gases from the abdomen to escape as a result of the combined effects of bacterial putrefaction and maggot feeding. The body deflates at this point, marking the start of the decay stage. Strong decompositional odours are noticeable at this time. Large feeding masses of Diptera larvae are the key characteristic of this stage. Internally, externally, and frequently spilling onto the ground next to the body, these are present. During the Decay Stage, some Coleoptera that have been settling during earlier stages of decomposition multiply and are frequently quite noticeable. While some predators, like the Staphylinidae, are visible during the Bloated Stage, others, like the Histeridae, also become more noticeable now. Necrophages are also noticeable in addition to the predators, and their numbers are growing as the process progresses. The majority of the Calliphoridae and Sarcophagidae will have finished this stage of development by the time it is over, leaving the remnants to pupate in the nearby soil. The majority of the body's flesh will have been removed by the end of the Decay Stage by Diptera larvae, leaving only skin and cartilage (Amendt *et al.*, 2011).

6.4 Postdecay stage (10-23 DAYS):

The Diptera stop being the dominant feature as the body is reduced to skin, cartilage, and bone. They will be replaced by different groups of Coleoptera in xerophytic and mesophytic habitats, with species from the family Dermestidae being the most frequently observed. These first appear as adults in the later stages of the decay Stage, but during the post decay Stage, they predominate as adults and larvae. Their feeding clears the bones of any remaining dried flesh and cartilage, and their mandible scraping gives the bones a clean, polished appearance. The Coleoptera typically do not thrive in moist environments (such as swamps, rainforests, etc.). Other groups of arthropods eventually take their place. Several

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Diptera families, including the Psychodidae, and their corresponding predator/parasite complexes are among these. An increase in the quantity and variety of predators and parasites is linked to this stage in both types of habitats (Horenstein *et al*., 2012).

6.5 Skeletal/Dry stage (18-90+ DAYS):

When only bones and hair are left, this stage has been reached. Normally, no taxa that are obviously carrion-frequenting are visible at this stage. Several soil-dwelling taxa, such as mites and Collembola, can be used to calculate the time since death during the earlier parts of the Skeletal Stage. During this stage, elements of the typical soil fauna gradually return as the pH of the soil gradually returns to its original level. There is no clear end to this stage, and the soil fauna may differ for several months or even years, indicating that a body was present at some point in the past (Horenstein *et al*., 2012).

7. Applications of forensic entomology

Insect developmental stages tell more regarding post morterm interval, place of death, cause of death, presence and position of wound sites, entomotoxicology, association of suspects with the death scene and abuse & neglect.

7.1 Post mortem interval:

Post-mortem interval (PMI) refers to the time between the death and discovery of a corpse (Catts, 1992). There are several natural processes associated with decomposition, such as rigor mortis or livor mortis, that can be used to estimate the PMI, but many of these are reciprocal functions and become inaccurate in application very quickly (Bourel *et al*., 2003). Furthermore, they are limited to the first 72 h after death. However, during that 72 h and well beyond, insects can be a very powerful tool for estimating the minimum time since death (Campobasso *et al*., 2001). There are three methods for calculating the PMI: isomorphen diagram, isomegalen diagram and thermal summation model.

7.1.1 Isomorphen diagram

The isomorphen diagram, which is the simplest of these models, plots the length of developmental events (egg hatching, first and second ecdyses, beginning of wandering, beginning of pupariation, and adult eclosion; X-axis) versus temperature (Y-axis). It is possible to determine an accurate PMImin from this model by using the min/max error bars to estimate the confidence window for PMImin and knowing the age of the oldest immature insects on the body as well as the average environmental temperature of the crime scene while the body was in place.

This model's benefit is that it is easy to apply and has concepts that can be clearly stated in court. However, this simplicity limits the output's accuracy, as the average

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environmental temperature of the crime scene used to calculate PMImin undermines the estimate in scenarios where environmental temperatures vary, such as outdoor scenarios.

In some instances, living specimens may be killed and kept before being examined, as entomological evidence is not usually collected while still alive. In these situations, the estimated age of insect specimens modelled from an isomorphen diagram will always be lower than their actual age because it must be based on the last developmental stage that has been observed (for example, the moult from 2nd to 3rd instar larvae for all 3rd instars, regardless of how long ago that moult occurred). This results in an underestimate of PMImin.

7.1.2 Isomegalen diagram

The isomegalen diagram is a more complex model that has the ability to account for and analyse data on changing environmental temperature. It can estimate PMImin using dead larvae. The isomegalen diagram is a 3D contour graphic that models the relationship between temperature (Y-axis), time (X-axis), and larval size (length, weight, or width) as a measure of age. Larval size, a more accurate age indicator than developmental event, reveals specifics of growth between two developmental events.

From egg hatching through the start of pupariation, the diagram shows the whole range of larval sizes, with each contour on the graph denoting a different size of larva. In order to estimate post mortem interval minimum (PMImin), the biggest larvae from the body and the crime scene are collected, identified, and measured. These data are then compiled and modelled along with the relevant environmental temperatures. In the procedure for determining PMImin from variable environmental temperatures is shown using an isomegalen diagram (Villet *et al.,* 2010).

7.1.3 Thermal summation model

Of the three accessible models, thermal summation models are the most complex. They are able to interpret information on size, developmental events, and changing ambient temperatures. The preferred model to use when predicting PMImin is this one since it can handle more complicated data sets and is less impacted by the constraints placed on the two iso-diagrams. For the analysis of developmental data, a recognised linear regression model is available (Higley & Haskel, 2001; Greenberg & Kunich, 2002). It has historically been modified for forensic entomology and used to determine development thresholds for application in biological control (de Re´aumur, 1735). The high variance in the upper and lower developmental temperature extremes is a criticism of this model because these values are the end points of the regression, and as such, heavily weighted values, they significantly influence the confidence of the regression coefficients, which compromises the precision of a

PMImin estimate (Ikemoto & Takai, 2000). Recently, a revised regression model has been used to model blowfly development (Richards *et al*., 2009).

7.2 Place of death:

Many fly species have specific geographic preferences. One species common in rural areas may not be found in a city and vice versa. Some flies prefer to breed indoors while others prefer an outdoor environment. Flies can also exhibit preferences for corpses in shade or sunlight conditions of the outdoor environment (Ghodake *et al.,* 2014).

7.3 Cause of death:

Dead body with external injuries is more attractive to insects than one having none. Insect colonization in or around the opening other than 9 natural opening. So, depending upon the degree of degradation brought about by maggots or growth and development of the maggot (larvae of flies), it may be possible to suggest the probable mode of death, i.e. strangulation or mutilation or drug / poison (Ghodake *et al.,* 2014).

7.4 Presence and position of wound sites:

Insect colonization occur primarily in or around the external injuries or opening followed by other 9 natural opening (Ghodake *et al.,* 2014).

7.5 Entomotoxicology:

Drugs ingested by the deceased individual may accumulate in the fly larvae that feed on carrion. It is challenging to search for toxicological substances on bodies that are skeletonized or in late stages of decomposition. When this occurs, it is possible to macerate the larvae feeding on the body and analyse the results using methods like thin-layer chromatography, gas chromatography, and/or mass spectrometry. The phases of the larvae's development can be affected by toxins. The presence of cocaine and heroin in the carcass can hasten larval growth. Carrion poisons like malthione can prevent insects from colonising (Chen *et al*., 2004).

7.6 Association of suspects with the death scene:

The DNA from human blood can be recovered from the digestive tract of a **blood-feeding insect** (like **mosquitoes**) that has fed on suspect. Since 1985, DNA typing of biological material has become one of the most powerful tools for personal identification in forensic medicine and criminal cases (Ghodake *et al.,* 2014).

7.7 Abuse & neglect:

Insects evidence may be used to determine the circumstances of death. Sometimes entomological evidence may involve living victims. The diverse applications of forensic entomology include the detection of abuse in children and neglect of the elderly. Insect

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evidence represents how long a person was abused/neglected. Elderly people of wounds are potential target for the insects**.** However, wounds and circu mstance of bad hygiene in elderly and very young person are attracted certain species of flies. Blowflies are valuable as forensic indicators in cases of abuse, rape and neglect**.** Sometimes victims that are bounded, helpless and drugged have associated urine soaked clothes. Such material is potential targets for flies (Anderson & Huitson, 2004). When sexual assault has occurred prior to death, blowflies will more likely to attract and oviposit in these regions (anus and penis or vagina*)*, and investigators can start to suspect a sexual crime. Insect evidence can yield many clues to both antemortem and post-mortem circumstances of the crime (Khimani, 2015).

8. Factors affecting Forensic Entomology

8.1 Fluctuating temperature:

In some insects the rate of development is constant at fluctuating temperatures but in some insects the fluctuating temperatures have a greater impact on development. Some species develop more rapidly under fluctuating temperatures and some insects take a longer time to develop under fluctuating temperatures. Negligence of fluctuating temperatures in legal cases can lead to distinctly wrong estimates of the post mortem interval. Insects are cold blooded, so their development is temperature dependent, as the temperature increases, they develop more rapidly (Niederegger *et al.,* 2010).

8.2 Effect of photoperiod:

Development time can be influenced by the photoperiod that larvae and pupae experience, which in turn may have an impact on post mortem interval estimates. However, at constant light there is an increase in variation in overall adult developmental time and it significantly slows down the development. Temperature appears to have a greater impact on development than photoperiod. Estimating the time since death correctly depends on understanding how light-triggered stimuli cause developmental rates to slow under constant light (Nabity *et al*., 2007).

8.3 Weather condition:

The insect flora varies according to location. Blow flies are the first creatures to colonize a corpse, and understanding their life cycle is crucial for calculating the passing of time. It is based on the local climate and environmental factors. The temperature at the crime scene and at the weather station are also taken into account. Although the microhabitat conditions might not be comparable even though the overall weather conditions might be quite similar. The conditions of the microhabitat are greatly influenced by vegetation cover, air

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drainage, and slope exposure, which may also have an impact on how the forensically significant flies develop (Catts, 1992).

8.4 Myiasis:

The feeding of maggots on living or dead tissues close to a wound is known as myiasis. Myiasis is most frequently connected to facultative parasites belonging to the families Calliphoridae, Sarcophagidae, and Miscidae in forensic contexts. Because the victims are typically helpless infants or elderly people, myiasis mostly occurs indoors and can develop hours to weeks before death. Myiasis can be a major source of confusion for the forensic entomologist if not fully understood, appearing to give an estimate of post mortem far longer than the actual period of time since death (Mozayani & Noziglia, 2010).

8.5 Antemortem condition of deceased:

The maggots discovered on the corpse may hold the key to what actually took place. The study of these insects can show how the deceased was before death or how they died. The presence of drugs and toxins consumed internally before death can affect how long has passed since the person passed away. Drug presence in tissues can result in an inaccurate or inaccurate estimation of the post-mortem period. When estimating the post mortem interval using entomological techniques, it is necessary to take potential drug interactions with tissues into account in order to account for insect growth rates (George *et al*., 2009).

8.6 Maggot mass effect:

The temperature of the corpse and the surrounding air may differ. This is a result of the gregarious fly maggots that are active on the corpse. Temperatures can rise by 1-3 °C above the ambient temperature as a result of increase maggot activity. The accuracy of post mortem interval estimates may suffer as a result of this temperature rise because it may promote the growth of maggots (Charabidze *et al.,* 2011).

9. Molecular techniques in forensic entomology

Polymerase chain reaction (PCR) enables amplification of suitable region of the genome, allowing identification of arthropods in cases where morphologically unsuitable or fragmented body parts of insects are obtained (Wells & Stevens, 2008). DNA barcoding aims to use the information of one or a few gene regions to identify all species of life (Campobasso *et al*., 2005). Restriction fragment length polymorphism (RFLP) analysis may restrict the analysis to only a selected area of a nucleotide sequence which may aid in eliminating false inclusion (Wells *et al*., 2007). DNA methylation regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factor to DNA. Random amplified polymorphic DNA (RAPD) may be used to determine taxonomic identity, assess kinship relationship, analysis mixed genome samples and create specific probes (Kader & Ghai, 2015).

10. Significance of Forensic Entomology

The presence of insects on the body that are not present in the area suggests that the body was moved, which could provide information about the murder's situation. If the insect cycle is disturbed, it might indicate that the murderer visited the crime scene a second time. The entomologist might be able to predict both the victim's death and the killer's return date. When a maggot grows outside of a natural opening, a wound might be present. For example, maggots on the hands' palms could indicate defensive wounds. If maggots feed on a body with drugs in its system, those chemicals accumulate and may be detected. If an insect is found from a specific site, it may place a suspect at the scene of a crime. If insects are found on a living individual (often young children or seniors), it may indicate neglect or abuse (Chaudhary & Ganguly, 2018).

11. Limitation of Forensic Entomology

Estimating the exact time of death depends on accurate temperature data but local weather patterns can be seen and data may come from stations located far from the crime scene. Insect abundance is essential to forensic entomology. There are fewer insects in the winter, so entomology is less useful. Forensic entomology cannot deliver results immediately because it takes time to raise insects. Estimates may be impacted by treatments that exclude insects, such as freezing, burial, and wrapping (Chaudhary & Ganguly, 2018).

12. Conclusions

Entomological techniques should be used carefully when determining the PMI from corpses infested with insects because forensic entomologists should be aware of the effects of insects on corpses. A reliable estimate of the minimum time since death can be obtained in court using insect development and succession studies, but this method requires rigorous statistical support. Particularly for the earliest developmental stages of insects, which can be challenging to identify to the species level by morphological techniques, DNA sequence analysis (for example, barcoding) is a very potent tool. Insects found on a body may be toxicologically and molecularly analysed to help determine the cause of death, identify the victim, or even connect a suspect to a crime. Forensic entomology can complement and supplement other traditional forensic procedures if it is investigated with the proper methods. The methods' accuracy is largely dependent on the forensic entomologist's knowledge and the availability of all necessary information about the relevant insects. Due to a lack of background information on the insects involved in the decomposition of dead animals and a

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shortage of qualified forensic entomologists, forensic entomology has yet to establish an undisputed place in legal proceedings in our country. Insect is the voiceless detective can reduce crime globally.

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