CHAPTER 1

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1.1. Insects as evidence for forensic investigation

Forensic entomology characterizes the implementation of certain techniques focusing on insects in forensic contexts belonging to the various classes of different arthropods (Goff, 2001). The general use of evidences from such organisms towards medico legal investigations definitely helps the scientific community to estimate the time since death that's called the PMI (Harvey et al., 2008).

The organisms of forensic importance belonging to the Phylum Arthropoda which includes the insects, crustaceans, arachnids, and scorpions. They are synanthropists and had been observed from crime scenes as well. During the past few years, the application of forensic entomology in determining the post mortem interval (PMI) has been extensively documented in Asia (Singh et al., 2022) and from other parts of the world including USA, Europe, and Australia with the help of various experimental works and numerous case studies (Campobasso & Introna, 2001).

A study by Amendt et al., (2004) discussed the various observations done by Smith, (1986) and found that there are mainly four categories of insects that can be found on the decomposing carrion: 1) Parasites and predators usually consume the necrophagous organisms: this cluster also encompasses the schizophagous species, which are previously known to consume the dead body first and in later stages, they become predaceous in nature; 2) Necrophagous organisms targeting the carrion for feed; 3) Omnivorous organisms typically consuming the carrion, wasps, some beetles and ants; 4) Organisms

including the spiders and springtails and which are primarily using the cadaver as an extension for their setting. They also reported that among the various analyzed groups, the first and second groups of the classification are found to be significant for forensic entomology. The organisms belonging to this category are primarily Coleoptera (beetles) and Diptera (flies).

As reviewed in the former part, the major species having forensic significance in this order are Calliphoridae (blow flies), Sacrophagidae (flesh flies) and Muscidae (house flies). Among them, the Calliphoridae species are typically found glossy with metallic colouring, usually with green, blue, and black thorax. They principally arrive at the cadaver immediately after the death happens (Amendt et al., 2004; Sukontason et al., 2022).

Arthropods located at a crime scene and the body of the victim or the carrion can assist several kinds of forensic aspects. Specifically, they can provide sufficient information regarding the time of particular incident, death environment, primary crime scene, concealment of the evidences following the incident and the use of drugs/toxic substances (Archer et al., 2005). It is worth introducing that such instances and experiments illustrate the potential implementation of the aforesaid perspectives in the forensic investigations not only for the determination of PMI but also for the detection of poisons involved in the crime (Marchetti et al., 2013). Notwithstanding being exceptionally informative, arthropods have the ability to strongly control and alter the crime scene along with the body of the victim. The forensic pathologists need to be aware of the impairment that arthropods can instigate along with the beneficial records, they are able to offer (Viero et al., 2019). Hence, to understand the complexity of the specific crime scene and to recognize it’s dynamics, the forensic entomological expertise is of utmost significance.

Literature review revealed that 55 insect taxa belonging to three insect orders such as Hymenoptera (sawflies, wasps, ants and bees), Diptera (flies), and Coleoptera (beetles) had been discovered to colonize human dead body (Lutz et al., 2021). The extensive species variety with 37 taxa and 13 families was found belonging to Diptera which indicated its forensic importance. Among the various investigated insect species, the blow flies (Diptera: Calliphoridae) had been the most leading insect group.

The following insect species were found to have significant roles in forensic entomology; Coleoptera; *Saprinus semistriatus* (Scriba, 1790), *Nicrophorus humator* (Gled, 1767) *Nicrophorus vespilloides* (Herbst, 1748), *Thanatophilus sinuatus* (Fabricius, 1775), *Necrodes littoralis* (Linnaeus, 1758), *Thanatophilus rugosus* (Linnaeus, 1758), *Thanatophilus* sp., Diptera: *Lucilia sericata* (meigen, 1826), *Calliphora vicina* (Robineau-desvoidy, 1830), *Lucilia ampullacea* (Villeneuve, 1922), *Protophormia terraenovae* (Robineau-desvoidy, 1830), *Calliphora vomitoria* (Linnaeus, 1758), *Chrysomya albiceps* (Wiedemann, 1819), *Phormia regina* (Meigen, 1826) *Lucilia caesar* (Linnaeus, 1758), *Lucilia illustris* (Meigen, 1826), *Lucilia silvarum* (Meigen, 1826), *Triphleba autumnalis* (Becker, 1901), *Megaselia scalaris* (Loew, 1866), *Megaselia abdita* (Schmitz, 1959), *Megaselia rufipes* (Meigen, 1804), *Conicera tibialis*(Schmitz,1925) and *Triphleba aequalis* (Schmitz, 1919) (Lutz et al., 2021).

Blow flies exhibit prominent significance in the veterinary, medical, agricultural, sanitary, and forensic researches. Primarily, they were known to have extensive ability in transmitting a plethora of bacteria, viruses, fungi, helminthes, protozoans, and other diseases causing pathogens responsible for diarrhoea, poliomyelitis, plague, bacillary dysentery, tuberculosis and cholera. The recent years have witnessed the presence of them

in the dead bodies, excreta and the carrion of various animals including the human population. This vital presence has made them a significant candidate for the future forensic research (Greenberg, 1991).

The blow flies (Diptera: Calliphoridae) are typically the prime group to initially colonize the carcass. The various developmental stages of them, the length and weight of the larval body followed by the morphological alterations at intra-puparial stages can deliver an accurate estimation of the PMI (Amendt et al., 2011). Hence, it is, in specific, vital to set up accurate primary developmental records for the blow fly species.

The precise identification of forensically vital species is encompassed as the initial step and also the most important element in the forensic investigation (Harvey et al., 2008). Identification of the insect species gives us the information regarding the developmental stages and the insect succession styles (Chen et al., 2004; Potapov et al., 2022). If the species identification is found to be inaccurate, then simultaneously the predicted PMI can also be incorrect, which justifies the significance of species identification and the choice of selection of the specific taxonomic approach in forensic entomology (Byrd & Tomberlin, 2019).

From the above-said facts, it is clear that the insect taxonomy sprawl at the coronary artery of forensic entomology. The identification of insect species were mostly based on morphological observations in the early works. Morphology of insects is a consistent strand upon which numerous dichotomous keys are based totally and has brought about the identification of over one million insect species (Chen et al., 2004; Stork, 2008). Be it though, still it is anticipated that hundreds of thousands of insect species are yet to be identified and labelled (Chen et al., 2004).

Molecular strategies are the precise techniques to discover and identify insect species (Potapov et al., 2022), supplying unique benefits compared with the conventional approach. Precise identification of insects up to species level in forensic entomology remains challenging in many instances (Hebert et al., 2003; Kjer et al., 2016).

In order to collect the blow fly specimens for forensic investigations, preference should be given to carrion. As introduced in the former sections, forensic entomology is specially implemented for estimating the minimal PMI i.e. revealing the time of insect colonization in the carcass, by means of analyzing the age of the target insect species inhabiting the dead body (Matuszewski et al., 2010, 2011). But, it can also be integrated into many more situations, e.g. cadaver repositioning or manipulation of a situation to escape from the crime. Moreover, the various developmental stages of the insects, especially the larvae and pupae have promising records for the investigation of sexual crimes including rape, particularly when the victim was discovered in a complicated stage of decomposition (Clery, 2001).

Google search for the term “forensic entomology” generated about 2.190.000 hits on 16.10.2020 which included research articles, interviews, films and newspaper articles evidencing the relevance of the research in the current scenario (Lutz et al., 2021). But notwithstanding the global positive improvement in this discipline mainly in the last 20 years, insects are nonetheless too rarely considered as significant elements in the practical implementation of strategies concerning the forensic field. Even in a developed country like Germany, it was found that, out of 41 country-wide crime investigation centers, only three of them have employed forensic entomologists (Lutz et al., 2021).

The development of such forensically important species, especially the blow flies, was precisely characterized in previous forensic entomology studies (Richards & Villet, 2009). The cosmopolitan species such as the *Phormia regina* (Meigen, 1826), *Calliphora vicina* (Robineau-Desvoidy, 1830) and *Lucilia sericata* (Meigen, 1826) were primarily used to develop the life cycle stages as a fundamental developmental record for unique provinces over the world. *Chrysomya megacephala*, usually referred to as the oriental latrine fly, formerly Pacific and Australasian in distribution, has expanded its range tremendously (Wells & Kurahashi, 1994).

The most common approach chiefly employed to calculate PMI is the thermal summation model, which is principally based on the growth of various developmental stages of immature insect species in context with the influence of temperature (Catts & Goff, 1992). This approach actually assumes a linear link between the rate of developmental stages and the surrounding environmental temperatures (Richards & Villet, 2009).

Although huge number of studies concerning the development of blowflies are available, data regarding the same aspect is found to be lacking for populations from specific geographical regions (Reibe-Pal & Madea, 2015). Also, specifically, no different is the case with the current study site of Kerala, India. Moreover, the differences in various seasons may also result in a discrepancy in the developmental time. Hence, it is of extensive significance to set up fundamental data concerning the above-said facts with special inference on the developmental stages of the flies in context with the seasonal perspective for different areas to enhance the easiness in the estimation of PMI.

Since morphological characters do not help us in confirming the identity of the species, it is imperative to choose technologies like SEM and Mitochondrial DNA (mtDNA) for the successful and precise identification of the larvae and the adult. The mitochondrial cytochrome c oxidase subunit I (COI) was known to encompass the advantageous effects of forensic entomology in species identification (Harvey et al., 2003). Molecular studies thus supplement the morphology based taxonomy internationally. However, studies focusing on the same aspect in the Indian regions, especially in the Kerala region are scanty.

According to Zajac et al., (2016), as mentioned in the former section, the COI gene analysis seemed to be more decisive for the discrimination of species than 28S rRNA for identification. Absence of overlap between the intra and interspecific genetic distances can also be noted as an additional factor that made the aforesaid gene a strong candidate for species identification.

From the above said forensic significance perspectives and the output from the study by Sontigun et al., (2018), it was perceived that the environmental and other factors linked with the seasonal fluctuation, daily activity and reproductive ability of the same are vibrant. For instance, the various environmental factors such as rainfall, relative humidity, altitude, land use types and temperature (Sontigun et al., 2018) can directly affect the abundance and the distribution of blow flies. A similar perspective in terms of seasonal fluctuations in blowfly populations was also verified by Lertthamnongtham et al., (2003). Perusal of literature reveals that the studies concerning the aforesaid aspect in Kerala is scanty. In this regard, it is essential to analyze the seasonal activity of the respective location or habitat of the blow flies for forensic entomology needs.

Among the various factors discussed, the temperature and the humidity have been recognized as significant factors in investigating the biology of blowflies, since such factors can directly affect the various developmental stages of the same including its behaviour, survival, population dynamics and the longevity (Sukontason et al., 2008).

In addition to the above-said factors, Sontigun et al., (2018) also found that fecundity plays a significant impact on blow fly population dynamics because it truly governs the population growth potential of the same. It has been observed that larval nutrition plays a major role in life-history characteristics including body size, growth, longevity and survival followed by reproduction (Li et al., 2014).

Many investigations have witnessed that the higher temperature and lower temperature enhanced and slowed down the developmental process respectively (Queiroz, 1996). In contrast to these discussed facts, some of the studies reported that the environmental conditions and specific geographic locations may found to be major probable factors behind this situation. However, many of them have reported the direct influence of temperature on the fast developmental stages and growth. In this regard, many earlier research works have been conducted on the following blowfly species on the same aspect as mentioned in the former parts; *Chrysomya albiceps, Chrysomya megacephala, Chrysomya rufifacies, Calliphora vicina, Chrysomya erythrocephala, Calliphora vomitoria, Calliphora dubia, Protophormia terraenovae, Phormia regina, Muscina stabulans, Eucalliphora latifrons, Lucilia illustris, Ophyra capensis, Ophyra aenescens* and *Muscina assimilis* (Sukontason et al., 2008).

Besides principally identifying the species of blow flies collected from a death scene, the developmental rate of collected sample specimens is also found to be essential in

order to use as a piece of evidence in forensic investigation, specifically for the PMI estimation. As discussed by many of the researchers, it is also clear that the development of insects including the blow flies is primarily temperature dependent. For this reason, the normal metabolic rate of the same upsurges with augmented temperature, thereby making the developmental process happen at a faster rate (Anderson, 2000).

As discussed in the former parts regarding the temperature and other factors, there exists a controversy regarding the exact influence of these factors on the growth rate of blow flies. Moreover, many previous studies have documented that the pattern of influence may be changed with the geographic location. Although the facts follow these patterns, as stated in the above-said parts, some of the studies have suggested that there exists no noticeable difference between various seasons while following the same experimental conditions. A very similar range of temperature existing during their experimental conditions (summer - 23.3–32.0°C; rainy- 23.3–29.6°C: and winter-21.0–25.8°C) may be recognized as the major reason behind the aforesaid circumstance.

However, the blow flies, showed local adaptation to the different provinces over the globe, and earlier studies have reported that the various stages in the development of blow flies from different geographic locations may be found different (Grassberger & Reiter, 2002) in many of the instances and this justifies the significance of investigating the forensic entomological aspects of blow flies from Kerala, India.

In fact, a previous study recognized that an established life table of a particular species of fly is extremely beneficial in forensic entomology since it can deliver significant information concerning the survival growth as well as the fecundity of the studied fly (Abou Zied et al., 2003). The rate of development of blow flies having forensic importance

has been previously described in Thailand under natural temperature .In the same study it was observed that specific photoperiod followed by extensive development occurred in the summer season, while the rainy season was reported with relatively lower development (Hadura et al., 2018).

The concluding remarks by Shiao & Yeh, (2008) revealed that, the contributing factors in other species of *Chrysomya*, the temperature and various other factors significantly and obviously affect the larval development directly and thereby primarily impact the PMI estimations. Many of the investigators have suggested that the complexity emerged during such research in a geographic location perspective view should be clarified and rectified through an effective way.

In addition to the blow flies belonging to the *Chrysomya* genus, other species belonging to different genera like *Lucilia* may also exhibit great significance in forensic entomology. The precise and accurate identification of such specimens having forensic significance is one of the very significant elements from an applied point of sight (Catts & Goff, 1992).

In southern India, studies relating to the time since death assessment have not been conducted so far. The estimates of PMI based on the known characteristics of the infesting fauna in the natural conditions of the specific geographical location are very important.

The studies focusing on the use of insects as evidence in forensic investigations in different geographical areas are often considered to be the initial steps in developing the reference base line data (Acosta et al., 2021; Dawson et al., 2022). In the collection of ecological data with regard to the decomposing model, insects are found to be a major element in forensic investigations. The pre-colonization phase of the forensically significant insect is usually witnessed in the following phases; a) detection b) finding the

remains c) evaluating the cadaver d) colonization and e) post-colonization (Tomberlin et al., 2011).

While discussing the practical implementation of various strategies in forensic entomology it was evident that, developing baseline data using various parameters like temperature, humidity, successional pattern, and developmental time, is essential for the PMI determination. In addition to this, it is also required to consider the fact that successional sequence and developmental rate may be strongly influenced by the geographic region, various seasons, climate change and life cycle pattern (Brundage et al., 2014).

The process of natural variation in different geographical locations significantly impacts the distribution of forensically significant taxa. In addition to this, it should be remembered that seasonal variations should also be considered, as different species are showing variations in their life cycle even if the species have been found within the same locality (Cammack et al., 2016).

There are many approaches that are likely to provide support for PMI estimation in the various decomposition stages. A number of extrinsic and intrinsic factors are previously witnessed as the core of forensic entomology research. The former consists of the environmental conditions such as concealment, temperature, insect activity, clothing, moisture and various seasons while the latter encompasses the elements linked with the cause and manner including gender, body mass, injuries, and age (Amendt et al., 2011).

While focusing on the various factors discussed, the significant variables influencing the life cycle stages of insect include the temperature, humidity and seasonal variations (rainy, winter and summer seasons) (Amendt et al., 2011). For the early determination of PMI, post mortem hypostasis followed by algor mortis and rigor mortis

are recognized to be the most reliable and applicable method. The influence of various parameters including the temperature, humidity and other significant factors on the insect life cycle stages would be recognized as the most efficient method for PMI determination (Pittner et al., 2020). The lifecycle pattern and succession of flies inhabiting the cadavers usually followed a particular pattern and can be used as a reference for forensic investigations.

In order to estimate the PMI based on the developmental stages, geographical differences and seasonal variations, it is essential to accomplish the proper identification of the species (Sukontason et al., 2022).

Recent studies have validated that the knowledge concerning the carrion-inhabiting insects found at the specific geographic location is vital for determining the PMI (Amendt et al., 2011). The various developmental stages of these carrion-inhabiting insects are influenced by many factors including the geographical region, climate, temperature, humidity and seasons and may also result in diapause. The PMI determination principally relied on the information concerning the life cycle of the chosen insect and the various factors linked with it. The growth rate and the developmental pattern of insects having forensic significance might vary in several regions over the world. Hence the seasonal variations in different regions may also illustrate considerable interest in forensic investigations (Verma & Reject Paul, 2016).

In this regard, it is essential to warrant studies concerning the above-said aspect in insect species belonging to Calliphoridae, since their forensic significance have been previously reported in various provinces over the world except in Kerala. The output gathered from this investigation could be used to estimate the PMI in the Kerala region, which would be recognized as the novelty of this investigation.

1.2. Relevance of the Study

Among the various previously described necrophagous insects, flies belonging to Calliphoridae are known to inhabit and colonize the cadaver as the first comers within a short period of time. For this reason, studies focusing on the determination of the age of the blow fly life stages with special inference on the PMI estimation have gained extensive significance in the past few years. Many of the previous studies have discussed that among the various studied flies, blow flies are prominently found to exhibit great significance in the current scenario. Moreover, the studies from different parts of the world clearly validate that the studies concerning the influence of various parameters on the development rate, growth rate and lifecycle stages of blow flies are of great forensic significance. From this point of view, the research gap found in India, especially in Kerala were the main reasons for the present investigations in the above said perspectives in blow flies. As we all know, the data obtained from forensic entomology studies with special inference on the PMI estimation is usually allied with the geographic location. In this regard, the data obtained from the current study can be explored to determine the PMI in India especially, Kerala.

1.3. Objectives of the present study

The prime objectives of the current study are as follows:

1) To generate data on blow fly fauna of Central Kerala
2) To assess the seasonal differences in blow fly population in Central Kerala
3) To study the life history of selected blow flies in carrion
4) To find out the relation between the temperature and humidity on the life cycle of calliphorid flies
5) To relate the life cycle of flies and time since death assessment for forensic application