Forensic entomology: an overview

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Abstract

Insects are the most important, in terms of number and diversity, group of animals on the earth. Insects have colonized all the world's environments and are associated with both human life and death. Although their economical and sanitary importance is well documented, in the past few years they have been used also in a forensic context. In forensic entomology, necrophagous insects have proved useful in; studying postmortem interval (PMI), postmortem transfer (the movement of a body from one location to another after death), presence of drugs or poisons, and in identifying the victim and/or the suspect. Many species can be used to estimate the minimum PMI (mPMI), according to the stage of cadaver decomposition, body exposure, geographical region, and season. The most important British flies of forensic interest are described.

Key words: Insects, Forensic Entomology, PMI, drug detection, Diptera

Introduction

The word ‘forensic’ comes from the Latin *forēnsis*, meaning ‘before the *forum*’, the place where ancient Romans used to discuss about social, political, economic and judicial matters. In such a context, criminal charges were presented before a group of public individual in the *forum*.

Nowadays forensic sciences involve the application of any kind of science to the investigation of legal matters. Actually discussions about the use of science in criminal and civil law typically concern the subject of forensic physical evidence as observation, identification and interpretation. Forensic evidence refers to items, facts or opinions proffered in a criminal case that have been generated or supported by the use of a group of forensic sciences routinely used in criminal prosecutions (Kiely 2001).

Even if it may not offer strong support in all cases, science often plays a special investigative role since together with technology it is used to serve as independent ‘witnesses’ in criminal or civil matters.

Long-standing forms of physical evidence include fingerprints, bloodstains, hairs, fibers, soils, and DNA, but any object can serve as physical evidence if it provides reliable insight into the activities associated with a death scene or crime. Forensic world is extremely heterogeneous and rarely forensic scientists specialize in all aspects of forensic science. Only an interdisciplinary cooperation between forensic disciplines (eg: archaeology, dactiloscopy, anthropology, odontology, biology, genetics, botany, entomology, ballistics, toxicology, psychology, etc) allows to a better reconstruction of a case. The contribution of these disciplines is fundamental in order to answer the ‘6Ws’ questions concerning a criminal event (Where, When, What, How, Who and Why?).

It is worth mentioning that in the last year some disciplines have shown their potential in providing useful information in the investigative contexts, and one of them, Forensic Entomology is increasing its impact, especially in the estimation of the time since death in decomposed or strongly compromised bodies (eg: dismembered, burned, etc)

Forensic entomology

Generally Entomology is the study of insects, involving their biology, locations, mutations, and their control in relation to the world’s environment. Forensic Entomology occurs every time that information about insects is used to draw conclusions in investigating legal cases relating to both humans and wildlife, and they are presented in the court as an evidence. Insects can be used in the investigation of a crime scene both on land and in water.

Between the thirteenth and the nineteenth century, a number of developments in biology laid the foundations for Forensic Entomology to become a branch of scientific study. One of the most notable was the experiment done by Redi (1668) that, using the flesh of a number of different animal species, demonstrated that larvae developed from eggs laid by flies and the spontaneous generation theory was confuted.

In the late twentieth and early twenty fist century Forensic Entomology started to be accepted as a proper forensic scientific discipline. Academics and practitioners working alongside the police and legal authorities are contributing to refine and develop it.

Forensic Entomology can be divided into three sub-fields depending on the context which it operates on:

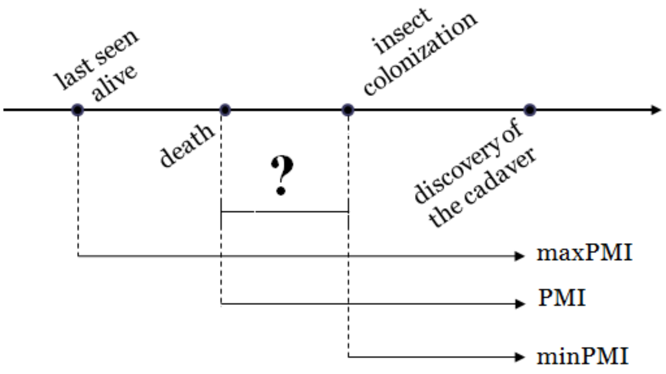
- Urban Forensic Entomology. It typically focuses on pests infestations that are related to litigation such as legal disputes between exterminators and landlords. Besides studying insects and other arthropods, Urban Forensic Entomology typically also involves toxicological studies as the appropriateness of pesticide application.
- Stored-product Forensic Entomology. It investigates cases of insects' infestation or contamination of commercial foods as aim to find evidence relevant for litigation.
- Medico-legal Forensic Entomology. This sub-field gathers evidence through the study of insects and other arthropods at a crime scene such as murder and suicide. It most often involves the study of different development stages of insects, in what order they appear and where on the body they are found. Because insects occur only in particular places and are active only at a particular season, their presence can reveal a lot about location and time of the crime as explained below.

Estimation of the time since death

After a murder has occurred, the estimation of the time elapsed since death until the discovery of the cadaver is the main task of Medico-legal Forensic Entomology. How long a dead body has been exposed to the environment is defined as *Post Mortem Interval* (PMI). PMI estimation is crucial in every legal investigation because it is the starting point to go back in time to reconstruct the criminal events and to define the circumstances of death. It also allows the identification of victims and suspects and provides the acceptance or rejection of suspect *alibi*. However, PMI is difficult to estimate because the decomposition process depends on several and extremely different variables. Over the short term, i.e. in the range of 0-96 hours after death, *mortis* triad *(algor, livor* and *rigor mortis)* and changes in vitreous humor and soft tissue components can be used by forensic pathologists to estimate the PMI. Other potential chemical analysis are under study but not clearly established yet (Hauther et al. 2015). As the decomposition process starts, medico-legal evaluations are compromised by thanatological events which include everything naturally acting on the death body. Moreover, the analysis becomes more complicated in cases of concealment, body dismemberment, explosion and burning. In these circumstances the morphological characteristics of the cadaver are often so damaged that there is no standardized methods based on experimental studies for deriving time since death in an accurate way (Vanin et al. 2013). For intermediate range of time (days or weeks) the study of cadaveric arthropod-fauna is a well-known and widely accepted method to estimate the time of death, and may support histological and chemical analysis. This kind of approach studies the decomposition process as a function of the time to estimate PMI of vertebrate remains. The development rate of blow fly larvae and changes in the communities colonizing the body are the main objects of the entomological approach.

The majority of the invertebrate fauna founded on corpses are insects, mostly Diptera (flies, eg: blow, house and flesh flies) and Coleoptera (beetles) which are selectively attracted by the decomposing status of the body. Insects form complex communities within necrophagous species and their predators, parasites and parasitoids (Turchetto and Vanin 2004). The insects' activity, especially the rate of they appearance and development on the cadaver, is specie-specific and temperature dependent. The Period of Insect activity (PIA), also known as Colonization Interval (CI), occurs from the eggs or larvae deposition until the discovery of the cadaver. It can be affected by the degree of physical connection with the dead body (e.g. outdoor or indoor location and clothes or lack of clothes) so it hardly overlaps to the real PMI. Actually, the forensic entomologist often takes care to evaluate the minimum *Post Mortem Interval*, mPMI, i.e the time elapsed between eggs deposed on carrion by flies and the cadaver finding. There is no experimental way today to establish the real PMI (Fig.1).

Figure 1. Schematic representation of maximum PMI, PMI and minimum PMI



Once a cadaver has been found, temperature data from the dead body and also from the environment are collected. All the insects found on the *scena criminis* must be collected regardless their stage of development and stored or breed as suggested by standard available protocols (Amendt et al, 2007). In the latter case, after the species identification, experimental laboratory work allows to study how long a species takes to develop once let growing up at specific temperature. Each developmental stage of insects' life cycle actually requires temperature values which are specie-specific. The subsequent comparison with temperature data of days prior the discovery of the cadaver makes the estimation of insects' age (i.e. PIA) possible. Among many validated methods to do that, Accumulated Degree Days or Hour (ADD-ADH) is a method widely used (see Amendt et al., 2007).

Decomposition is a variable, dynamic, ecological and biochemical process depending on biotic and abiotic factors. Scavenging, insect activity, microbial communities, climatic conditions and physical modulators such temperature and moisture drive the decomposition process over time. Vertebrate and invertebrates activity depend also on physical barriers preventing their access, while climatic conditions are related to seasons and geographic locations (Carter et al. 2007). Biotic and abiotic factors acting on dead bodies are closely related. In body breakdown biotic factors' activity is actually significantly affected by temperature and moisture: at warm temperature insects and microorganisms are more active and chemical reaction rates are more rapid as well. As a consequence a corpse typically decomposes faster at warm temperature rather than at low values. Furthermore, egg deposition by adult female flies requires a high degree of moisture and an easy way to enter the cadaver. Weather conditions having specific temperature and humidity values are obviously related to seasonality in a strong way. As documented by Carter and coworkers (2007), significant differences were observed between the decomposition of swine carcasses, used as human cadaver surrogates, in summer and winter. Carcasses during the winter trial were frozen for approximately 20 days *post mortem*, which resulted in little visible decomposition until 30 days *post mortem*. Although some maggot activity was observed in the mouth during the first few days of the winter trial, there was no other insects' activity during the winter trial until approximately 25 days *post mortem*. In contrast, insects' activity was rapid and significant during the summer trial. As a result, carcasses decomposed rapidly in the summer; a state of advanced decomposition was observed by 9 days *post mortem*. Carcasses in both seasons decomposed to a similar extent by 60 days *post mortem*, with the majority of decomposition in the winter occurring after 15 days *post mortem*. Conversely, the majority of decomposition during the summer trial occurred prior to 15 days *post mortem.*

The body decomposition process can be divided for didactic purposes in several stages based on the physical appearance of the remains and associated arthropod arrival patterns also known as “waves of colonization”. However the stages are not always observed and may be totally absent, depending on the taphonomy of the corpse. Here we describe the six stages of decomposition and the insect associated, as proposed by Payne in 1965. Several experiments carried out around Europe and America in the last years confirmed this pattern of colonization

Fresh Stage

This stage starts from the death to the first signs of bloating of the body and overlaps to the autolysis previously described. The first wave of insect colonization is given by the arrival of blowflies, family Calliphoridae. In Europe *Lucilia* is the predominant genus during summer while *Calliphora* is typical of spring and autumn. Muscidae families are usually present on cadavers lying in an indoor environment. The flesh flies Sarcophagidae are part of the second wave of colonizers looking for a cadaver as a suitable site for the development of their offspring. Fly oviposition is a vital step in the breakdown of a cadaver as maggot activity is the main driving force behind the removal of soft tissue in the absence of scavengers. Indeed, Linnaeus (1767) stated that ‘three flies could consume a horse cadaver as rapidly as a lion’.

Bloated Stage

The depletion of internal oxygen also creates an ideal environment for anaerobic microorganisms (e.g. *Clostridium, Bacteroides*) originating from the gastrointestinal tract and respiratory system transforming carbohydrates, lipids and proteins into organic acids (e.g. propionic acid, lactic acid) and gases (e.g. methane, hydrogen sulphide, ammonia) that result in color change, odor and bloating of the cadaver. This process is putrefaction and leads to the onset of the ‘Bloated’ stage. Internal pressure from gas accumulation forces purge fluids to escape from cadaveric orifices (mouth, nose, anus) and flow into the soil. Initially the abdomen swells but later the whole body becomes stretched like an air-balloon. This effect would be similar to the formation of discrete ‘islands of fertility’ observed in association with plant and faecal resources. Eventually, putrefactive bloating and maggot feeding activity cause ruptures in the skin which allow oxygen back into the cadaver and expose more surface area for the development of fly larvae and a renew aerobic microbial activity. At this stage fermentation products such as propionic acid are powerful attractive for Piophilidae families (cheese skipper flies) starting the third colonization waves. At the same time more and more flies keep to be attracted by the smell of putrefaction gases deriving from tissue liquefaction which become suitable also for Fanniidae (little house flies), bee flies like, Syrphidae (hover flies) and Sphaeroceridae (small dung flies), the fourth invertebrate colonizers.

Active Decay

This stage is characterized by rapid mass loss resulting from peak maggot activity and a substantial release of cadaveric fluids into the soil via skin ruptures and natural orifices. This flux of cadaveric material into the soil will connect any islands of fertility resulting from purge fluid and, thus, lead to the formation of a single cadaver decomposition island (CDI) characterized by changes in soil carbon, nutrients and pH values. Active decay matches with the advanced stage of putrefaction fermentation including ammoniacal fermentation of the body, which a different cohort of insects are attracted to. Silphid beetle *Nicrophorus humator*, the Histerids *Hister cadaverinus* and *Saprinus rotundatus*, but also the Muscid fly *Hydrotaea capensis* form the fifth wave of colonization. The Phorid *Megaselia scalaris* (coffin fly) is the first colonizer in indoor environments but one of the last outdoor (Gennard, 2007). ‘Active Decay’ will continue until maggots have migrated from the cadaver to pupate.

Advanced Decay

The lateral extent of a CDI during ‘Advanced Decay’ is determined by the size of the cadaver, the lateral extent of the maggot mass (including the path of maggot migration) and soil texture. Soil texture and cadaver size also affect the vertical extent of a CDI. The transition from ‘Advanced Decay’ to ‘Dry’ to ‘Remains’ is difficult to identify (Payne 1965).

Dry

Increased plant growth around the edge of the CDI might act as an indicator of the dry stage.

Remains

Increased plant growth within a CDI might indicate the beginning of stage six. Anyway in the later stages of decay, all that remains of the body are skin, cartilage and bones with some remnants of flesh including the intestines. Any remaining body tissue can be dried. The biggest indicator of this stage is a reduction in the dominance of the flies (Diptera) on the body and an increase in the presence of beetles like larder beetles (Coleoptera: Dermestidae) but also clothes moths (Lepidoptera: Tineidae) which conclude the colonization waves earning the seventh and eighth (Gennard 2007). These final stages of cadaver decomposition correspond to a second period of slow cadaver mass loss which is probably due to the depletion of readily available nutrients and moisture (Carter 2007).

Species identification

In Forensic Entomology, the correct identification of the species is extremely important to estimate the mPMI because insects' development is specie-specific and depends on temperature. Two methods are used to reach this aim: the morphological approach uses specific phenotypic keys to discriminate individuals belonging to different species (Szpila 2012). Microscope techniques are used in order to look for specific characteristics of the insect; it can be applied to all the developmental stages of flies in an easier way for the adult but for the immature stages, larvae and pupae, the available keys are really few and not well established yet.

Because of this reason, another approach has been developed in the last years and it has been used daily to confirm the observations resulting from the morphological analysis. The molecular approach consisting in the DNA extraction, amplification and sequencing based on standard Sanger method is applied. The amplification step usually targets specific nuclear or mitochondrial regions which can be used to identify the species in an accurate way. A good molecular target is a DNA sequence which consists of constant regions, usable to design the oligonucleotide for the amplification, and variable regions in length or sequence which allow to distinguish a species among all the others. According to these requirements the most used molecular target for insect species identification is COI gene: it is a mitochondrial gene, so despite nuclear genes it is present in many copies in the cells, and it codifies for the subunit I of the Citochrome Oxidase. This is one of the main protein involved in the respiration process occurring in mitochondria, a well-known and evolutionary conserved mechanism among the invertebrates (Fig. 10). Many pairs of oligonucleotides annealing to mtCOI were designed in the past years and new of them are being developed according to the needs of researchers who study different species of insects. A list of the main COI primers is here reported (Fig. 11, Tab. 2, 3). Universal primers LCO/HCO were the first to be used (Folmer et al. 1994): LCO anneals to light strand of mtDNA which is the external one and it's defined as 'light' because its G+C content is smaller than G+C content of 'heavy' strand which HCO primer is specific for. Together they amplify a region of COI which is 658 bp long. In the last year, the usage of molecular approach in order to identify the species has been growing up but unfortunately its efficiency is going down. Actually more and more research groups keep going to design new couple of primers to amplify different regions of COI, and most of them and the resulting amplified regions almost overlap. As a result, instead of making clear the situation and making easier the identification they are creating confusion. Obtaining reliable molecular results for species identification and phylogenic analysis is becoming more and more difficult because of the fact that new sequences are kept going to be placed in databases in an unchecked manner.

Most important species of forensic relevance in UK

Insects are the most numerous and diverse organisms on the planet, comprising more than half of all the living species on Earth, exceeding all other groups of organisms combined. Such big quantities come from their ability in adapting at almost every kind of environments ranging from terrestrial to aquatic, from coldest to hottest, from driest to wettest habitats. The United Kingdom has a temperate climate, with plentiful rainfall all year round. The temperature varies with the seasons seldom dropping below −11°C or rising above 30°C.The prevailing wind is from the south-west and bears frequent spells of mild and wet weather from the Atlantic Ocean, although the eastern parts are mostly sheltered from this wind since the majority of the rain falls over the western regions the eastern parts are therefore the driest (http://www.metoffice.gov.uk/). Atlantic currents, warmed by the Gulf Stream, bring mild winters; especially in the west where winters are wet and even more so over high ground. Summers are warmest in the south-east of England, being closest to the European mainland, and coolest in the north. Heavy snowfall can occur in winter and early spring on high ground, and occasionally settles to great depth away from the hills. In this kind of climate four species of blowflies (Calliphoridae) are well known to have a role in forensic investigations: *Calliphora vicina, Calliphora vomitoria, Protophormia terranovae* and *Lucilia sericata*.

Figure 2

Calliphora vicina: Common name: European blue bottle fly. Adult in lateral view (Photo S. Vanin).

This is a large blowfly, 9–11mm in length. The thorax is black and the top of the thorax (dorsum) is covered with a dense greyish micropubescence. The abdomen is blue with a silvery chequerboard effect (tessellation) (Fig. 4). *Calliphora vicina* is generally considered a spring and fall species in the temperate zones. In UK is largely present during spring-autumn period and primarily favors shady situations and urban habitats, where it is often the dominant species on human cadavers.

Figure 3

Calliphora vomitoria: Common name: Holarctic blue blow fly. Adult in lateral view (Photo S. Vanin).

This blow fly species is Holarctic in distribution, as the common name implies and it ranges from 7 to 13 mm in length. Like *C. vicina*, the thorax of this species is dark blue to black and adorned with four dark longitudinal stripes on the dorsal thorax between the wings. The thorax is covered with fine hairs and appears to have a light gray dusty coating, giving it a silver coloration. The abdomen is bright metallic blue and patterned with a silver-gray powder (Fig. 5). This species is similar in appearance to *C. vicina*, except the head appears almost entirely black. Overall, the body appears very stocky and bristly. *Calliphora vomitoria* is common and widespread all over the UK and it can be seen mainly during the spring season between April and October, it is common in wooded rural as well as suburban areas, where it prefers shaded locations.

Figure 4

Protophormia terraenovae: Common name: Birds nest screwworm fly Adult in lateral view (Photo S. Vanin).

Adult Holarctic blow flies of *P. terranovae* are 7 to 12 mm in length. This species has a dark blue to black body coated with a silver-gray powder. The abdomen is a greenish blue to blue, but with its powdery coating appears tessellated. This species is rather large in size and appears more hairy than most of the Calliphoridae (with the exception of *Calliphora* spp.). Generally it is most common during the spring months. Cool weather favors development, and this species is the most cold tolerant of all bottle flies.

Figure 5

Lucilia sericata: Common name: Sheep blow fly Adult in lateral view (Photo S. Vanin).

Historically, this species has been Holarctic in distribution, but now it is nearly cosmopolitan in range. Worldwide this may be the most common species of bottle fly, and it is most abundant in the temperate zone of the northern hemisphere. Adult sheep blow flies are 6 to 9 mm in length. This fly is a brilliant metallic blue-green, yellow-green, green, or golden bronze.

The larvae of this species can successfully develop in a wide variety of food substrates, but they are best suited to carrion. Along with other species of the genus, this fly is one of the earliest arriving fly species on human and animal remains. Commonly this fly will deposit eggs on carrion only a few hours after death. The adults prefer carcasses located in bright sunshine and open habitats; however, they will seek shaded areas of the body in which to deposit their eggs. There have been reports of this species anticipating death and laying eggs on the wounds of the dying. However, there are also reports that the larvae develop most rapidly on decomposing (not fresh) carrion. The larvae of *L. sericata* have been routinely employed in maggot therapy for the removal of necrotic tissue from wounds with great success. In UK it is widespread and fairly frequent typically in outdoor locations during summer but can persist indoors into the colder months.

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