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Biology of insect interaction on vertebrate corpse of forensic significance

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Abstract
Forensic entomology relies on natural processes occurring in insects which form evidence in solving medicolegal cases. Post Mortem Interval [PMI] calculation is a major part of forensic entomology. Insect successional wave and maggot development stages are mostly used for PMI calculation. A basic knowledge of the lifecycle of insects associated with a corpse and their succession is vital to this. The paper deals with the biology of major carrion insects of Diptera, Coleoptera, Hymenoptera and their interactions with the corpse. The insect attractants like apneumones emitting from a corpse, synomones from microbes on a corpse and various volatile compounds that form during the decaying process are discussed. Various stages of corpse decomposition and the factors affecting it are also reviewed.

Keywords: forensic entomology, insect succession, post mortem interval, diptera, coleoptera, insect attractants

1. Introduction
In forensic science, corpse analysis is conducted based on abiotic phenomenon and transformative phenomenon. Abiotic phenomenon following the end of vital functions have been a subject of thorough research for years [4]. Transformative phenomenon follows in a timeline and includes extensive modification and change in the corpse or the decomposition of the cadaver [2]. Various constructive and destructive changes occur at physical, chemical and biological levels during decomposition and these can be divided into five stages: fresh, bloated, decay, post-decay and skeletal [3, 5]. Corpse analysis in a crime scene draws out information regarding many events like time elapsed since death, mode of death, and whether the corpse have been moved or disturbed. Various methods based on body cooling, rigor mortis, changes in the eye, putrefaction, supravital reactions and contents of stomach used to determine the time elapsed since death in olden times were crude and inaccurate so the focus has been shifted to biochemical methods based on pathophysiological changes [1]. These methods demand laboratory procedures, pose difficulty in practical result utilization and inaccurate results if time after death is more than three days. Forensic entomology can give an accurate measurement even in later stages of the post-mortem interval even when other methods would fail to do so.
Often animal evidence collected from and around a crime scene may be in different stages of their life cycle as eggs, maggots, pupa, empty pupal cases, cast skins or as adults. These organisms should be considered as physical evidence in the crime scene and should be processed as any other biological material following recommended procedures for collection, preservation, transport and analysis [6]. Though many organisms including bacteria, fungi, annelids, molluscs, arthropods like millipedes, collembola, diplurans, spiders and vertebrate scavengers are potential organisms [27] in several crime scenes, the most exploited group for the same is insects. Forensic entomology gives information regarding the original place of the commitment of a crime, whether the corpse has been moved or disturbed after death, the time elapsed after the crime, the season of death in case of a reinvestigation, presence of wound which would have been obscure due to decomposition, presence of any drugs via bioaccumulation in insects. As per the circumstances, either the insect successional wave or maggot developmental stages is made used for determining the time elapsed since death [39]. The first method is employed when the corpse has been dead for between a month up to a year or more, and therefore the second method is employed when the death occurred less than one month before discovery. Though many studies have been conducted on the suitability of insects for forensic analysis less studies have been conducted on the insect biology and insect -corpse interactions in the successional wave which is dealt with in detail in the paper

2. Insect Interactions Associated with Various Stages of Corpse Decomposition
Vertebrate corpse decomposition serves as an important stage in nutrient cycling on land [26]. In nature, the decomposition process is accelerated by the action various organisms of which insects are the most dominant group. Different insects are attracted to the nutrient island formed from the decomposing cadaver at different stages of decomposition wherein each of their unique needs are met. These biological interactions in nature are exploited by forensic scientist to get information on the cadaver.
Corpse decomposition process may range in time from weeks to years depending on various external and internal factors of the corpse like the climatic conditions, temperature, access to the body, clothing, animals feeding on the corpse and the body fat. The stages of corpse decomposition are fresh, bloated, active decay, advanced decay and skeletal stage [28].

2.1 Insects in Initial stage of decomposition
The initial stage or the fresh stage lasts upto three days. Autolysis of cells leads to early post-mortem changes like

algor mortis, rigor mortis and livor mortis [5]. There is no strong odour of decomposition in the fresh stage but enough for blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*) which are the first insects (Diptera) to arrive in most cases within minutes. Carrions offer a high protein food source for both species of flies which they require for attaining reproductive maturity. Female blow flies lay eggs while flesh flies lay their first instar larva on the carrion since their larva are scavengers. The larva of both species goes through three instars feeding on the flesh and these maggot activities might not be visible from outside [30,31]. The third instar larva is a voracious feeder and seen in aggregation, this is due to clustering of eggs in nutrient-rich spaces and social aggregation which creates a larval mass effect (local heat emission) and accelerated larval growth [32,33]. The complete cycle is predictable and may take around three weeks to get completed and if the temperature is low it might take a longer period. After leaving the larval stage, the 'Prepupa' wanders away searching for suitable sites to pupate in the surrounding soil or even hair and clothes of corpse [32]. The newly emerged fly can't fly and they can also be used for PMI estimation. Adult ants (Hymenoptera) may be also seen at this stage of decomposition to feed on the flesh as well as the eggs and larvae of the flies [30].

2.2 Bloating stage entomofaunas

The Bloat Stage (4-10 days) is the swelling of the body due to accumulation of gases from microbial activity which begins from the stomach and expands all over the body. This accumulation continues till the body collapse followed by the release of the contents outside which renders the surrounding soil alkaline and thus the natural soil fauna leaves area under the body setting stage for the invasion of organisms associated with decomposition. Other changes like marbling, degloving phenomenon, discoloration of the skin is also pronounced in this stage [28]. The internal temperature of the corpse arises significantly above 50°C and it becomes a distinct habitat with respect to the external environment attracting many insects [5].

Maggots start spreading digestive enzymes, tearing tissues with their hooks in the mouth and spreading bacteria throughout the body. As the rate of decay starts increasing and the smells of the body fluids and pungent smell of ammonia spreads, creating a surge in attracted adult flies along with beetles and mites. Adult and ovipositing individuals of *Calliphoridae, Sarcophagidae* and Muscidae could be seen. Muscidae are considered to arrive at cadavers and carrion just after the blow flies and flesh flies or as primary colonizers in some cases to lay eggs and the larvae feed on dead tissue or on other dipteran larvae [35].

The members of the *Piophilidae* (Skipper flies), and *Fannidae* whose larvae feed on carrion are also occasionally seen during this stage. Species of *Staphylinidae* (rove beetles), *Silphidae* (carrion beetles) and *Cleridae* (Checkered Beetle) including the first species of Coleoptera also arrive which predate on dipteran larva. Beetle species of family Histeridae are often hidden beneath remains. Then parasitoid wasps (Hymenoptera) come and lay their eggs inside dipteran maggots and pupae [36,37].

2.3 Active Decay Stage and Insects

In the active decay (10-20 days) stage, the body turns to be flattened with a creamy consistency flesh, there comes a strong smell of decay and the exposed body parts are black in colour. Liquefaction of soft tissues starts with the presence of froth follows [28]. The body fluid that drains into the soil becomes food for some microorganisms, mites and insects. The number of Dipteran larvae will be maximum at this point often spilling around the body [38]. One of the major indicators for this stage is the mass migration of Dipteran larvae for pupation [5]. Adult flies are usually found in fewer numbers and are not usually found to be mating. The first members of *Sepsidae* (ensign flies) arrives and some Coleoptera including rove beetles and *Histeridae* may be present [5].

2.4 Entomofauna of Advanced Decay Stage

Around 20 -50 days, the remaining flesh removes off during this time exposing bones and some tough tissues like hair, cartilage are left. The odor of decaying smell or cheesy odor weakens in this stage and a strong smell of butyric acid develops which attracts new organisms. The Cheese fly eats up all the remaining flesh which is moist. Parasitoid wasps, their larvae and predator beetles are present at this stage also. Necrophagous species will arrive to feed on beetles and flies and to consume dead flesh. Rove Beetles are predatory on carrion feeders. Adult *Dermestidae* (skin beetles) may be common, whereas larvae are not usually found. The soil organisms increase in number and diversity during this stage. Another major event is the mass migration of the third instar larva of dipterans for pupation. There is a clear faunal succession of Coleopterans over Dipterans [28,5].

2.5 Insects in Dry Decay Stage

Over 50 days of death, bone exposes and no flesh remains. The highest number of species is noticed at this stage of decay. Animals like Tineid moths arrive to feed on the hair as the body is dry now and mites and collembola arrive to feed on micro-organism like bacteria present. Scarab beetles arrive when the body is totally dry. *Dermestidae* also occur at this stage, where members of *Histeridae* may also arrive to feed on *Dermestidae* larvae. Gradually, soil pH restores and soil fauna like Centipedes, millipedes, isopods, snails and cockroaches are seen around the corpse [7-21].

3. Insects and Carrion cues

Odours arising from corpse due to microbial action are the major attractants that aids insects to locate the carrion. Sarco-saprophages like *Sarcophagidae, Calliphoridae, Muscidae* and *Dermestidae* feed on decomposing flesh and imbibe in the blood and body fluids. The *Sarcophagidae and Calliphoridae* locate the corpse by apneumones emitted from the corpse that prompt them to adult feeding, mate finding and breeding. Apneumones are made up of a complex of ammonia and Sulphur rich compounds [39]. Oligosulphides and other volatile sulphurous compounds produced by bacterial activity on carcases are known attractants of *Calliphoridae* [29]. Gases like methane, ammonia, putrescine and cadaverine along with other chemicals attracts flies to corpses. Bacteria acting on corpse also send signals that attract blowflies. Some of the male dipteran flies use their pheromones to advertise the corpse- a rich protein source- to attract females [39]. Coprophages like *Scarabaeidae, Muscidae* are attracted to herbivores' rumen material. Dermatophages like *Dermestidae, Tineidae* feed on dried skin, hair, ligament and bones. Predaceous species like Histeridae and *Staphylinidae* feed on Dipteran larvae.

Different carrion insects are attracted to various biological, chemical and physical changes the body undergoes in the process of decomposition.

4. Forensic Interpretations Using Entomofauna

Forensic entomology uses the biology of insects to solve medico-legal cases. Insect successional wave can be used to analyze PMI as various levels of corpse decomposition involve different organisms. Flesh flies and blow flies are among the first insects to colonize on the corpse and have a definite lifecycle. This maggot developmental stages can be utilized to calculate PMI. Migration of dipteran larva for pupation is an indicator of advanced decay stage. Dipterans prefer moist epithelial linings of body openings to lay eggs unless there is the presence of an open wound. So even at stages when body tissue is damaged to such an extent that the wound is undiscoverable, we can locate a wound by looking for the pattern of maggot colonization [5].

Every ecosystem has a distinct and unique fauna, the presence of which can be used to know whether the body has been moved. The nativeness of fauna on a corpse can also give a hint to where the crime occurred. Recovering a Gunshot residue or presence of any toxic substance can be tedious from a decayed body. But since maggots bioaccumulate, they can be ground and analyzed to detect these [39].

Wasp host preferences and seasonal occurrences can reveal if the corpse had been disturbed. Foraging behaviour of adult wasps can be used to locate concealed bodies [36]. Muscidae are more attracted to bodies with faecal contamination. Some species of Muscidae colonize wounds of living people. The period of colonization of a wound can be analyzed and this would shed light on whether the person was abused or neglected. High diversity of Muscidae among arthropods are the major ones to get attracted towards the decomposing carrion in rural and forest areas of Central Europe [22, 40].

5. Factors Affecting Corpse Decomposition

The factors affecting the process of decomposition is extremely important in death investigations. These may be intrinsic or extrinsic factors. Intrinsic factors include age, cause of death, the integrity of the corpse etc. In the case of fetuses and newborns, due to the sterile condition of the body, the rate of putrefaction is slower. Obese corpses decompose rapidly due to the excessive fluid found in tissues and since they can retain more heat insect colonization is accelerated [18].

Extrinsic factors include temperature, climate, access to body, clothing, animal predators etc. Temperature is a major factor for bacterial growth, mainly the temperature between 25°C and 35°C. Low temperature slows down the activity of blow-flies and their colonization. High temperature results in large maggot masses on carrion. Mummification of corpse occurs when the body had been exposed to dry and windy climatic conditions. Dryness causes less bacterial growth due to the less availability of the nutrients. If the corpse is in a water environment, decomposition slows down due to the soaking up of tissues [22, 18].

Access to the body can limit the number and type of insects getting into the body to feed and lay eggs. Clothing supports cooling of the dead body which speeds up putrefaction process. Clothing also acts as a barrier between the body and insects that might result in the decomposition delay. Darkness, cold, and rain limit the quantity of insects. Fish, crustaceans, aquatic insects and bacteria would be found in a corpse found in water. Similarly, insects getting attracted to dark and light exist, so the light conditions where the body lies is a major factor. Time of death could be altered when Scavengers and carnivores such as wolves, dogs, cats, beetles, and other insects feed on dead bodies. Therefore, the decomposition process speeds up. Corpses having wounds, decompose because of easier insect access [22, 23,18].

Bodies that are covered in pesticides or a neighborhood surrounded in pesticides could also be slow to possess insect colonization, thus slowing down the speed of decomposition. Drugs present in the body can affect the speed of insects degrading the corpse as cocaine sped up and arsenic drugs slows down the degradation. The effects of medicine and toxins also affect on the insect colonization [22, 23].

6. Limitations of Forensic Entomology

Like any other technique, forensic entomology also has its limitations. When estimating PMI based on the duration of the insect activity with the corpse, each case is unique in presenting variables that may affect the development rate of fly larvae and decomposition rate of the corpse. The variables like include geographical region, temperature, humidity, season and presence of toxins in the corpse must be considered in data analysis for accurate interpretations [15].

The temperature of the death site is a vital factor [14]. Extreme temperatures hinder corpse decay and slow down insect development which may lead to false results. Forensic entomology is commonly used when the insects are abundant as in spring, summer, and fall. In winter insects are found less [18].

If the body was frozen after death for a period before being placed outside, insect interaction starts with the body from the time it was kept outside, so it can be misleading towards the date of crime [22]. Many insects might be not found if the body is buried deeply. Most insects will reach down to the body even if the corpse is buried. As the main aim of the burying is hiding the corpse, the burying would not be so deep. So, insect evidence can still be used. If the body is wrapped, insect activity might not take place [1]. Insect species, activities, colonization time varies according to the region. The presence of drugs may alter the insect cycle. As many dipteran species possess some resemblance, difficulties in identifying species occurs, particularly during the larval stage. Correct identification can also be support by the introduction of foreign species from all over the globe [24].

7. Conclusion

Forensic entomology is preferred for cases where the time since death has exceeded 72hrs and as a feasible technique in comparison to other biochemical, histological or molecular tests [22].

Insects also can provide other important information a few crime or victim and also about the person's life before death. Insect behaviour also can be helpful in knowing the incidents that happened around the time of death. Forensic entomology has stood for ages as an efficient technique in estimating PMI [Post Mortem Interval] since it relies on the natural action of biological decomposition in the environment. Further studies into the biology and

interaction of insects with a corpse can increase the estimation accuracy.

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