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## TABLE OF CONTENTS

1. Learning Outcomes
2. Introduction
3. Forensically relevant Insects
4. Current Perspectives of Forensic Entomology
5. Entomotoxicology
6. Equipment and Tools for Evidence Collection at Crime Scene
7. Common sites for sampling
8. Evidence Collection: Practical Guidelines
9. Summary

## 1. Learning Outcomes

After studying this module, you shall be able to know about-

- What is forensic entomology and their divisions
- What are various roles of forensic entomologists
- The factors which affect the growth rate of insects
- How the Post-mortem interval can be determined with forensic entomology.
- Equipment and Tools for Evidence Collection at Crime Scene

## 2. Introduction

**Forensic Entomology** is the use of insects and other arthropods that feed on decaying remains to aid legal investigations.

### Divisions of Forensic Entomology

- Urban: It concerns with litigations arising from Bedbugs and Termites affecting manmade structures and other aspects of human environment.
- Stored-Product: It covers the litigations arising from grains and other food contamination by insects.
- Medico-Legal: It involves the analysis of necrophagous insects to gain insight into Time of Death (TOD).

### Role of Forensic Entomologists is

- 1) Identification of insects at various stages of their life cycle.
- 2) Collection and preservation of insects as evidence.
- 3) Determining an estimate for the post-mortem interval or PMI (the time between death and the discovery of the body) using factors such as insect evidence, weather conditions etc
- 4) Testifying in the court to explain insect-related evidence found at a crime scene.

## Historical Perspective of Forensic Entomology

The earliest known reference about Forensic Entomology dates back to 13th century in a Chinese manuscript 'The Washing Away of Wrongs' written by Sung Tzu. The first application of Forensic Entomology in determination of PMI in a child death in France by Dr Bergeret d' Arbois was done in 1850. Jean Pierre Megnin, a French veterinarian, did revolutionary work to give the theory of predictable waves, or successions of insects onto corpses. Bernard Greenberg is regarded as the father of modern Forensic Entomology.

### 3. Forensically relevant Insects

Insects are ubiquitous in nature. Even if we don't see them, they are likely to be involved in crime scene. Entomologists can help forensic pathologist in determining TOD, by looking at the insects that are feeding on and around the body.

**Flies:** Flies are the first one to get attracted towards the dead bodies. Carrion Flies includes Calliphoridae (blow flies), Sarcophagidae (flesh flies) and Muscidae (house flies) and belong to order- Diptera. These are the most wide-spread and accurate insects for determining TOD.

**Beetles:** These are often found on old cadavers, or in dry conditions. Beetles includes Silphidae (Carrion beetles), Staphylinidae (Rove beetles) and Dermestids (Carpet beetles) and belong to order- Coleoptera.

**Ants:** These generally consume smaller cadavers and belong to order- Hymenoptera.

All these insects belong to the Class- Insecta of the Phylum 'Arthropoda'.

### 4. Current Perspectives of Forensic Entomology

#### (1) Time of death (Larval development):

The first approach in estimation of time since death is the estimation of maggots developing in the body. For this purpose, the knowledge of life histories of flies of families Calliphoridae (blowflies), Sarcophagidae (flesh flies) and Muscidae (Houseflies) has an important application in forensic medicine.

Fly Life Cycle can take anywhere from few days to several months depending upon several factors. For many fly species, the precise timing of their life cycle has been calculated and can be used to calibrate time of death or at least time of infestation.

**Blow Fly Metamorphosis:** Blow flies are the Gold Standard forensic indicators. These are most useful in estimating TOD. These are the ones that come in first, immediately after the body is dead and start to decompose. They have an incredible sense of smell. They have a complete life cycle which consists of egg, larva, pupa, and adult stages, known as Complete Metamorphosis.

### **Blow Fly Life Cycle:**

1<sup>st</sup> – Adult flies lay eggs on the carcass especially at wound areas or around the openings in the body such as the nose, eyes, ears, anus, etc.

2<sup>nd</sup> – Eggs hatch into larva (maggots) in 12-24 hours

3<sup>rd</sup> – Larvae continues to grow by feeding on corpse and molt (shed their exoskeletons) as they pass through the various instar stages

1<sup>st</sup> Instar stage– is 5 mm long after 1.8 days

2<sup>nd</sup> Instar stage – is 10 mm long after 2.5 days

3<sup>rd</sup> Instar stage– is 14-16 mm long after 4-5 days

4<sup>th</sup>– The larvae (17 mm), once it gets satiated develop into pupa after burrowing in surrounding soil.

5<sup>th</sup> – Adult flies emerge from pupa cases after 6-8 days.

Insects have hard exoskeletons, so when they grow they have to shed their skin. Larvae are all about growing and getting bigger, so they grow as fast as they can and molt when they need to (when their skin gets too tight). Once a larva reach full size, it goes through metamorphosis, a complicated rearrangement of body parts and tissues that leads to the development of adult characteristics. Therefore, insects have so many stages in their life cycle.

### **Factors that affects growth rate of insects are:**

- 1) Temperature: Higher the temperature, faster the insect will grow and develop into an adult.
- 2) Food Quality: Eating rich, nutritious food help larvae grow faster.
- 3) Oxygen Levels: Increasing oxygen concentration increases growth rate of insects.
- 4) Day Length/Season: Many insects co-ordinate their developmental cycles with the seasons.

## **(2)Time since Death (Faunal Succession):**

Faunal Succession is the term used to describe the transition of colonising species from one insect species to another, through the different stages of decay. Succession is a less precise science than PMI relying to some extent on the forensic entomologist's prior knowledge of species over a wide range of families and is also reliant on knowledge of local fauna to assess the significance of the presence or absence of a particular species. The faunal succession can vary significantly between individual cadavers and the arrival times of certain species are influenced by local factors.

Since insects invade bodies in "waves". Estimation of time since death requires the ability to identify each species in all stages of their life cycles and knowledge of the time occupied by each life stage under various conditions.

### **Typical Succession of Insect Colonization:**

Flies arrive within minutes or hours of death as the body begins to give off the odours of decomposition. Carrion beetles arrive within few days and dig underneath the body and begin feeding. Carpet beetles arrive once the corpse is dried out sufficiently and begin to consume skin and hairs.

### **Factors affecting insect colonization:**

- 1) Weather: when it's raining insects are less active, so colonization is slowed.
- 2) Temperature: during cold, when temperature is less, insects are less active and grow slowly.
- 3) Burial/Exposure: Even a partially buried corpse decomposes slowly; degree of exposure also affects how accessible the body is for colonization.
- 4) Location: bodies in dry environment will desiccate before insect colonization; bodies in wet places will attract a different set of insects like aquatic beetles.

### **Temperature Dependent Development:**

Insects are cold blooded organisms. Rate of development is more or less dependent on ambient temperature. For each species there is a threshold temperature below which no development occurs. As temperature rises above this threshold, a certain amount of time is required for the insect to pass through each life stage. Because this heat is accumulated as "thermal units," it can be calibrated and described as "degree-days" or "degree-hours".



### **Problems with the faunal succession:**

The differences in weather, location, season and the extent of interference by other animals makes it very difficult to predict by a forensic entomologist what species will arrive when.

### **Post Mortem Interval (PMI):**

PMI is the time elapsed since death. Estimation of accurate PMI can help to identify both the criminal & victim by eliminating suspects. The estimated age of an immature insect that has fed on a body provides a minimum PMI because; offspring are not deposited on a live host.

Basic assumptions that a Forensic Entomologist make before calculating TOD are:

1. Most homicides occur at night, under cover of darkness when flies are presumably inactive.
2. Flies will begin ovipositing as soon as they discover the body.
3. Faunal succession in and under the corpse will follow a predictable pattern.
4. Weather conditions recorded at a site distant from the death scene reflect the conditions at the scene.
5. Ambient air temperatures are the major factors influencing the rate of maggot development.

### **Estimating Post mortem interval:**

Collect & preserve a sample prior to use in estimating PMI. Rear others to adult stage for identification. The moment of preservation is the point in time from which you calculate backwards to death. Use known developmental times at constant temperatures to estimate PMI.

### **(3) Place of death:**

Since some of the species appear in a particular geographic area or a particular locality, from the examination of the insects, the particular geographic and ecologic place can be known.

#### **(4) Determine neglect of patients or children:**

Cases where wounds and bedsores arising out of physical abuse or neglect become infested with insects, forensic entomology may be useful.

#### **(5) Manner of death:**

Actual event that led to death is especially important in advanced putrefied bodies. If putrefied body has wounds infested with insects, it could be Homicide/Trauma. In cases of death due to suspected poisoning, by using arthropods in a corpse or at a crime scene, investigators can determine whether toxins were present in a body at the time of death.

### **5. Entomotoxicology**

Recovery of heavy metals from bodies in the 1970s and 1980s led to the development of forensic research at the boundary between entomology and chemistry: this became known as entomotoxicology. Entomotoxicology is the analysis of toxins in arthropods (mainly flies and beetles) that feed on carrion. This technique is a major advancement in forensics; previously, such determinations were impossible in the case of severely decomposed bodies devoid of intoxicated tissue and bodily fluids.

Entomotoxicology can be divided into two main categories:

1. Entomological analysis of substance-induced changes to insect development rates with subsequent effects on PMI estimation.
2. Toxicological analysis of drugs within insects: substances may be more easily detected because of storage excretion and bioaccumulation in insect metabolism, resulting in concentrations of substances higher than in the surrounding tissues.

Substance-induced changes can be caused by substances external to the body (for example fire accelerants, oil, paint, organophosphate poisons) or those internal, such as ingested or injected narcotics, alcohol consumption or pharmaceuticals. Most important in this context is the presence of pharmaceutical or narcotic drugs in the deceased and the differential affect these can have on insect growth, depending on where in the body these drugs and their metabolites concentrate and whether some (or all) of the maggots have fed on those tissues.

We know for example that over-the-counter medicines such as paracetamol, is one of the foremost drugs used in suicide typically resulting in a level of 250mg/kg drug to body weight concentration. At this concentration, growth period of blow-fly larvae is differentially increased by up to four days. This critical piece of evidence suggests that the growth pattern diverges from the normal linear relationship we more commonly use.



A commonly prescribed drug for anxiety and sleep disorders, Nordiazepam (and Oxazepam, its metabolite in the human body) cause an increase of at least 24 hours, especially on or around day 4 of drug accumulation. Amitriptyline (a tricyclic antidepressant) prolongs the post feeding and pupa stages, resulting in death during either, thus preventing complete development to adulthood. Another prescription drug, Zopiclone (for the short term treatment of insomnia) prevents pupariation, the process by which blow-fly maggots turn into pupae. Acting on the peripheral nervous system, it has the effect of seriously disrupting the pupation process.

Among the narcotics, cocaine seems to have little effect, but ecstasy (MDMA, i.e. 3,4-Methylenedioxymethamphetamine) speeds up development and reduces both the larval and pupal growth periods, sometime resulting in insect death; while methamphetamine on its own appears to have little effect other than possibly terminating insect growth at the pupa . On the other hand, human lethal dosage of morphine results in a 24-hour increase in development, while heroin at human lethal doses, increases the larval growth period and can dramatically alter the pupa duration by 18-36 hours. Organophosphate poisons, such as Malathion, do not necessarily result in death of the flies breeding in bodies containing a human lethal dose; instead Malathion extends the larval growth period by about 72 hours.

Clearly, such alterations of the growth in different stages of the insect life cycle impact on the assessment of PMI. It therefore becomes critical that the entomologist is informed of drug or alcohol usage in cases requiring PMI assessment. In cases where the body is extensively decomposed and tissues are no longer available to analyse, it may be possible to process the gut contents from particular developmental stages of insects collected from the death scene, that have fed on the body. This may be the only recoverable evidence of drug presence in the decedent.

### **Entomological DNA Assessment:**

Species determination and assessment of human DNA from the crops of haematophagous (blood feeding) species are two aspects of DNA studies particularly important in the forensic context. The insect crop is the portion of the digestive tract, forming the bulk of the foregut and capable of considerable expansion. It is distal to the oesophagus, serving as a storage vessel, retaining undigested food prior to its movement through the valve into the midgut for digestion.

Purified and PCR amplified human DNA can be extracted from the guts of species feeding on human remains. In addition, within a given post-feeding period, human DNA remains viable and can be isolated from blood-meals of haematophagous insects including: lice, bed-bugs, assassin bugs, mosquitoes, fleas, non-insect arthropods such as ticks and mites, and other invertebrates, such as leeches. Species with limited dispersal (such as bed-bugs) and longer post feeding storage of crop contents offer the most promising results.

## 6. Equipment and Tools for Evidence Collection at Crime Scene

- Fine paintbrush for collecting eggs
- Spoons for collecting maggots
- Fine and medium forceps (for collecting adults and the more fragile immature insects)
- Hand net for catching flying insects
- Ethanol (70%) for storing dead specimens
- Protocol sheets for writing down what specimens were collected
- Labels
- Vials and storage boxes of different sizes for preserving living and dead insects
- Sawdust or tissue paper for handling eggs and living larvae in vials or storage boxes
- Shovel and Robust plastic bags for soil samples and leaf litter
- Thermometer for measuring the body and ambient temperatures, as well as the larval mass temperature
- Camera for photographic evidence

## 7. Common sites for sampling

- a. The natural orifices and eyes
- b. Traumatic wounds
- c. At the corpse–substrate interface and under the body
- d. In the folds of clothes and pockets, shoes, socks, etc.
- e. From the carpet, bag or material in which the body might have been wrapped
- f. From the plastic body bag in which the corpse or the remains have been enclosed for transport to the place of autopsy and storage

## 8. Evidence Collection: Practical Guidelines

Insects can be collected from a corpse or its surroundings in three groups, namely maggots, insects from soil and other insects.

**Maggots:** Maggots are immersed and killed in very hot (almost boiling) water and transferred to a solution of acetic alcohol that is 3 parts 70% alcohol and 1 part glacial acetic acid.

Killing in hot water over immediate immersion in preservative has two advantages:

- (i) Death of maggot is instantaneous so exact moment of death can be known
- (ii) Hot water kills maggots in a "relaxed state" in which they are fully stretched. Measurements of maggots can then be compared with measurements from experimental work

If maggots are immersed in preservative to kill them, they will shrink and any measurements will be difficult to interpret.

Rearing of maggots can be done, i.e. they can be kept alive on some meat or liver and reared to adult stage.

Any puparia either empty or with the pupae inside them should be preserved in a specimen tube.

All the containers carrying live specimens must be perforated for gaseous exchange, so that live organisms get the proper oxygen.

Collect specimens from everywhere on the corpse. Try to collect specimens of every type, shape and size. Sample size will vary depending on the number of larvae found (from all the larvae, where fewer than 100 are available, to 1–10% of the larvae, where thousands are available).

**Soil insects:** The soil samples should be collected in specimen bags from which insects can later be extracted in the laboratory. Each soil sample should be as much as would fit into a 'pint' bottle. Soil should not be compacted.

Storage in ethanol ensures that a later DNA analysis for identification is still possible. This may be necessary if a morphological identification is not possible

## 9. Summary

The use of insects in criminal investigation is well established. Usually, the knowledge and experience of a professional entomologist is required to establish species identity and to understand and explain the biology associated with those species. Consequently, the potential use of insects in the forensic context is extensive.

The most frequent application of insects to criminal investigation is the estimation of a post mortem interval (PMI): the minimum period since the first eggs were laid. Considerations affecting this estimation include the ambient temperature, weather, time of day, presence of drugs, amount of clothing or attempts to conceal or destroy evidence, indoors or outdoors, to prevent investigation.

Other important applications of forensic entomology include the assessment of drug use and the extraction of human DNA from the crops of haematophagous species. Significant developments are expected in both these areas of research.

In the wider forensic context, insects are used in many aspects of forensic investigation, sometimes of less medical significance such as: veterinary cases, misapplication of pesticides, conservation, import violations, food contamination & insurance disputes.

Forensic entomology is an emerging field in forensic sciences. It has become an important tool in criminal investigations. Increased instances of forensic entomologists being involved in criminal investigations, as part of the forensic team, have necessitated the need for an increase in awareness of emerging sciences like forensic entomology and its applications.

“The insects will tell you everything, people lie, but insects don’t lie”