Small Size, Big Impact: Insects for Cadaver Examination

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Abstract

The fields of forensic entomology and entomotoxicology are developing areas of interest in this new era. These two fields are fascinating and are capable of accruing curiosity among researchers. Forensic entomology allows the investigator to estimate the post-mortem interval (PMI), and entomotoxicology enables us to find out if the decedent was intoxicated before death when body tissues are not available for toxicological analysis. The province of forensic toxicology divides toxic substances into special categories according to their chemical and analytical properties. This system of classification helps the investigator in identifying the actual toxic substance present in the biological or entomological samples. The present review is aimed at these two parameters while also focusing on the factors affecting the life cycle of insects feeding on the cadavers.

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3.1 Introduction

Small insects usually cause a big effect in the field of forensic science. Whenever the insects are encountered in any kind of crime scene or involved in legal issues, they are dealt with under forensic entomology, a specialized branch of forensic science. Forensic entomology is a vast field, and both entomology and legal science interact in this particular province. Usually, insects are found inside packaged foods, poisoning cases, termite damage, murder-related cases, and other such medicolegal cases. Cases of negligence in hospitals or due to myiasis might crop up. Pets can also get infected from myiasis due to negligence [1]. Myiasis is an infection caused by fly larvae and is more severe than the other general infections as the maggots are formed in a part of the body and they start feeding on the area. Insects generally come from the open fields and cause such infections.

Many disputes arise when insects and/or insect parts are found in stored products and issues of negligent preservation, packing, etc. crop up. Larvae and flies are commonly found in juice cans or other packaged food products. Insects are sometimes planted in restaurants and other eating places to defame a business house or to blackmail for money/take revenge, etc.

Insects are of great value in criminal cases like violence and murder and also in those cases where the cause and time since death are not known. Medicolegal entomology is concerned with insects which can be used as evidence to solve a crime. It also helps in estimating the minimum post-mortem interval (PMI) [2–7] and any drug intoxication before death [2, 3]. The PMI estimation depends on many factors like temperature, exposure of the body to the external environment, decomposition rate, geographical location, humidity level, and season [8, 9]. Many cases have been reported where the time of death is unknown, and insects of forensic importance have proved to be of great value. When only skeletal remains are found, then maggots can be used for drug analysis [10].

3.2 History of Forensic Entomology

Several species of insects get attracted to a cadaver at different time intervals. These insects feed, reproduce, or live according to the condition and preference of the cadaver [11–22]. The study of forensic entomology was first started and documented in China by the lawyer and investigator of the death of Sung Tzu in the thirteenth century [23, 24]. He described a case of stabbing in an open field of rice with the help of blowflies. After that, many painters, sculpture artists, etc. started observing the decomposition stages of various corpses. Many paintings depicted the maggots eating the body after death. These articles show the insect-based decomposition of the body perfectly [25]. A poem was written by the French poet Charles Baudelaire, titled *Une Charogne*, which depicts the decay of human bodies, and even the sound caused by maggots on cadavers was also mentioned perfectly [26].

In the year 1767, a biologist, Carl Von Linne, stated that as little as three flies can destroy an adult horse with as much rapidity, as an elephant can do [27]. A mass disaster occurred in France and Germany between the eighteenth and nineteenth centuries, after which, for academic purposes, the graves were exhumed. Investigators found that the dead bodies were being eaten by insects of different species. One of these investigators, Dr. Orfila, observed that the insects play a huge role in the whole decomposition process of the cadaver [28, 29]. A French doctor, Bergeret, gave the first-ever report of forensic entomology in the year 1855 [30]. He estimated the post-mortem interval (PMI) from the life stages of flies from two species, i.e. blowfly pupae and larvae of the moth, feeding on the dead body of a child. There was a mistake in his case study as he supposed that female flies only lay their eggs in the summer season and the larvae will grow into a pupa in spring, which would hatch in summer. Because of this mistake, he thought that two generations of insects were eating the body and that the post-mortem interval was more than a year.

A German doctor named Reinhard gave a systematic report of forensic entomology in the year 1881 [31]. At that time, he was working on exhumed bodies. He found phorid flies, which were identified by a taxonomist Brauer. He concluded in his case study that the bodies in graves were older than 15 years. These phorid flies are now commonly known as coffin flies.

A sub-speciality of forensic entomology, forensic entomotoxicology, is a more advanced and rapidly developing field. This field is concerned with the maggots that feed on the cadaver of a person who has consumed drugs, etc. The first case of entomotoxicology was reported by Beyer and his colleagues in 1980. They used maggots for drug analysis when no tissues were available [32]. This field becomes very important when the body becomes skeletonized, either completely or partially. When maggots feed on such a cadaver, the drugs present inside the body transfer to maggots' gut. These drugs cause an effect on the development rate of insects, creating problems in estimating the post-mortem interval (PMI) [33–36].

3.3 Flies in Direct Relevance with the Corpse

Many different species of flies get attracted to the corpse at different intervals of time. These flies are generally from order Diptera, [32, 37–68], Coleoptera [69, 70], Hymenoptera [70–72], and others [70] (Table 3.1).

Different facts can be revealed while examining the insects scavenging on a cadaver, of which time since death estimation and drug detection are of most forensic value. Different insects have different life cycle durations, and so identification of the insect species needs to be done before any other examination can be undertaken. Blowfly is the most common to be found on a dead body, but other flies and mites can also be found. The job of a forensic entomologist is not an easy one, they have to examine insects that cause diseases like myiasis, and other skin and organ injuries can also occur. Sometimes, the drugs are ingested by maggots, and even the dead maggots can also cause severe problems to the health of the workers.

Table 3.1 Showing different insects that are generally found on the corpse

S. No.	Order	Species/family	Reference
1.	Diptera	*Calliphora vicina*	[33, 44–51]
		Lucilia sericata	[42, 52–59]
		Chrysomya megacephala	[60]
		Chrysomya albiceps	[48]
		Chrysomya putoria	[48]
		Megaselia scalaris	[61]
		Phormia regina	[62]
		Cochliomyia macellaria	[32]
		Calliphora stygia	[63, 64]
		Protophormia terraenovae	[51]
		Chrysomya ruffifacies	[65]
		Piophila casei	[66]
		Calliphora dubia	[67]
		Hermetia illucens	[68]
2.	Coleoptera	*Dermestes maculatus*	[61]
		Dermestes frischi	[35, 55]
		Thanatophilus sinatus	[35, 55]
		Staphylinidae family	[70]
		Scarabaeidae family	[70]
		Carabidae family	[70]
		Histeridae family	[70]
		Silphidae family	[70]
3.	Hymenoptera	Mymaridae family	[72]
		Formicidae family	[71]
		Apidae family	[70]
		Halictidae family	[70]
		Mutilidae family	[70]
		Vespidae family	[70]
4.	Hemiptera	Coreidae family	[70]
		Gelastocoridae family	[70]
5.	Blattodea	Blattidae family	[70]
6.	Dermaptera	Forficulidae family	[70]
7.	Lepidoptera	Hesperidae family	[70]

3.4 Importance of Insects in Criminal Investigation

3.4.1 Estimation of Post-mortem Interval (PMI)

Whenever the remains of a human body are found, the main question that arises is the estimation of the post-mortem interval. Most of the time, the body may be found in a partial or completely skeletonized condition, and the post-mortem interval is hard to estimate without the help of insects [73–78]. Generally, the insects found on the dead body can be either of 1-day-old infestation or even up to 1 month. The growth rate of the insects always depends upon climatic conditions. This method of estimating the post-mortem interval can only give a rough estimate without much accuracy because the lifecycle of insects is greatly influenced by climatic conditions [79].

The basic protocol to be applied for estimating PMI from insects is to identify the insect and its life stage. The first method generally used to estimate PMI from insects is the life cycle observation, and the second method is to observe the succession pattern of the insects. The species recognition involved, arrival time and the pattern of succession of adult insects on the cadaver, and the information of their rate of development can give an estimation of the death time. Mostly, experimental data obtained from animal cadavers are utilized to estimate the PMI and to apply the findings to real-life investigations concerning human remains [76].

3.4.2 Drug Detection from Insects

The pharmacokinetics of drugs of abuse or prescription drugs in insects depends on the developmental stage and feeding activity of different species. Bioaccumulation has also been observed in parasitoids, predators, and omnivorous species. In investigations related to entomotoxicological samples, species of insects belonging to Coleoptera (beetles) and Diptera (flies) are generally recommended because they come first to attack a corpse and are commonly encountered in crime scenes. Blowflies from order Diptera are forensically important insects because they attack first and deposit their eggs on the corpse within minutes following death. Blowflies identify corpses mainly through the natural odour of decomposing tissues. Other insects like Sarcophagidae (flesh flies) from order Diptera generally reach after the blowflies and houseflies (Fig. 3.1).

The forensic toxicologist generally analyzes the biological and non-biological samples recovered from a crime scene to investigate the cause of death. In day-to-day casework, tissue and body fluids are available as samples. But in cases like unknown dead bodies, which are recovered after a long time following death, no conventional soft tissues and fluids are available. Carrion feeding insects, dipteral, and other arthropods are used as alternate specimens for toxicological investigation [3, 80]. Dayananda R et al. put forward various advantages of knowing these flies and beetle's life cycle, distribution, and ecological behaviour in solving the legal matters [81].

World Drug Report 2020 points out that worldwide, about 0.5 million deaths are attributable to the use of opiates, cocaine, cannabis, amphetamine-type stimulants, and new psychoactive substances (NPS) [82]. In this context, Goff M. L. et al. highlighted the importance of using chemical and instrumental techniques for entomological samples in determining the cause of death in drug overdose cases [83]. Table 3.2 demonstrates several studies that, despite being cumbersome work, entomological evidence is proved as a valuable tool for toxicological analysis of

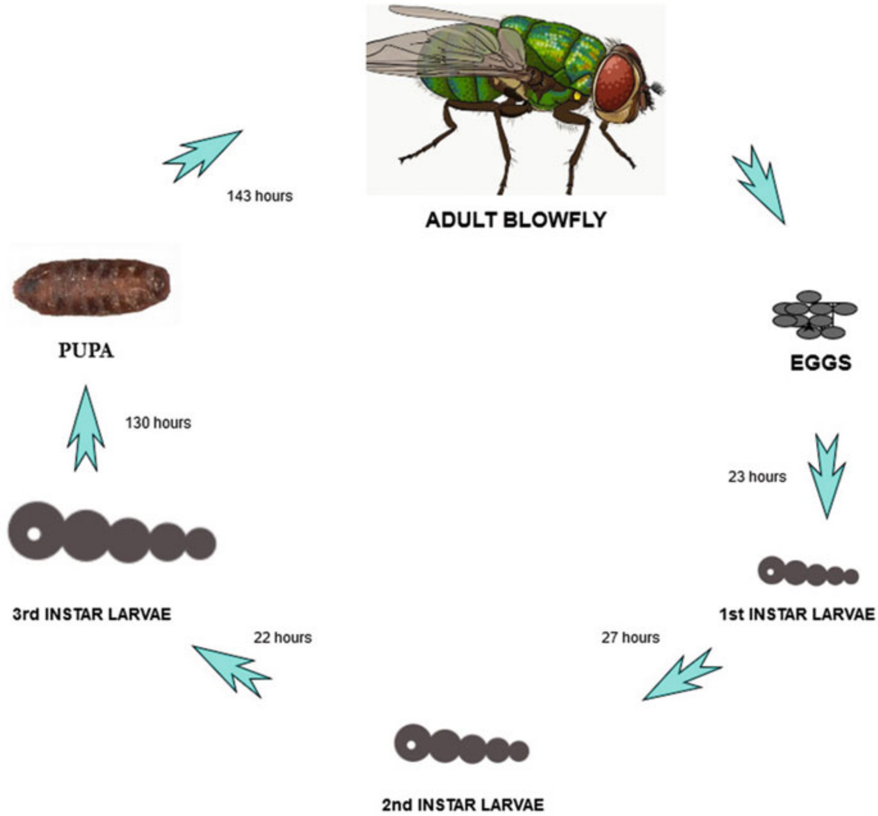

Fig. 3.1 Life cycle of blowfly [75]

numerous drugs of abuse and psychotropic substances overdose cases. Many flies and beetles belonging to Calliphoridae and Sarcophagidae families are used frequently in the determination of drugs and overdose of other prescription drugs and [33, 52, 63, 84–90] pesticides [60, 91] as well as in ante-mortem ethyl alcohol poisoning cases [92, 93] by identifying the ethyl sulfate as a biomarker of ethyl alcohol.

This identification of drugs and pesticides from larvae, pupae, and imago of blowflies and beetles needs an interpretation of chemical and instrumental setup such as TLC, GC, GC-MS, LC-MS, LC-MS-MS, HPLC, and immunoassays [94].

In a case study, the toxicological analysis of human tissues, including blood, and the different fly species /maggots collected from several sites on and in the body showed the presence of drugs in all samples. (See Tables 3.3, 3.4, and 3.5.)

Table 3.2 Studies showing the results of detection of drugs from specimens of blowflies and other arthropods

S. No.	Entomological evidence	Detected toxic substance	Technique used	References
1.	*Calliphora vicina*	Amitriptyline, temazepam, and trazodone + trimipramine	GC-MS	[33]
2.	Lucilia sericata	Barbiturates, opiates, cocaine, clomipramine, amitriptyline, nortriptiline, levomepromazine, and thioridazine	Immunoassay and GC-MS	[52]
3.	*Protophormia terraenovae*, *Calliphora vicina*	Morphine	Radioimmunoassay	[84]
4.	Chrysomya albiceps, *Chrysomya putoria*	Diazepam	GC-MS	[85]
5.	*Calliphora vicina*	Nordiazepam and its metabolite oxazepam	HPLC and LC-MS-MS	[86]
6.	*Chrysomya albiceps*	Amitriptyline, citalopram, and morphine detected. Diazepam—not specified	GC-MS and LC-MS-MS	[87]
7.	*Calliphora vomitaria*	Methamphetamine	GC-MS	[88]
8.	*Calliphora stygia*	Morphine	TLC, immunoassay, and HPLC	[89]
9.	*Lucilia sericata*	Codeine and norcodeine were detected, but morphine was detected only in treated meat	LC-MS	[90]
10.	*Calliphora stygia*	Morphine	Flow injection analysis, chemiluminescence detection coupled with HPLC	[63]
11.	*Calliphora vomitaria*	Endosulfan detected only in meat spiked	GC-MS	[91]
12.	*Chrysomya megacephala*	Malathion	GC	[60]
13.	*Calliphora vicina*	Ethyl sulfate larvae and puparia as promising biomarkers for acute and chronic alcohol abuse	LC-MS-MS	[92]
14.	Calliphoridae and Sarcophagidae families	Amphetamine and alcohol	GC-MS	[93]

Table 3.3 Organic compounds detected in analyzed samples

Compound	Species/family	FL	PFL	Blood	Urine	Liver	Kidney	Reference
Amphetamine	Calliphoridae and Sarcophagidae	+		0.48 mg/L		0.35 μg/g	0.21 μg/g	[93]
Alcohol	Calliphoridae and Sarcophagidae	–		0.45 μg/g		–	–	[93]
Opiate concentrations (μg/mL) in morphine equivalents	*Lucilia sericata*	0.75	0.14	0.72	26.75	2.45	–	[52]
Cocaine concentrations (μg/mL) in benzoylecgonine Equivalents	*Lucilia sericata*	1.99	0.40	1.34	38.75	3.30		[52]

FL Feeding larvae, *PFL* Post-feeding larvae

Table 3.4 Prescription drug concentrations (μg/mL)

Compound	Species/family	FL	PFL	Blood	Urine	Bile	Liver	Reference
Phenobarbital	*Lucilia sericata*	1.70	5.20	15.00	14.90	36.80	32.70	[52]
Levomepromazine	*Lucilia sericata*	0.04	0.06	0.01	0.12	0.53	0.29	[52]
Amitriptyline	*Lucilia sericata*	0.65	0.01	0.40	0.56	13.16	9.42	[52]
Nortriptyline	*Lucilia sericata*	1.44	1.26	1.32	2.14	11.62	120.43	[52]
Thioridazine	*Lucilia sericata*	0.36	0.37	2.06	1.28	1.62	1.26	[52]
Clomipramine	*Lucilia sericata*	5.40	8.73	2.86	0.38	19.70	3.90	[52]

FL Feeding larvae, *PFL* Post-feeding larvae

Table 3.5 Organic compounds identified in arthropod larvae (Medico-Legal Institute of Strasbourg 1988–2002)

Compound	Concentrations (pg/mg)	Reference
Benzodiazepines		
Nordiazepam	21–228	[95]
Oxazepam	44–200	[95]
Lorazepam	155	[95]
Bromazepam	810	[95]
Alprazolam	27	[95]
Triazolam	204	[95]
Barbiturates		
Phenobarbital	500–2250	[95]
Amobarbital	15–0	[95]
Antidepressants		
Amitriptyline	133	[95]
Clomipramine	28	[95]
Dothiepin	280	[95]
Fluoxetine	16	[95]
Venlafaxine	59	[95]
Phenothiazine		
Chlorpromazine	55.116	[95]
Cyamemazine	103–489	[95]
Levomepromazine	45	[95]
Alimemazine	22	[95]
Opiates/opioids		
Morphine	90–182	[95]
Codeine	12–59	[95]
Pholcodine	13	[95]
Propoxyphene	867	[95]
Miscellaneous		
THC-COOH	16–39	[95]
11-Hydroxy-THC	11	[95]
Meprobamate	718–4439	[95]
Digoxin	21	[95]
Nefopam	880	[95]

3.5 Factors Affecting the Growth of Decomposers

There are some factors or parameters which affect the growth of decomposers. These are:

(a) **Temperature**—Temperature is the most critical factor among all the environmental factors affecting the growth rate of decomposers. The arthropods are cold-blooded because the body temperature is directly related to the surrounding environment; consequently, they show variations in the metabolism and growth rate. The growth of insects is temperature-specific, and it has a low and high point where the development stops, which is termed the threshold temperature level. The species collected for the rearing purpose are kept at room temperature. The rearing temperature varies from species to species and with the different stages of the same species. The species should be kept inside the rearing cages placing the cages in direct sunlight should be avoided. The extreme heat will kill the specimens [96].

The primary environmental factor affecting the growth or metabolic activity of decomposers that are cold-blooded is temperature because at the lower temperature, they develop slowly and at higher temperatures, rapid growth is observed [97]. Rodriquez and Bass performed a study in which four cadavers, of which one was female and the rest of three were male, were exposed to decay in May, June, October, and November. The study concluded that the decomposition rate occurred more rapidly when the temperature was higher and the carrion insects' colonization was great [98].

Numerous studies were conducted to examine the effect of temperature on the growth cycle of insects. And it was observed from those studies that the growth rate was slightly longer at varying temperatures as compared to the mean and constant temperature conditions [99].

(b) **Drug**—Another factor affecting the growth of decomposers is the drug. The toxic substances or the drugs in or the corpse can affect the growth rate of the decomposers or the feeding larvae. For example, heroin and cocaine can drastically increase the growth rate of larvae and simultaneously affect the accuracy of PMI estimation. Similarly, the insects might take a longer time to colonize and decompose a body [97]. According to the in vitro study performed by Monthei on *Phormiaregina*, the low amount of ethanol (0–0.1% w/v) does not affect larval development but can produce some characteristic growth differences such as lower pupal and adult weight, whereas the entomotoxicological effects of oxycodone produced affect the developmental rate of maggots [100]. Ethanol produces significant changes in the maggots' length for the third instar which may further alter the PMI estimation. There is less literature available on the maggots feeding on ethanol-treated flesh [99]. Drugs or toxins can be encountered in the larvae when the absorption rate goes beyond the metabolism rate. But the fact is not yet clearly known that how the metabolism of drugs occurs in larvae and how the larval development gets affected. The changes produced in the growth rate of the necrophagous insects can further produce significant variations or errors in PMI estimation if not considered during the death investigation because most of the drugs delay insect colonization. A rapid growth rate was observed in the larvae of *Calliphora vicina* eating tissues contaminated with PCM (paracetamol) and *Lucilia sericata* larvae feeding on the tissues contaminated with morphine while affecting the size and shape of *Chrysomaalbiceps* feeding upon the tissues contaminated with Diazepam [101].

Drugs in larvae—Numerous experiments were performed to find out the concentration of drugs and controlled substances (for example, opiates, cocaine, antidepressants, derivatives of amphetamines, etc.) to achieve useful information on the cause of death. The studies have demonstrated that heroin speeds up larval growth and the pupal stage remains for a longer period. So, the total time required for the metabolic growth from the larval stage to adult is of longer duration when the colonies are feeding on the heroin–containing tissues [102].

Drugs in pupae—Miller et al. detected the presence of drugs (drug extracted was amitriptyline) in chitinized insects. The drug concentration was observed higher in pupae as compared to the casts of the beetle's skin (exuviae) [102].

Drugs in beetles (frass and exuviae)—Only a few drugs were extracted by following the method of hair-drug extraction. The concentration of drugs was found higher in fly pupae, whereas, in the faecal materials of the beetles, only a few drugs—cocaine and amitriptyline—were extracted [102].

(c) **Season**—Most of the insects synchronize their cycles according to the seasons. Higher temperature increases the insect activity, while the lower or colder temperature slows it down. The dead bodies exposed to the higher temperature or brighter sunlight will heat up easily and further provide a warmer place to the insects for development, hence reducing the time of growth of the insect colony. In contrast, the humid environment increases the reproduction and growth rate of most insects. On the other hand, high winds will also affect the activity of decomposers by creating a problem to locate and land over the body [98].

As the insects are cold-blooded, they adapt themselves according to the surroundings to survive accordingly within the temperature or seasonal variations. In places with extreme cold weather conditions, there is at least one stage in the growth cycle of insects that is resistant—the stage which survives in low temperatures. Such resistant stage could be any one of all the growth stages, i.e. egg, larva, nymph, pupa, or adult [96].

(d) **Location**—The bodies in damp locations will attract a variety of insects, while those in dry locations will become dehydrated before the insect colonization starts [103].

The moisture needs of insects and mites vary. The location from where the specimens are collected tells about the moisture requirement, as some of the insects in their pupal stage were found to be drought resistant. Apart from it, some require little moisture, while some produce water. Outdoor species have more moisture needs than indoor species. This is because even a minor interruption in the photoperiod can disturb the whole developmental process. In places with extreme cold weather conditions, there is at least one stage in the growth cycle of insects that is resistant, which means the stage which survives in low temperatures. Such resistant stage could be any one of all the growth stages, i.e. egg, larva, nymph, pupa, or adult [96] (Table 3.6).

Table 3.6 Entomotoxicological findings and various drug concentrations in pupae [102]

S. No.	Drugs	Techniques used	Drugs concentrations found
1.	Barbiturates (phenobarbital)	GC/MS	100 μg/g
2.	Opiates (morphine)	Radioimmunoassay, GC/MS	8–1208 ng/g
3.	Bromazepam; levomepromazine	EIA, HPLC	Bromazepam 0.81 μg/g; Levomepromazine 45 ng/g
4.	Cocaine and its metabolite	RIA, GC/MS, GC/NPD	Cocaine 0.49 μg/g Metabolite 0.3 μg/g
5.	MDMA, MDA (metabolite)	HPLC/MS	MDMA 6.6 μg/g MDA 1.8 μg/g

3.6 Conclusion

Forensic entomology is a huge field to discuss due to its relevance with cases where evidence is not even expected. This field is fast developing due to the fascinating behaviour and curiosity of researchers. Numerous researches have been conducted in the past few years, but the relationship between the drug amount in a substrate and the insects reared on it is still doubtful. Different qualitative analyses have been performed with different drugs, but the question arises always for quantitative analysis from entomological samples. The reason for not finding the exact relation between drug concentration of substrate and maggots can be one or many. There is a shortage of literature available for behavioural studies and feeding habits of insects particularly decomposers. Future researches need to be more focused on the ADME of the drug inside the insects as this can help in the quantification of drug from predators. Recent researches finding the DNA material and other crime-related evidence inside maggots gut are of great emphasis in forensic science province because these findings are arcane in their type. The field of forensic entomology and entomotoxicology are accruing and of great value for the judicial system as justice can be delivered to decedents even after a long time.

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