8

Forensic entomology

Martin Hall, Amoret Whitaker and Cameron Richards
Department of Life Sciences, Natural History Museum, London, UK

8.1 Introduction and current state of the discipline

Forensic entomology is the study of insects and other arthropods in a legal context (Hall, 2001), which can be broadly divided into three main areas of application (Lord and Stevenson, 1986): urban entomology (e.g. civil actions relating to insects and human environments), stored products entomology (e.g. civil actions relating to insects found in food products), and medico-legal entomology (e.g. criminal proceedings in cases of violent crime or unexpected death). The latter, sometimes also referred to as medico-criminal entomology (Hall, 1990), is the most high profile area of forensic entomology and the subject of this chapter. It can be utilised in many situations, from isolated domestic incidents to large-scale atrocity crimes, and for many different types of investigation: neglect of people in care, such as the elderly (Benecke, 2004); child abuse (Benecke and Lessig, 2001); wildlife poaching (Anderson, 1999; Samuel, 1988); detection of gunshot residue (Roeterdink, Dadour and Watling, 2004); detection of drugs, also called entomotoxicology (de Carvalho, 2010); movement of vehicles through identification of insects impacted on windscreens; transport and relocation of human remains (Smith, 1986); and estimation of minimum time-since-death (Hart, Whitaker and Hall, 2008).

Insects are ubiquitous in nature and it is almost inevitable that they will be associated with a crime scene, either because it is a part of their natural habitat or because they have been introduced or attracted to it. Since publication of the first manual of forensic entomology (Smith, 1986) there has been a surge of interest in the subject and major efforts have been made to increase the robustness of the interpretation of insect evidence. These activities have been reviewed extensively (Amendt *et al*., 2010; Byrd and Castner, 2009; Haskell and Williams, 2008; Erzinçlioğlu, 2000; Gennard, 2007; Goff, 2000; Greenberg and Kunich, 2002).

The insects associated with human cadavers belong to one of four ecological groups: necrophagous species (feeding on the body); predators and parasites (of the necrophagous species); omnivorous species (feeding on the body and its

Forensic Ecology Handbook: From Crime Scene to Court, First Edition.
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Figure 8.1 Bluebottle blowfly *Calliphora vicina,* surrounded by larvae of different sizes/ages. (To see a colour version of this figure, please see Plate 8.1.)

inhabitants); and adventive species (using the body as an extension of their environment) (Smith, 1986). The necrophagous species provide the information of greatest evidential value because of their direct and obligatory association with the body. Of greatest forensic importance in this group are the blowflies (Diptera: Calliphoridae) (Figure 8.1) because they are usually the first group to colonise a body and are found in greatest numbers, consequently they can provide the most accurate information regarding the minimum time-since-death (Greenberg, 1991). Blowflies have four developmental stages: egg, larva (maggot), pupa and adult (Figure 8.2). The larva is the main feeding stage and it passes through three instars (1st, 2nd and 3rd) which are separated by a moult (shedding) of the cuticle (skin) allowing for growth. Once larvae have finished feeding they move off the body and burrow into the soil, if outdoors, or under furniture and carpets, if indoors. Metamorphosis from larva to adult fly occurs during the pupal stage which takes place within the puparium, the hardened and darkened cuticle of the 3rd instar larva. In addition to blowflies, other insects collected from a body can have value in estimating a minimum time-since-death if their development rates are known, for example species of hide beetle (*Dermestes*) which are common on dried bodies (Kumara *et al*., 2009; Midgley, Richards and Villet, 2010).

'When did the victim die?' can be a crucial question in cases of untimely death (Wells and Lamotte, 2001) and the estimation of time-since-death, also referred to as post-mortem interval (PMI), is the most frequent task required of a forensic entomologist. Of course what an entomologist actually establishes is the time of colonisation of a body by the insects that arrived first. This is, therefore, termed the minimum PMI (PMI_{min}) because there could be many reasons for a delay in colonisation (see Section 8.6.5). Many other methods have been applied to establish

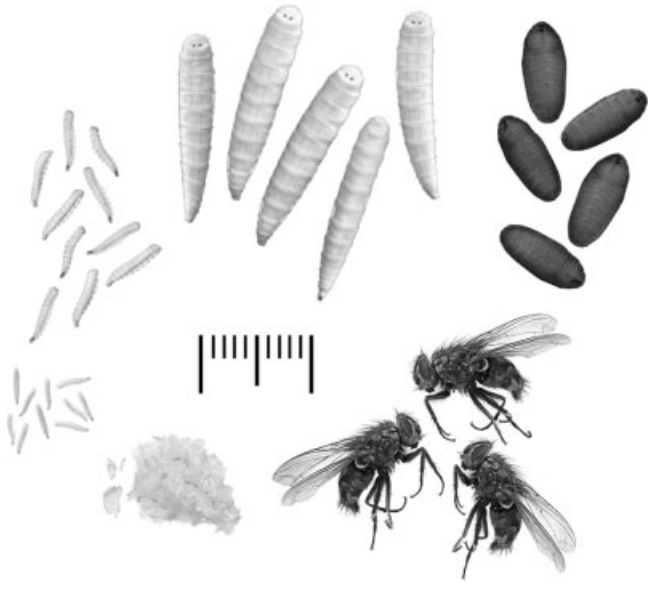

Figure 8.2 Life cycle of bluebottle blowfly *Calliphora vicina*. Clockwise from bottom left: eggs, 1st instar larvae, 2nd instar larvae, 3rd instar larvae, puparia (containing pupae), adult flies. Scale bar in millimetres. (To see a colour version of this figure, please see Plate 8.2.)

time-since-death, such as chemical, histological and bacteriological (Easton and Smith, 1970). However, most post-mortem changes in a cadaver measured by pathologists to estimate PMI (e.g. rigor mortis, algor mortis, livor mortis) are not reliable because of the inability to accurately account for the variability in these processes, and the reliance upon subjective evaluation rather than objective measurement (Henssge, Madea and Gallenkemper, 1988). In addition, most post-mortem changes used to estimate PMI occur within the first 72 hours of death, so the medical examiner's estimate is limited to these first three days. Swift (2010) concluded that, 'It remains debateable whether there is any single, reliable and accurate means of estimating the time since death during the early post-mortem interval' (p. 107).

Nevertheless, the first blowflies to lay eggs on a body start a biological clock ticking so that, using objective methods to study the colonisation and development of these insects, the time of death can be estimated over periods of a few weeks in summer and a few months in cooler seasons. If the discovery of a body is delayed beyond this period then the succeeding colonisers (mainly flies and beetles) will still provide a useful but less accurate time of death (Greenberg and Kunich, 2002). This is done by interpreting the changes in insect fauna composition that accompany decompositional changes. Insect succession was first observed by Mégnin (1894) and is now the subject of much research (e.g. Matuszewski *et al*., 2011). However, it can be difficult to interpret and is subject to considerable local and seasonal variation, therefore it will not be considered in detail here. Measurement of changes in

the chemical composition of empty puparia over time might become another tool in estimating longer PMIs (Drijfhout, 2010).

8.2 Applications

8.2.1 Introduction

As indicated above, there are many diverse applications for forensic entomology. Even the absence of insects on a body can be of value, for example confirming that a concealed body had not been exposed to fly activity before concealment (Erzinçlioğlu, 1996). However, the main applications relate to insect infestation or colonisation of living or dead humans and animals.

8.2.2 Live bodies

When fly larvae develop on living humans or other vertebrates they cause a disease condition known as myiasis (Hall and Smith, 1993; Hall and Wall, 1995). Although some species of myiasis-causing flies are obligatory parasites and cannot develop on dead animals, the majority of fly species associated with myiasis in a forensic context are the same species as are found on dead bodies. On live hosts they feed mainly on dead tissues within sites of trauma that have been neglected. Usually that neglect occurs in a domestic situation with either young children (e.g. as a result of soiled nappies) or the elderly (e.g. in infested bed sores). Occasionally neglect can occur even in a hospital or other medical environment leading to a hospital acquired (nosocomial) infestation. One example is that of a young boy who was admitted to hospital in a coma following a traffic accident. The emergence of two- to three-day-old larvae of the greenbottle blowfly *Lucilia sericata* from his nostrils four and five days after admission was consistent with infestation within the hospital (Hira *et al*., 2004). In addition to being evidence of neglect in live humans and animals, myiasis infestations can cause problems in estimating PMI_{min} if overlooked. For example, if a victim of myiasis dies but it is assumed that the larval infestation began only after death, then the PMI_{min} estimate will be greater than it actually is. This can be shown by reference to a case in the United Kingdom, when the body of a woman was found indoors infested by large numbers of blowfly larvae, mostly dispersing from pressure sores in the anal region and thighs. The pathologist estimated that death had occurred about one day before collection of the larvae, but these were estimated by the entomologist to be two to three days old. The conclusion of an ante-mortem infestation was supported by the observation of a complete absence of any eggs or larvae in the exposed head orifices, where blowfly eggs are normally first laid. This ante-mortem myiasis infestation was, therefore, evidence of neglect by the carers of the deceased. Similar infestations of animals can be used as evidence of cruelty or neglect (Anderson and Huitson, 2004). For example, a case of infestation of the

wound created by a wire snare around the neck of a trapped but still living badger in Scotland, with larvae estimated to be two to three days old, showed that the person who had set the snare had neglected to check it at least every day as was required by law.

8.2.3 Dead bodies

The major objective of a forensic entomology investigation is to determine the PMI_{min}. However, insect evidence can also help to indicate the manner of death (e.g. by directing a pathologist to knife wounds on bones below larval infested tissues in which any knife marks had been obliterated by larval feeding activity), the place of death and post-mortem movements of the body (e.g. through a knowledge of insect distribution and finding larvae on a body that were of a species not found in the locality of recovery), and can assist in toxicology studies when the body tissues are too degraded for analysis, that is, the larvae act as a reservoir for drugs in tissues they ingest (de Carvalho, 2010).

The value of insects in providing a PMI_{min} is exemplified by a case presented by Hart *et al*. (2008) in which the body of a young man was found behind a building in northern England in mid February. He had last been seen alive the previous November but pathology tests suggested he had been dead for just two weeks. However, aware of the potential for forensic entomology the pathologist asked for the insect evidence to be analysed. This gave a PMI_{min} consistent with death having occurred soon after the last sighting of the deceased. The body had lain in a shaded trench behind the building since death and the low winter temperatures had markedly slowed the rate of decomposition leading to the pathologist's underestimate of PMI. The low temperatures had also slowed the rate of insect development, but this was accounted for in estimating the PMI_{min} using forensic entomology techniques.

8.3 Pre-scene attendance

8.3.1 Information required pre-scene attendance

The forensic entomologist is generally notified by telephone that a body has been found and that their involvement is required. On some occasions, the Senior Investigating Officer (SIO) or Crime Scene Investigator (CSI) simply wants advice as to whether or not a forensic entomologist is actually required at the scene and, if not, how to collect the samples. In these situations, it is important to glean as much information about the scene and situation of the body as possible, and to provide instructions in a clear and concise manner. If it is possible for photographs of the body and scene, preferably prior to recovery, to be e-mailed to the forensic entomologist, this can help to give a clearer picture of the case.

However, attendance of a forensic entomologist at the scene or mortuary is often required to ensure an accurate evaluation and collection of the entomological evidence, in particular to ensure that the most relevant insect evidence is collected in an appropriate manner. If the body is discovered late in the day, attendance may be requested for the following day, but often the request is urgent and the entomologist is asked to attend as soon as possible, so that the body can be examined *in situ* prior to its removal from the scene. It is therefore advisable to have the entomological sampling equipment ready prepared and able to be accessed at short notice.

The first question to be asked of the SIO, CSI or Crime Scene Manager (CSM), is whether the body is indoors or outdoors, as additional equipment and clothing will be needed if the body is in an outdoor environment. An indication of the state of decomposition of the body and what insect activity is visible provides information on what might need to be collected so that preparations can be made before arrival at the scene. Ideally, early attendance at the scene of recovery of the body is preferable, to ensure that the insect evidence has not been disturbed so, if possible, a request should be made to the CSM not to move the body until the entomologist is in attendance.

8.3.2 Preparation pre-attendance

A clean, solid toolbox should be prepared and stored ready for use with the following recommended equipment:

- Forceps of varying sizes, both pliable and firm.
- Teaspoon (for collecting larvae when abundant).
- Sealable and aerated storage tubes of varying sizes (e.g. 10 ml, 30 ml, 50 ml, 150 ml).
- Specimen labels.
- 80 % ethanol (ethyl alcohol).
- Small sieve.
- Hand-held insect capture net.
- Protective clothing: disposable suits, gloves, shoe covers, facemasks.
- Disinfectant wipes and gel.
- Tape measure.
- Notebook, pens and pencils.
- Tin/pouch of moist dog food.
- Digital camera.

- Measuring scale for inclusion in photographic images.
- Hand-held infra-red and/or probe digital thermometer (calibrated and certified).
- Two electronic temperature dataloggers (calibrated and certified).
- Cooler bag and ice packs for live specimen transport.

Ethanol ($\geq$80 %) is recommended as a preservative because, compared to most other preservatives such as formalin, it has relatively few health and safety concerns, it does not degrade DNA (analysis of which might be required) and it has less impact on the physical appearance of the samples (Adams and Hall, 2003). Additional equipment needed for outdoor scenes includes:

- Trowel.
- Plastic basin.
- Large soil sieve.
- Plastic sheet, e.g. 2 m $\times$ 2 m (to search soil samples on).
- Strong resealable bags, e.g. 25 cm $\times$ 35 cm (for holding soil samples).
- Stevenson screen (to protect datalogger from direct sunlight).
- Crime scene indicator flags.
- Compass or hand-held GPS.

Prior to attendance at the scene, two electronic dataloggers should be pre-set to record temperatures to match local weather station recording intervals (usually at hourly intervals, on the hour). More information regarding temperature recording is given in Section 8.6.4.

8.4 Scene attendance

8.4.1 Sampling protocols

Before proceeding with the collection of insect evidence, clear agreement for a sampling strategy should be reached with the CSM, pathologist and other forensic experts present at the scene to minimise the risk of inadvertently disturbing or destroying other evidence. Detailed and widely accepted protocols for sampling insect evidence have been published (Amendt *et al*., 2007; Catts and Haskell, 1990) and are summarised here, with emphasis given to potential problems that can arise from common sampling errors made by untrained investigators, especially due to the lack of recognition of insect evidence, suboptimal sample size and incorrect labelling, handling and preservation. It is imperative that entomological evidence is collected

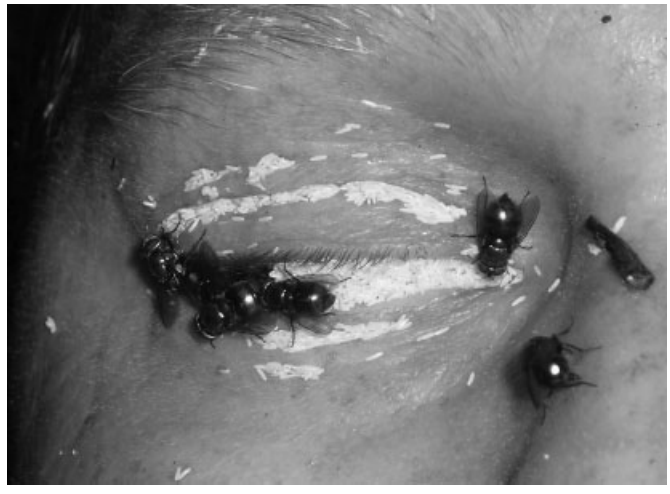

Figure 8.3 Blowfly eggs laid in the eye. (To see a colour version of this figure, please see Plate 8.3.)

in the correct manner, otherwise its value will be compromised and an accurate analysis will not be possible.

8.4.1.1 Sampling from the body

Insect eggs are at risk of predation and desiccation and are therefore usually laid at sites which minimise this, and where hatched larvae can gain ready access to soft tissues. In the majority of cases, blowfly eggs are first laid in and around the body orifices, usually beginning around the head, particularly in the mouth, nose, eyes and ears (Figure 8.3) and then the genital and rectal area if accessible. The body-soil interface and the scalp are also common sites for egg-laying. Eggs may also be concentrated at sites of trauma, for example where there are knife or gunshot wounds, and their location in such circumstances might provide information on the manner of death. Eggs can also be laid on clothing, especially in folds away from direct sunlight and if contaminated with blood or other body fluids. Eggs are fragile but can be collected without damage by using a fine paint brush or fine forceps, especially when they occur in large masses.

Larvae can be readily collected using pliable forceps or a small spoon, taking care not to inflict post-mortem wounds on the body of the deceased. It is important to record the sites from where larvae were collected. Specimens from different sites should not be combined but should be stored separately with detailed labelling. Records can be enhanced by photography of the sites of collection, ideally including a measurement scale, calibrated in mm.

It is essential to collect a sample of larvae for analysis that accurately reflects their abundance and diversity on the body (Hall *et al*., 2008), that is, 50–100 larvae from each sample site. Although the entomologist is generally most concerned with

the oldest insects, which are often the largest visible larvae, it is important to collect an accurate subset of all larvae. On occasion there may be a small number of larvae that appear to be significantly bigger than others – this could be due to their being precocious larvae, that is, having been laid as a 1st instar larva instead of as an egg (Wells and King, 2001), or their development may have been accelerated significantly, for example by feeding on tissues that were contaminated with drugs (Lord, 1990). Also, different species of fly may be present in the same larval mass, for instance many Sarcophagid (fleshfly) larvae are generally larger than blowfly larvae of the same age; conversely, the small larvae of some muscid species (e.g. the housefly, *Musca domestica*) could be older than the larger blowfly larvae. Therefore a sample should be collected that is representative of the complete range of larval sizes – a sample of six larvae from a mass of thousands will not provide confidence in PMI estimations.

Most people will easily recognise a writhing mass of larvae, but some life-stages of blowfly are more difficult to see, or if not moving, they may not be recognised as being of insect origin. Blowfly eggs are very small, about 1.5–3 mm long, immobile and are generally laid in dark and hidden areas such as the nose, mouth, ears, eyes, scalp and folds of the clothes as discussed above, and therefore may easily be overlooked (Figure 8.3). Likewise, as larvae enter the pupal stage they contract, harden and become dark brown and immobile (Figure 8.4), so they may also be overlooked, especially as they are often found some distance from the body. Therefore the possibility that the oldest specimens might have dispersed from the body should be considered (see Section 8.4.1.2). If adult flies have emerged, the empty pupal cases will remain in the environment as evidence of completed immature development (Figure 8.5) and these can also be easily overlooked resulting in an underestimate of PMI_{min} if only the younger larvae or unemerged puparia are collected.

Figure 8.4 Post-feeding larvae (white) and young (pale) and older (dark) puparia. (To see a colour version of this figure, please see Plate 8.4.)

Figure 8.5 Adults with unextended wings (left), newly emerged from pupal cases (right). (To see a colour version of this figure, please see Plate 8.5.)

The insect fauna on a buried body may differ from that found on an exposed body (Payne, King and Beinhart, 1968), but collection of specimens from a buried body should proceed in a similar way to those described above, with a careful assessment of the layers of soil above, around and below the body to identify any evidence of insects moving towards or away from the body. While even a shallow burial (10–20 cm depth depending on soil texture) will deter most blowfly species, other groups of fly are more adept at colonising buried bodies. Species of *Muscina* (Diptera: Muscidae) (Gunn and Bird, 2011) and *Eumacronychia persolla* (Diptera: Sarcophagidae) (Szpila, Voss and Pape, 2010) will colonise bodies in shallow graves and species of scuttle flies (Phoridae) will colonise bodies buried under 1–2 m of soil (Disney, 1994; Gaudry, 2010).

8.4.1.2 Sampling of dispersed stages

During the post-feeding stage the larvae of most blowfly species leave the body (Figure 8.6) and search for a suitable pupariation site (Greenberg, 1991). If outdoors, larvae may disperse some distance from the body and then burrow down into the soil or under logs, stones and other ground cover. Therefore a search for dispersed insects should be conducted at several compass positions up to 5 m from the body, using a trowel to sample soil to a depth of about 15 cm. Care should be taken to exclude potential competing sources of larvae (e.g. dead wildlife) and control samples should be taken 10–20 m from the body to determine the background level of insect activity in the soil. Soil can be sieved and searched on a plastic sheet at the scene or can be bagged for inspection later in the laboratory.

At indoor scenes, dispersing larvae may be found under furniture and rugs, especially around the edge of the room, and even in rooms adjacent to that in which the body was found. On hard floors they can disperse much further than on soils and in blocks of flats larvae have been recorded to disperse under doors, into communal corridors and even down stairwells to lower floors. Care should therefore be taken to search some distance from the body.

Figure 8.6 Post-feeding larvae dispersing from a body. (To see a colour version of this figure, please see Plate 8.6.)

8.4.1.3 Handling of samples after collection

Samples should be collected in two main batches:

- those to be killed and preserved at the time of collection to provide a record of the age of the insects at the time of collection (see Section 8.4.1.4);
- those to be kept alive for transport to the laboratory where they can be subsampled, some to be reared to the adult stage (see Section 8.6.1) and, where needed, some to be frozen for toxicological analysis and others to be frozen or preserved in ethanol for molecular studies, of both insect and human DNA (Wells *et al*., 2001).

Eggs, larvae and pupae are living organisms and therefore they need to respire, so they should be placed in aerated sample tubes. These can be purchased or made simply by punching holes in lids, or by putting gauze or thick tissue held in place by elastic bands over standard specimen tubes, ensuring that the insects inside (such as small larvae) cannot escape through any openings. Also, if the larvae have been collected directly off the body it is likely that they are still feeding and therefore need a food source in the transport container in order to continue to develop. It is best to use the same food as that used in experiments that generated the development data being used for analysis. This is often liver, but if that is not available then alternatives such as minced meat or canned moist dog food should be used. In addition, live insects will die if they become either too cold or too hot, so they should not be placed in a freezer or left in a hot environment such as the boot of a car.

Ideally the samples should be placed in a cool bag for transport to the laboratory. If they need to be stored for a short period (ideally no more than 24 hours)

then they should be kept in a fridge or left at room temperature with a temperature datalogger or some other record of their temperature (e.g. a simple thermometer will suffice).

8.4.1.4 Correct preservation

If fly larvae are not killed and preserved in the correct manner, their physical shape (length, curvature) will be changed which will make diagnostic characters difficult to see and affect the analysis to be carried out (Tantawi and Greenberg, 1993; Adams and Hall, 2003). Larvae placed directly into a tube without preservative will die and rapidly start to decompose or dehydrate making them difficult to analyse even soon afterwards, whereas samples properly preserved will retain their evidential value for many years (Figure 8.7). Eggs can be placed directly into 80 % ethanol. However, larvae will shrink in size if placed live into ethanol, so they need to be first killed in boiled water ($\geq$80°C) by immersion for up to 30 seconds, before being sieved out and placed into ethanol. This will produce good-quality specimens, but if the immersion period is too short or the water not sufficiently hot, then preservation will be poor, resulting in deformed and discoloured larvae. The time and date of killing and preservation should be recorded. It is sometimes difficult to obtain boiled water at a crime scene, but this can usually be taken in a thermos flask or fetched from a catering van, a local cafe/restaurant, or from a nearby house. If ethanol is not available, a spirit such as vodka (*c*. 40–50 % alcohol) can be used instead, as long as this is noted and the samples are transferred into 80 % ethanol at the first opportunity.

Ideally, puparia should be killed in hot water prior to immersion in ethanol, but if hot water is not available, the cuticle of live puparia should be pricked to enable preservatives to penetrate readily. There is evidence that if not killed first, development of puparia can continue for some hours in ethanol (Richards, unpublished) – they can also survive for up to four days in water (Singh and Greenberg, 1994) – resulting in an incorrect estimate of PMI_{min}.

Any adult insects collected at the scene can be killed in the laboratory by placing into a deep freezer for one or more hours. After thawing they can be pinned for taxonomic study or stored in 80 % ethanol. Dried insects are very fragile and, if not pinned, are easily damaged when stored in bulk, losing hairs, wings and legs. Dead adults stored in humid conditions that prevent them drying will quickly decompose. For long-term storage dried insects should be stored in insect proof containers to prevent destruction by small beetles that feed on dead insects (e.g. museum or carpet beetles, *Anthrenus* species).

8.4.1.5 Correct labelling

It is important that samples are correctly labelled so that their provenance is known. Ideally, a label should be placed inside the tube with the preserved samples, written in pencil or archival quality micro-pigment inks, because most pen inks will

Figure 8.7 Blowfly larvae in 80 % ethanol, preserved badly (left – by immersion while still alive) and correctly (right – by first killing in hot water).

dissolve in ethanol. A label should also be attached to the outside of the container in both preserved and live samples. Information contained on the label should include: case name/location, police force or other client identifier, date, time of collection, where on the body or environment the insects were collected from, who collected the sample, and a unique sample number (the latter two are often combined). Examples are given below:

High St, Brentford 12th July 10 13:15 Inside shoe under bed APW/03	Operation Maggot July 12 2010 13:15 Scene/left nostril APW/05	Met Police/Brentford 12/07/10 at 1:15 pm Mortuary/right sock APW/10

8.4.2 Recording at the scene

Comprehensive notes should be taken during scene attendance to aid in preparing the final report. Details should be taken of date and time of attendance, location and a list of names and roles of all other people at the scene, including the pathologist, CSM, SIO, police photographer and any other forensic experts. It is also useful to exchange contact details with all these people in case you wish to communicate at a later date regarding the case.

Comprehensive notes and supporting photographs, if possible, should be taken regarding the scene:

- Indoor/outdoor.
- Rural/residential.

If indoor:

- Flat/house/shed or other description of the property.
- Curtains/windows/doors – open/closed/ajar.
- Number of rooms/floors.
- What room the body is in; its state (furnished, clean, littered); is the body exposed to direct sunlight or not?
- What other potential larval food sources are available, e.g. meat pies, dog food?

If outdoor:

- Environment, e.g. woodland/field/road/water.
- Local flora, e.g. trees, shrubs, grassy with identifications if possible.
- Soil type, e.g. clay, chalky.
- Aspect, e.g. shaded, exposed to sun.

Comprehensive notes should be taken regarding the body:

- Male/female.
- State of decomposition, e.g. fresh, bloat, active decay, advanced decay, dry/skeletal (Anderson and VanLaerhoven, 1996).
- Clothed/semi-clothed/naked.
- Wounds and their locations, e.g. knife, gunshot.
- Position, e.g. lying/sitting/hunched, face up/down.
- Direction of body, e.g. head facing north/south/east/west.
- Substrate, i.e. what is the body lying on?
- Contaminants/toxins – are any present on or in the body that could affect insect development?

An example of a scene/mortuary attendance form is provided in the Appendix to this chapter (based on Amendt *et al*., 2007) and has a useful body outline diagram on which the location of insect evidence can be recorded.

In addition to collecting insect evidence, temperatures should be recorded at the scene and mortuary (if attended – see Section 8.5) of air, ground, body (on, in and under the corpse) and larval masses. A temperature probe may be used, but as this may cause the mass to disperse, the use of a non-invasive infra-red thermometer is recommended.

8.5 Mortuary attendance

Often a request is made to visit the mortuary and/or attend the post-mortem examination or autopsy. This may occur if the body was removed from the scene prior to the entomologist being called, or even after scene attendance, for instance if the body was removed before all sampling was completed. Movement of the body during its recovery can provoke larvae to disperse from their feeding sites and so present a distorted picture of the larval infestation when viewed at post-mortem. Therefore, although it is usually easier to collect insect samples directly from the body in the mortuary, scene attendance is still recommended so that insects can be collected from the body prior to disturbance, and also from the surrounding environment.

In the mortuary, it is important to follow instructions from the forensic pathologist and the mortuary technicians. If the body is clothed or wrapped, insect samples should be taken as it is being undressed or unwrapped to ensure that any important evidence is not lost. A record should be kept of where the samples are collected from.

It is important to record details of the mortuary temperature and, if used, that of its cold storage facility so that a record of temperatures to which the insect evidence was exposed is kept. This is because development of blowflies can be effectively suspended at the low temperatures (3–4°C) commonly found in mortuary cold stores (Johl and Anderson, 1996), although this effect will be less pronounced in a large larval mass. The mortuary technician will usually have details of mortuary temperatures together with the dates and times when the body was taken from one environment into another. In short, it is important to obtain the full ‘thermal history’ of the body including periods of transport and storage.

8.6 Laboratory analysis

8.6.1 Rearing of insect evidence

Live insect evidence is collected for rearing in a laboratory incubator for two principle reasons. Firstly, adult insects are easier to identify than larvae, so it is a useful

way of confirming the identifications of the preserved immature stages. Secondly, if the insects are reared through at a known temperature, this thermal input can be subtracted from their known total developmental needs in order to ascertain how long they had been developing prior to collection, hence when they were deposited as eggs on the cadaver. This estimate of PMI_{min} can provide support to that calculated from larvae killed at the time of collection.

Insects should be reared through to adults in an incubator, set to a known constant temperature. If the temperature is too cool, the insects will take a long time to develop which might be critical if there are deadlines in the investigation to meet. On the other hand, if the temperature is too warm, the larvae may expire through dehydration. Ideally the temperature should be constant at around 18–23°C for temperate species of blowfly. It is recommended that a temperature datalogger is put into the incubator to obtain an accurate record of the temperature.

Blowfly eggs should be kept on moist tissue paper to maintain humidity because they are very susceptible to death by desiccation. As soon as larvae have hatched they should be placed into small pots (e.g. 50 ml disposable plastic cups) for rearing on a food substrate such as liver or dog food (see Section 8.4.1.3). Feeding larvae should be checked daily to ensure that they have enough food, and that they are not able to escape. Once the larvae are nearing the pupal stage, the pots should be placed into larger containers with sawdust or sterilised soil, into which they can disperse. The pupae will remain in their immobile state for a number of days, but care should be taken to ensure that the pots remain covered but still aerated (e.g. with gauze) to ensure that when the adult flies emerge they cannot escape. While some blowfly species (e.g. *Calliphora vicina*) will pupariate in a relatively dry environment such as sawdust, others are less tolerant of low humidities (e.g. *Lucilia caesar*) and will not pupariate unless the substrate is moist.

It is important to ensure that all rearing pots are clearly labelled with their original data, including case and sample number, and that different samples are not mixed. Insects being reared through to the adult stage should be checked on a daily basis, and a record of visit times and stage of the insect development on each occasion noted. It is especially important to note down the date/time when the adult flies emerge. Once emerged, the adults should be removed from the sample, so as not to confuse these adults with ones that emerge over the following days, killed by placing in a freezer for one or more hours, and then pinned and identified. Each pin should also be labelled with the appropriate information for that specimen, including sample number and time/date of adult emergence.

8.6.2 Insect identification

Identification of all specimens, both immature and adult, is the essential first stage in any investigation of insect evidence. It should be undertaken by a trained entomologist, using a binocular microscope and reference to published identification keys (Greenberg and Kunich, 2002; Smith, 1986; Szpila, 2010; Thyssen, 2010). It

is crucial that all samples are correctly identified to ensure an accurate estimation of PMI_{min}, because different (even closely related) species develop at different rates (Richards, Crous and Villet, 2009). If morphological identifications are not possible then molecular biological methods can be used to identify all life stages (Stevens and Wall, 2001). These should be carried out by someone familiar with molecular techniques to ensure accurate DNA extraction, analysis and interpretation.

8.6.3 Insect measurement

The life-stage of larvae should be determined (i.e. 1st, 2nd, 3rd instar, feeding/post-feeding) and they should also be measured in length using an eyepiece graticule or computer-based measuring software. There are an increasing number of publications which provide information on development of blowfly species in terms of development stage and/or larval length (Table 8.1). Less commonly, weight is used as a measure of age, however this parameter is much more variable than length and is therefore not recommended.

8.6.4 Estimating scene temperatures

Insect development occurs at a rate that is proportional to temperature such that between the upper and lower thermal limits for development of any species, cool temperatures will result in a longer period of development while warm temperatures

Table 8.1 Publications that contain developmental data on different blowfly species (Diptera: Calliphoridae) based on three or more temperatures.

Species	References
Calliphora vicina	Reiter, 1984; Williams and Richardson, 1984; Greenberg, 1991; Davies and Ratcliffe, 1994; Anderson, 2000; Marchenko, 2001; Donovan *et al*., 2006
Calliphora vomitoria	Greenberg and Tantawi, 1993; Davies and Ratcliffe, 1994
Chrysomya albiceps	Queiroz, 1996; Al-Misned, 2001; Grassberger *et al*., 2003; Richards *et al*., 2008
Chrysomya megacephala	Nishida, 1984; Richards and Villet, 2009
Cochliomyia macellaria	Melvin, 1934; Byrd and Butler, 1996
Lucilia cuprina	Melvin, 1934; Buei, 1959; O'Flynn, 1983; Dallwitz, 1984; Williams and Richardson, 1984
Lucilia sericata	Melvin, 1934; Ash and Greenberg, 1975; Wall *et al*., 1992; Davies and Ratcliffe, 1994; Anderson, 2000; Grassberger and Reiter, 2001; Marchenko, 2001; Bourel *et al*., 2003
Phormia regina	Melvin, 1934; Nishida, 1984; Byrd and Allen, 2001
Protophormia terraenovae	Greenberg and Tantawi, 1993; Grassberger and Reiter, 2002

Figure 8.8 Blowfly larvae of the same age, 48 hrs old, which developed at 21°C (above) and 27°C (below).

will result in a shorter period of development (Figure 8.8). Therefore it is essential to estimate the temperatures at which the insects collected from the body were developing. Significant temperature variation can be recorded between different locations even only a short distance apart due to environmental features, for example open grassland, under a hedge, in a ditch. Because of this the forensic entomologist cannot rely on simply using temperature data from the nearest weather station. Likewise, uncertified weather data such as that available on some web sites should not be used. Instead, hourly temperatures should be recorded at the scene for a period of approximately 10 days using a digital temperature datalogger placed at the location where the body was found, ideally in a portable Stevenson screen if outdoors. A regression analysis should then be carried out of scene temperatures and those at a nearby weather station for the same period (Figure 8.9). The temperature at the scene before discovery of the body, usually back to the time of last confirmed live sighting of the victim, can then be estimated by applying the regression formula to the hourly data from the nearby weather station for that same period.

8.6.5 Estimating the age of insect evidence

It is important to always bear in mind that forensic entomology does not estimate the actual PMI or time-since-death but instead estimates the minimum (PMI_{min}). This is because what is actually determined from the age of the oldest insects is when they were first laid as eggs (for some species as larvae) on the body, which is not necessarily when death occurred. If the body was lying outdoors in summer

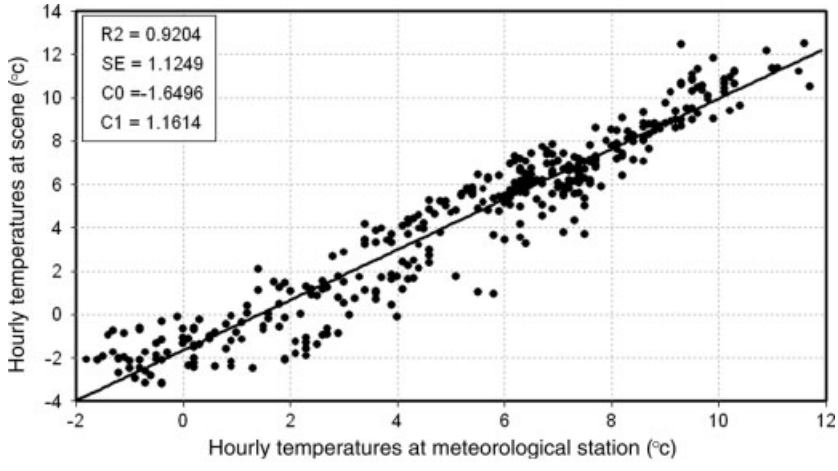

Figure 8.9 Linear regression of hourly temperature data of scene against local meteorological station, for a 16-day December period in the United Kingdom.

conditions then the primary colonisation could indeed occur within hours, or even minutes of death (Anderson, 2011). However, there can be many reasons for delayed colonisation if the body is concealed in some way, such as having been wrapped (Goff, 1992) or if it is first exposed during inclement weather (rain, cold) or in a season of low fly activity (e.g. winter or dry season). Even fires may not destroy insect evidence, although oviposition rates may be affected (Anderson, 2005). An indoor scene is an obvious example with potential for delayed colonisation and studies have shown that colonisation is indeed delayed, with fewer insects on indoor pig cadavers and an extended decomposition and insect colonisation period compared to outdoor bodies (Anderson, 2011; Reibe and Madea, 2010).

It is beyond the scope or objective of this chapter to go into detail regarding how insect age is determined from the available evidence, but more detailed information on this can be found in Byrd and Castner (2001, 2009). Estimation of PMI_{min} can be a relatively straightforward procedure by measuring the size or stage of the oldest insects and then checking databases of development (Table 8.1) to determine how long it would take them to reach that size or life stage at the temperatures estimated for the scene (Villet, Richards and Midgley, 2010). However, many highly variable factors need to be considered. One of these is the potential elevated temperature of a larval mass (Cianci and Sheldon, 1990; Rivers, Thompson and Brogan, 2011). The raised temperatures (Figure 8.10) would be expected to lead to more rapid development, but measurement of this is complicated because the larval mass temperature for the oldest specimens will not be a constant, increasing gradually from the 2nd instar stage, and the larval specimens will not necessarily be exposed to the highest temperatures for all of their development because these can approach the upper limits for survival. At present, although some compensation factors can be applied, there is no simple method to account for the effects of larval masses (Charabidze

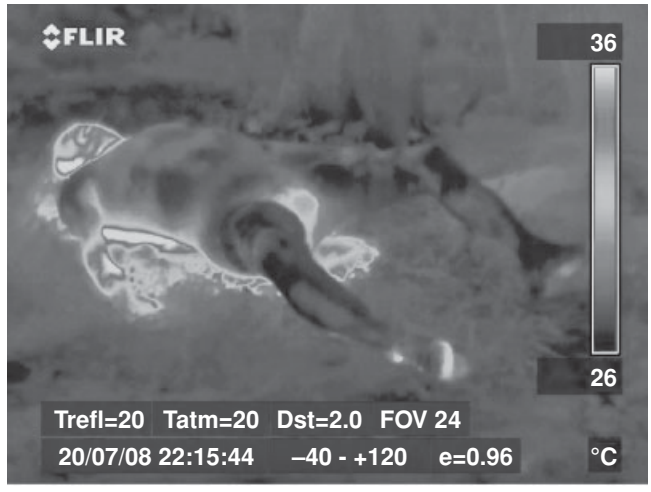

Figure 8.10 Infrared thermal image of human body showing increased temperatures at sites of larval masses, that is, on the head, the body–soil interface, and the anal-genital region. (To see a colour version of this figure, please see Plate 8.10.)

et al., 2011). However, in many cases a significant period of development within larval masses for the oldest insect stages is not observed and forensic entomology can still provide a very powerful tool for estimating PMI_{min}. Much basic and applied research is under way to further improve the manner in which insect evidence can be presented to meet the increasingly strict criteria required by courts since the *Daubert v. Merrell Dow Pharmaceuticals, Inc*. case of 1993 (Amendt *et al*., 2010; Greenberg and Kunich, 2002; Tomberlin *et al*., 2011).

8.7 Reporting and court appearance

Attendance in court is usually required only when the PMI is central to the case or in contention. However, the entomologist should be fully prepared to be called to court to give evidence, and it is therefore wise to have completed an Expert Witness course, and this might be a prerequisite for being considered for casework. If required to attend court, the entomologist should be suitably attired, arrive in good time and follow the instructions of the clerk of the court. Although a copy of the entomology report will usually be provided at court, it is advisable to take a copy that can be referred to in the witness stand.

The forensic entomologist will usually be asked to explain to the jury what forensic entomology is and how it can be used in criminal cases, and then to sum up their findings, as outlined in the report. Care should be taken to use language that will be understood easily by non-scientists, and to explain any unusual terms or processes. The entomologist will then be asked questions first by the prosecution and then the defence. Likely questions will focus on how the samples were collected, whether

they met peer reviewed protocols, (e.g. Amendt *et al*., 2007), why temperatures are so important to PMI estimation, the accuracy of forensic entomology estimates and what results were obtained.

8.8 Conclusion

Forensic entomology can make a highly effective contribution to criminal investigations, but in order for it to be of robust evidential value strict protocols should be followed with regard to collection and preservation of insect evidence, subsequent analysis and reporting. Ideally this should be conducted by an appropriately qualified forensic entomologist or under the guidance of such.

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Appendix 8.1 Entomological collection scene sheet (based on Amendt *et al*., 2007).

Collected by:_____Date_____Case No_____

Entomological Collection Scene Sheet

Client Name_____

Contact Name_____

Client Address_____

_____

_____

Telephone Number_____

Police Force (if not client)_____

Time of arrival:_____ Time of departure:_____

Scene of Recovery / **Mortuary** (delete as applicable**)**

Persons Present

Name	Job Title	Company

Address of Scene/ Mortuary _____

_____

_____

_____

_____

_____

Approved by: Name ..... Signature ..... Date .....

Appendix 8.1 (*Continued*)

Collected by:_____Date_____Case No_____

Specifications of deceased (Known / Estimate)

Age _____Gender _____Height_____ Weight _____

Position (tick more than one if appropriate):

Indoor ☐ Outdoor ☐ Aquatic ☐ Buried ☐ (estimated depth:_____)

Concealed ☐ Hanging ☐ (In contact with ground) Yes / No

Clothing:
Fully clothed ☐ Partial ☐ (record partial clothing on body sketch) Naked ☐

Covering:
Fully covered ☐ Partial ☐ (record partial cover on body sketch)

Body covered with:_____

General state of decomposition:

Fresh ☐ bloated ☐ active decay ☐ advanced decay ☐ dry/skeletal ☐
(if more than one decomposition state, record position on body sketch)

Mark the positions and provide details for evidence of scavenging or trauma on body sketch

Scene of recovery (tick more than one box where appropriate)

Urban ☐ Rural ☐

Outdoor:

Vegetation:

Woodland/Forest ☐ Grassland ☐ Heath ☐ Marshland ☐ Agricultural ☐

Other _____

Substrate:

Clay ☐ Sand ☐ Stony ☐ Man-made ☐ (define:_____)

Other_____

Exposure: Sun ☐ Shade ☐ Partial shade ☐

Indoor:

Building zone: Residential ☐ Commercial ☐ Industrial ☐ Agricultural ☐

Building type: Apartment/Flat ☐ House ☐ Garage/Storage ☐ Stable/Barn ☐

Other: _____

Approved by: Name ..... Signature ..... Date .....

Appendix 8.1 (*Continued*)

Collected by:_____Date_____Case No_____

Room: Kitchen ☐ Bedroom ☐ Bathroom ☐ Living Room ☐

Dining room ☐ Passage/Hallway/Entrance Hall ☐ Staircase ☐

Store room ☐ Study ☐ Loft ☐ Cellar ☐

Other:_____

Record details of dispersing insect evidence on Sampling Sheet

Floor level: _____

*Floor type (carpet, wood, concrete, etc.):*_____

Heated: ☐ *Thermostat setting:* _____

Insect access: Windows – Open ☐ Closed ☐ Ajar ☐

Doors – Open ☐ Closed ☐ Ajar ☐

Miscellaneous (e.g. car):_____

_____

_____

_____

Temperatures

Ambient 1 (2 m above ground): _____

*Ambient 2 (5 cm above ground):*_____

Body surface: _____

(If present) larval mass 1*:_____ larval mass 4*:_____
larval mass 2*:_____ larval mass 5*:_____
larval mass 3*:_____ larval mass 6*:_____

* please mark the positions on body sketch

*Interface between body and ground:*_____

Soil(at a depth of about 20 cm): _____

Water: _____

Record air temperature at scene of recovery for 5–10 days after discovery of body

Datalogger left at scene? **Y** / **N** Serial no._____ Stevenson Screen? **Y** / **N**

Datalogger with live samples? **Y** / **N** Serial no._____

Approved by: Name ..... Signature ..... Date .....

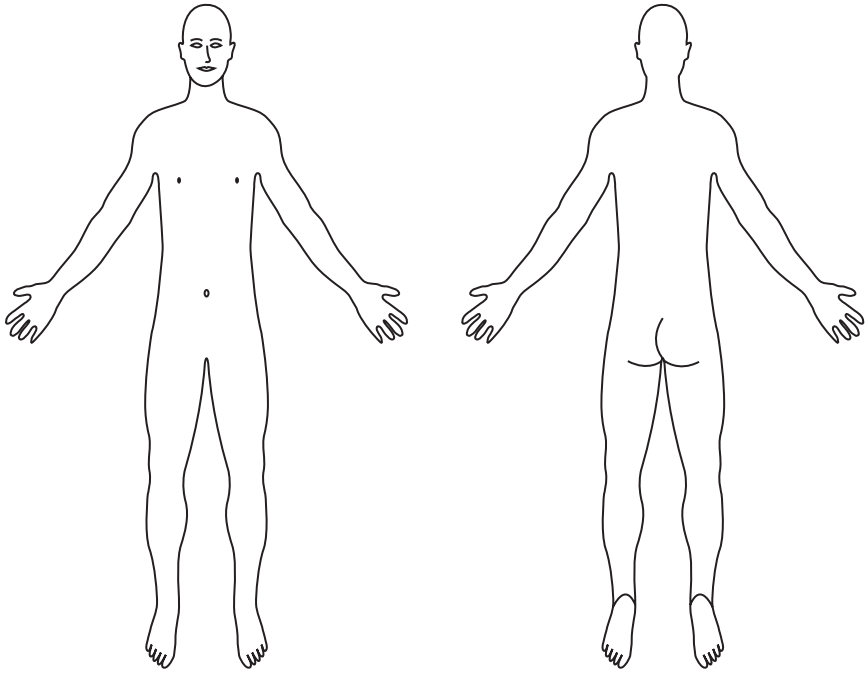
Appendix 8.1 (*Continued*)

Collected by:_____Date_____Case No_____

Key

Shade for partial clothing/cover ☐

F	fresh	EM_1, EM_2	egg masses
B	bloated	LM_1 , LM_2	larval masses
AC	active decay	W	wandering larvae
AD	advanced decay	P	pupae
D	dry/skeletal	EP	empty pupal cases
SC	scavenging	F	flies
T	trauma sites	B	beetles



Approved by: Name …….. Signature …….. Date ……

Appendix 8.1 (*Continued*)

Collected by:_____Date_____Case No_____

Sampling Sheet

E = Eggs **L** = Feeding Larvae*
W = Wandering larvae** **P** = Pupae
EP = Empty pupal cases **F** = Flies
B = Beetles

Sample N°	Approx. number	Type	Preserved (P) / Alive (A)	Location on body/scene ^
1		E □ L □ W □ P □ EP □ F □ B □		
2		E □ L □ W □ P □ EP □ F □ B □		
3		E □ L □ W □ P □ EP □ F □ B □		
4		E □ L □ W □ P □ EP □ F □ B □		
5		E □ L □ W □ P □ EP □ F □ B □		
6		E □ L □ W □ P □ EP □ F □ B □		
7		E □ L □ W □ P □ EP □ F □ B □		
8		E □ L □ W □ P □ EP □ F □ B □		
9		E □ L □ W □ P □ EP □ F □ B □		
10		E □ L □ W □ P □ EP □ F □ B □		
11		E □ L □ W □ P □ EP □ F □ B □		
12		E □ L □ W □ P □ EP □ F □ B □		
13		E □ L □ W □ P □ EP □ F □ B □		
14		E □ L □ W □ P □ EP □ F □ B □		
15		E □ L □ W □ P □ EP □ F □ B □		
16		E □ L □ W □ P □ EP □ F □ B □		
17		E □ L □ W □ P □ EP □ F □ B □		
18		E □ L □ W □ P □ EP □ F □ B □		

Preserved = killed in boiling water and preserved in 80% ethanol
Alive = keep them alive e.g. for rearing
* Larvae feeding on the body
** Larvae no longer feeding on the body, i.e. dispersing from the body, or hiding in clothes, in soil, under furniture and so forth
^ mark the positions on the body sketch

Approved by: Name ..... Signature ..... Date .....