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Best practice in forensic entomology—standards and guidelines

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Abstract Forensic entomology, the use of insects and other arthropods in forensic investigations, is becoming increasingly more important in such investigations. To ensure its optimal use by a diverse group of professionals including pathologists, entomologists and police officers, a common frame of guidelines and standards is essential. Therefore, the European Association for Forensic Entomology has developed a protocol document for best practice in forensic entomology, which includes an overview of equipment used for collection of entomological evidence and a detailed description of the methods applied. Together with the definitions of key terms and a short introduction to the most important methods for the estimation of the minimum postmortem interval, the present paper aims to encourage a high level of competency in the field of forensic entomology.

Keywords Forensic entomology · Quality assurance · Guidelines · Standards · Postmortem interval

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Introduction

Forensic entomology is the name given to the study of insects (or even other arthropods such as mites and ticks) that form part of the evidence in legal cases [25], but it is mainly associated with death enquiries. Knowledge of the distribution, biology and behaviour of insects found where a body has been discovered can assist many types of forensic investigation by providing information on when, where, and how, under certain conditions, a crime was committed or a person died [5, 11, 12, 23, 28]. The most important application is in the estimation of the postmortem interval (PMI, i.e. the time since death) [2, 22, 34].

Insect specimens, such as blowfly larvae (maggots) or adults, must be considered as physical evidence just as blood stains, fingerprints, hairs, fibres, or any other biological material [31]. Therefore, insects should be processed as evidence at the crime scene examination as well as at the autopsy [12, 26]. In the recent past, several publications have highlighted the important role of quality assurance in medicine in general (e.g. [30, 34]) and particularly in forensic science (e.g. [7, 17, 35, 37]). A systematic, quality-assured approach should also exist for collection, preservation and even packaging and transport of entomological samples, not only to prevent contamination or destruction of evidence and to guarantee the chain of custody, but also because forensic entomology deals with living organisms, which should be treated with care.

This paper represents the opinion of the board and the members of the European Association for Forensic Entomology (EAFE) on minimal standards in forensic entomology (FE), ratified at the third annual meeting in April 2005 in

Lausanne, Switzerland. It is intended to assist entomologists, investigators and pathologists involved in the practice of FE in death investigations. Its main purpose is to encourage a high level of competency in the field of FE, to promote and establish common standards of practice, especially with respect to the collection and preservation of entomological evidence and corresponding temperature data.

The following definitions are important to the development, understanding and use of the present minimal *standards* and *guidelines*.

- ***Standards*** (STD) are generally accepted and mandatory principles for practice in FE. Practical variation due to ambient or cadaver-specific factors is not expected. Because of their importance, they will be highlighted by italics and bold letters.
- ***Guidelines***, representing the main part of the present paper, are recommendations for practice in FE that identify a particular management strategy or a range of management strategies. Practice variation can be acceptable if motivated or based on the circumstances of the particular case since the adherence to such guidelines is voluntary.

It should be understood that adherence to minimal standards or guidelines cannot guarantee a successful outcome, but it will certainly minimise the risk of error and allow the authorities and possible external consultants to audit the quality of the presented expertise and to understand how the conclusions were reached. As the development and practice of FE is dynamic, the following standards will be subject to continual revision based on advances in scientific knowledge and methodology.

Equipment, tools and preservatives

The collection of entomological evidence at the crime scene, especially from the corpse, requires the wearing of protective clothing, mainly to avoid any contamination of the scene with fibres or other material from the investigator. In particular, it is strongly recommended that FE practitioners wear overalls, gloves and shoe covers or boots. For the collection of insects and additional information at the scene, the following equipment is recommended:

- Tool box
- Protocol sheets for writing down what specimens were collected, when and where (see Appendix)
- Dark graphite pencil or pen with waterproof and alcohol-proof ink (do not use standard inks, because they will dissolve in wet surroundings or if splashed with ethanol)
- Labels
- Fine and medium forceps (with different levels of spring tension for collecting adults and the more fragile immature insects)
- Spoons for collecting maggots
- Fine paintbrush for collecting eggs (after moistening the brush)
- Vials and storage boxes of different sizes for preserving living and dead insects
- Shovel or trowel for taking soil and leaf-litter samples and searching for buried larvae/puparia
- Robust plastic or paper (double bagged) bags for soil samples and leaf litter
- Sawdust or tissue paper for handling eggs and living larvae in vials or storage boxes
- Thermometer for measuring the body and ambient temperatures, as well as the larval mass temperature
- Ethanol (70–95%) for storing dead specimens
- Camera/video for picture documentation (photographic evidence should include a measurement scale)
- Material for sealing the samples (different in each country: e.g. sticker, sealing wax)
- Cooler bag with re-usable ice packs for storing living insect samples
- Handheld insect capture net for catching flying insects, if necessary
- Temperature data logger for measuring the scene temperature for the 5- to 10-day period after body recovery

Methods for collecting entomological evidence

If collecting specimens that are already *dead*, regardless of whether they are adult or immature, store them in 70–95% ethanol.

STD ***Use 70-95% ethanol to preserve specimens and, for health and safety reasons and because tissues are less well-preserved for morphological and molecular identification, do not use formalin/formaldehyde.***

If collecting *living* specimens:

Eggs Place on moistened tissue paper in vials. The vials should allow entry of air, but prevent the escape of hatched maggots. Transfer the samples to an expert for rearing within 24 h. If this is not possible, kill and preserve all specimens by placing them in 70–95% ethanol.

Larvae If possible, store the majority of individuals of each sample in vials under controlled conditions (i.e. temperature/humidity known); cool temperatures are most suitable (2–6°C). The vials should allow entry of air but prevent the escape of maggots. They should be lined with coarse sawdust or tissue paper to absorb fluids produced by the maggots. The living samples should be transferred to an expert for rearing within 24 h.

Kill the remaining specimens with very hot (>80°C), but not boiling water; immersion for 30 s is ideal to achieve best preservation [1]. Afterwards pour off the water and rinse once with ethanol before storing them in a vial with 70–95% ethanol. All procedures should be undertaken as soon after collection as possible and with all times recorded. If hot water is not available at the scene, the larvae should be killed as soon as possible at the laboratory, or the use of hot water could be replaced by

killing the larvae in a deep freezer (ideally −20°C) for 1 h. Do not insert living larvae directly into ethanol because they will darken within a few days due to putrefaction, and they will also shrink. Shrinkage will make the estimation of real length difficult and can hide some morphological characters.

STD ***Killing is recommended for ALL sampled specimens when the use of the insects for the enquiry is ambiguous at the time of collection and/or rearing is impossible.***

Pupae If possible, transfer pupae for rearing within 24 h; otherwise, store each sample in vials under controlled (temperature and humidity known) conditions. Cool temperatures of about 2–6°C are most suitable. The lid of the vials should be punched with small holes to allow entry of air.

STD ***Maintain living insect samples under known temperature conditions.***

Adults Live adults should be killed by placing into a vial, which is then put into a freezer (ideally set at −20°C) for 1 h; afterwards, store the dead specimens in 70–95% ethanol. If facilities are available for pinning adult specimens, then this can be done in addition, because it often simplifies identification. Adults that are newly emerged from their puparium should only be killed after allowing the wings to fully harden and the colouring to develop (after ca. 6 h).

Insect remnants Remnants such as pupal skins, empty puparia or beetle faeces, which document previous insect activity and presence, should be stored, when completely dehydrated, in dry conditions in vials or in 70–95% ethanol.

Where to collect entomological evidence

STD ***Always check the corpse AND the surroundings for entomological evidence.***

(A) On the corpse (at the death scene and during autopsy)

It is fundamental that collection of insect evidence and temperature data from the body requires the cooperation of the Scene of Crime Officer and/or the forensic pathologist, depending on the person in charge at the scene or at the autopsy. Nothing must be moved or taken from the corpse without a specific authorisation of the responsible person. In every case, extreme caution should be exercised when using forceps or other tools for collecting insects to avoid inadvertently inflicting postmortem artefacts on the corpse.

STD ***Sample different regions of the corpse and collect the evidence into different labelled vials:***

– Sites for sampling should include:

1. The natural orifices and eyes (preferred oviposition sites)
2. Traumatic wounds (preferred oviposition site)
3. At the corpse–substrate interface and under the body
4. In the pleats of clothes and pockets, shoes, socks, etc.
5. From the carpet, bag or material in which the body might have been wrapped
6. From the plastic body bag in which the corpse or the remains have been enclosed for transport to the place of autopsy and storage

– *Collect from everywhere on the corpse*, not only from the locations of most concentrated insect activity.
– *Collect specimens of every shape and size* of larvae/pupae, using different vials for different types and sizes. Try to collect specimens of every type, shape and size.
– *Sample size* will vary depending on the number of larvae found, but as a rough guide, it should range from all of the larvae, where fewer than 100 are available, to 1–10% of the larvae, where thousands are available.

– *If present, sample adult insects*:

1. Obviously recently emerged blowflies, which are recognised by a silvery appearance and shrivelled wings, with the behaviour of creeping or running on the ground.
2. Dead flies or beetles lying on the ground or, for example, on the window sills.
3. If possible, collect flying insects with an insect net.

(B) Around the corpse at the death (crime) scene

It is strongly recommended to collect entomological specimens from the surrounding area before removal of the remains and also from directly under and in close proximity to the remains after removal.

Search the area away from the body intensively up to at least 2 m and even further (up to 10 m), depending on the circumstances, for insects that might have dispersed from the body. Be aware of possible sources of dispersed insects other than the corpse, e.g., a dead rat or rabbit. Take a control sample at a distance from the body, (beyond the range at which insects are likely to have dispersed from the body, to obtain a background level of insect abundance). It

is especially important to look under stones, rocks and fallen logs outside and under carpets, pillows, skirting boards, etc. to ensure that insects are not missed:

1. At an **outdoor** scene, it is advisable to take soil samples up to 2 m away from the corpse, from more than one compass point up to a depth of at least 10 cm or even more depending on the circumstances and, if available, also collect some leaf litter or other soil-covering detritus.
2. At an **indoor** scene, check different rooms. Larvae can leave the corpse and disperse widely before pupariation (metamorphosis) and could, therefore, be found in different rooms.

STD ***Collect the most mature insect specimens that have developed on the corpse (adults, puparia, pupae, post-feeding and feeding larvae, or eggs) and remnants, such as empty fly puparia or beetle exuviae.***

Microclimatic conditions and ecological features of the scene

1. Describe the condition of the corpse (position, sunshine or shade, clothes, wounds, etc.) using protocol sheets and photographs/video.
2. Describe the scene in detail using protocol sheets and photographs/video.
3. Collect temperatures: ambient, body, ground surface, soil (up to 10 cm depth) and of any larval masses.
4. Request weather data for the general area of discovery of the body from the nearest meteorological station, for the period from last sighting of the deceased up to discovery of the body.
5. If possible, and depending on the availability of the crime scene, the temperature for the next 5–10 days should be recorded with a temperature data logger at the position of the corpse (ideally in a Stephenson screen, a special protective yet weather-permeable box) to correlate the data of the nearest meteorological station with the conditions at the actual site of discovery of the body. It is preferable that these measurements be made hourly, using an electronic data logger. If this is not possible, daily maximum and minimum temperature records should be obtained.
6. Temperature inside larval masses could be 10–20°C above ambient temperature; therefore, you should consider that the heat generated from larval aggregations could prevent the cooling effect of the body in refrigeration units.

STD ***Only use reliable temperature data to estimate a time of death based on the developmental stage of the insects.***

Documentation and the chain of custody

1. Document the name of the instructing authority and principal contact and the time and type of approach.
2. Document date and time of sample collection.
3. Specify the whole sample clearly with A SINGLE code (number and/or name); this code will be your reference to the case in the future, and it has to be placed on any sample.
4. Label each vial you use and note the position of sampling (e.g. head, leg, carpet) on a protocol sheet.
5. Seal each sample to guarantee chain of custody.

Processing entomological evidence at the laboratory

It is strongly recommended to send the insect evidence to an expert in forensic entomology, who is familiar with local species and in processing the specimens at the laboratory. In general, this laboratory should use operating procedures that ensure that all necessary samples and information are documented in a way that can be clearly traced in an audit.

(A) Arrival of specimens at the lab

– If the samples were not collected personally, make sure to document the following upon arrival of the samples:

1. Who is the instructing authority and what is the time and type of approach?
2. When were the samples received?
3. How were the samples transported (e.g. parcel post, courier)?
4. In what condition were the samples received (e.g. did some larvae obviously die during the storage/transport? Were there any seals? If yes, were they whole or damaged)?
5. Specify the whole sample clearly with A SINGLE code (number and/or name); this code will be the reference of the case in the future, and it has to be placed on every sample.
6. All of these parameters and data must be mentioned in the report.

– Label any storage box and vial with the specific code before removing the seal or opening the containers.
– Decide if specimens should be reared up to the adult stage. If yes, what quantity of specimens will be reared to the adult stage?

(B) Rearing immature stages

Any living, immature specimen that is not going to be killed should be placed immediately into a rearing environment (see [8] for an introduction on rearing of necrophagous insects).

STD ***Rear all stages under conditions of temperature and humidity that are controlled and recorded.***

This means that:

1. This is done ideally by using a certified, calibrated incubator with constant temperatures or, at least, by maintaining a careful documentation of the temperature patterns by using a certified temperature data logger inside the incubator.
2. If an incubator is not available, it is advisable as a minimum measure to rear larvae at ambient temperature, to be able to document the remaining part of the life cycle of the samples and to aid identification, because adults can be easier to identify than those in the immature stages. Assure a careful documentation of the temperature patterns, for example, with the use of a certified thermometer.
3. Monitoring larval growth should be done to ensure successful emergence of adults and to document the respective dates of moulting, pupation and hatching.

(C) Treatment of soil and leaf-litter samples

Because different stages of development show different levels of mobility the following is recommended:

1. Examine the samples visually by spreading them on a specially prepared area, such as a framed panel on the bench. Here, the main targets for search are immobile pupae and living larvae for further rearing. A sieve and water flotation can sometimes be helpful to sort soil samples, depending on the type of soil and the type of particulate matter, including leaf litter.
2. If you are not able to examine the soil samples immediately, store them in a refrigerator (~4°C) to avoid further development of immature stages and the growth of mould/fungus.

(D) Preparation and identification of insects

It goes beyond the scope of the present paper to give an introduction into the morphological features of necrophagous insects and their terminology. Valuable introductions exist (e.g. [10, 38]), but of course each geographic region has its own characteristic fauna and, very often, an appropriate identification key, which enables the investigator to identify the insects relevant to their case (for an overview, see [23]).

1. Label any identified or examined specimen, microscope slide, or other sample with the laboratory code for the individual case AND with the code of the specific vial. It must be clear, without any ambiguity, which individuals were the basis for the analysis. This will guarantee that a possible future specialist will be able to identify the specific sample on which the analysis was based.
2. If you are not able to examine and identify the insects, immature or adult, in an appropriate manner, keep them well-labelled in a vial filled with 70–95% ethanol.
3. 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 (e.g. with larvae of Sarcophagidae) or where morphological determinations have to be confirmed in complex situations (e.g. in the case of so-called sister or sibling species, which are morphologically similar or identical, especially in the young larval stages).

STD ***Identify immature specimens (especially larvae) using reliable identification keys and establish the most mature individual available in the sample. For larvae, for example, record the stage of development (first, second, third, post-feeding, pre-pupae).***

Period of insect activity and estimation of postmortem interval

Every death investigation relies on an accurate estimation of the time of death or the period since death, termed as the postmortem interval. PMI estimation is essential in every suspicious death investigation to reconstruct events and circumstances of death, to link a suspect to the victim, and to establish the credibility of statements made by witnesses. It is not just useful in criminal cases but also in civil cases. Indeed, even when the death is natural, accidental, or a suicide, such estimates can have judiciary implications in questions of insurance and inheritance [23].

Different methods exist for estimating the PMI. A pathologist usually determines time since death in the early postmortem period based on the postmortem changes in soft tissues such as stiffness (rigor), settling of blood (livor), body cooling (algor), and stages of decomposition [27]. However, often the sequence of such changes can only crudely approximate how long the individual has been dead, because most of these methods are to a degree unreliable and inaccurate. Many factors affect human decomposition, some directly associated with the body (e.g. age, constitution, integrity of the corpse, cause of death) and others associated with the environment (e.g. temperature, ventilation, air humidity, clothing, access of the body to animals). There are some mathematical models or statistical tools that include all of these variables for PMI calculation [14]. Using temperature methods alone or body fluids chemistry, some authors claim a maximum precision within a statistical confidence limit of 95%, but the

accurate assessment of death chronology is not possible more than 48–80 h after death, depending on the availability of equipment, the nature of the circumstances of the death and the environment [2, 14, 15]. In every case the longer the actual PMI, the less accurate the estimate of the interval. Even if the thanatological method can produce accurate results during days 1–3 postmortem (i.e. the early postmortem period), beyond that period, the entomological evidence associated with and around the corpse becomes much more important and can indicate the time elapsed since death up to a period of several weeks or even months.

Definitions

Postmortem interval

PMI is the time interval between death and recovery of the body.

Period of insect activity

A great diversity of insects arrive at decomposing human corpses, both to feed and to lay their eggs or larvae, but blowflies are usually the first group of insects to arrive after death. Therefore, blowflies represent the insects of greatest forensic importance, because they have the potential to give the most accurate information regarding the time since death. Periods of colonisation and rates of development and, therefore, the time of insect activity on and around the corpse depend on environmental parameters, especially temperature.

Minimum postmortem interval

It is crucial that investigators are aware that the period of insect activity (PIA) does not always correspond to PMI. This is because PIA could either be shorter than PMI, in the case of delay in access of the body to insects (e.g., initial burial, wrapping the corpse, or by concealment of the body in a sealed room or in a freezer), or longer as in the case of myiasis (larvae initially feeding on living tissues, that continue feeding on necrotic tissues after death). Nevertheless, such estimates of PMI can be very useful to investigators or magistrates. The entomological estimation of the PMI during the first weeks after death is based on the age determination of the immature stages of necrophagous flies [4, 18–20, 24, 32]. The calculation indicates when insect colonisation of the corpse took place. Therefore, aging the oldest insects on a body gives the time when the adult female insect first gained access to that body after death and, hence, a minimum time since death, often referred to as the minimum postmortem interval.

Upper development threshold

This is the temperature value above which insect development is adversely affected; higher temperatures are usually lethal. The upper development threshold (UDT) is species-specific. It can be neglected in most forensic PMI estimations because it is not normally exceeded within the ecological limits of the species; however, it can be exceeded within a large larval mass.

Lower development threshold or base temperature

This is the temperature value below which insect development stops. The lower development (LDT) threshold is also species-specific and can vary between life stages.

Accumulated degree hours/degree days

Because the rate of development of the immature stages is a function of the ambient temperature, the age of specimens is positively correlated with the summed thermal input they accumulate during growth. The standard technique used to estimate the rate of development of insects over a period of time with temperature compensation is termed accumulated degree hours (ADH) or accumulated degree days (ADD), which are a summation of temperature (°C) above the LDT multiplied by time (hours or days). The ADHs or ADDs that are required for certain species of insect to reach specific developmental stages have been determined by experimentation.

Larval mass temperature

The temperature inside a dense larval mass can be significantly greater than the ambient temperature due to the metabolic heat produced by frenetic feeding activity. Larval masses can be especially prevalent in large carcasses. The heat generated from such larval aggregations could have a major role on the development rate of larvae and, consequently, on the estimation of the PMI.

Methods for postmortem interval estimate

The application of the entomological method to determine the time of death consists essentially of two main procedures:

1. During the early postmortem period, the estimate is based on a direct age assessment of the oldest individuals that have developed on the body (minimum PMI).
2. During the late postmortem period, the estimate is based on the composition of the arthropod community as it relates to expected successional patterns.

Age determination

STD ***Before aging the specimens, they must be identified to the correct species using reliable identification keys.***

Age determination is made by comparing the stage of the oldest immature specimens sampled from the cadaver and the environmental conditions, to which it is estimated that they were exposed, with known growth rate data, recorded from baseline rearing of Diptera from the same zoogeographical area at known temperatures.

Because the rate of development depends on the genetic characteristics and geographic adaptation of the single species, as well as the environmental factors, as a reference for age calculations, it is mandatory to use experimental data dealing with the same species identified or, as an alternative and with appropriate caution, species genetically related (from the same genus, such as *Lucilia*) and coming from, as near as it is possible, the same zoogeographical area.

The crucial element in the determination of larval age is the progressive increase in total body size (length and weight) with time. However, length and weight are not the only criteria useful for aging larvae. The larval instar, and hence approximate age, can be determined by a microscopic study of the morphology of larval mouthparts and posterior spiracles. In addition, study of the size of the crop of a mature third instar larva can indicate whether it has completed feeding. It is known that larval growth is a function of the temperature to which they are exposed during development. As a consequence, it is critical that estimates of the temperatures to which the larvae were exposed are as accurate as possible.

STD ***To age fly larvae, it is essential to:***

1. ***Measure the size of the larvae (length and/or weight), their stage of development (first, second or third instar) and determine whether they are feeding or post-feeding.***
2. ***Accurately determine the temperatures to which the larvae were exposed during their development either on or off the body.***
3. ***Relate the size and feeding stage of the larvae to their age using suitable experimental references or a database of size and stage against age at different temperatures.***

The more mature stages, puparia and adults, can also be aged with varying degrees of accuracy by measuring how far their development has proceeded and, again, by comparing this to tables relating development to temperature. For convenience, Tables 1, 2, 3 and 4 review the related development periods (in hours and days) calculated

Table 1 Development periods in hours and in days (in parentheses) of *Calliphora vicina* calculated by several authors at different constant temperatures (°C)

T (°C)	Kamal (1958) from oviposition to emergence	Reiter (1984) from hatching to pupariation	Greenberg (1991) from oviposition to emergence	Anderson (2000) from oviposition to		Marchenko (2001)[a] from oviposition to	
				pupariation	emergence	pupariation	emergence
10			1,647 (68.6)				
10–12							
12						(19.1)	(38.8)
12.5			1,069 (44.5)				
14–16							
15.8				294.0–440.3 (12.2–18.3)	719.7–874.6 (30–36.4)		
16						(13.6)	(27.7)
18–19		(10)					
19			583 (24.3) (22.8)[b]			(11.2)	(22.8)
20.6				213.0–233.0 (8.9–9.7)	514.8–572.0 (21.5–23.8)		
22			(19.4)[b]			(9.6)	(19.4)
22–23		(8)					
23.3				202.8–279.0 (8.4–11.6)	454.0–499.5 (18.9–20.8)		
25			460 (19.2)			(8.3)	(16.9)
26.7	508 (21.2)						
27						(7.6)	(15.5)
30		(6.5)					

*See the original paper for possible ± SDs

[a]Marchenko (2001) has calculated a heat constant of 388 ADD for the development from oviposition to emergence and a lower development threshold of 2.0°C

[b]Data available in Greenberg and Kunich (2002)

Table 2 Development periods in hours and in days (in parentheses) of *Lucilia sericata* calculated by several authors at different constant temperatures (°C)

T (°C)	Kamal (1958) from oviposition to emergence	Greenberg (1991) from oviposition to emergence	Grassberger and Reiter (2001) from oviposition to		Anderson (2000) from oviposition to		Marchenko (2001)[a] from oviposition to emergence
			pupariation	emergence	pupariation	emergence	
15.8					382.3 (15.9)	775.0–917.2 (32.3–38.2)	
16							(29.6)
17			400 (16.7)	842 (35.1)			(25.9)
19		(16.3)	271 (11.3)	564 (23.5)			(20.7)
20			242 (10.1)	451 (18.8)			(18.8)
20.7					245.7–356.9 (10.2–14.9)	486.2–647.8 (20.3–27)	
21			221 (9.2)	379 (15.8)			(17.3)
22		345 (14.4)	202 (8.4)	339 (14.1)			(15.9)
23.3					264 (11)	468.5–624.5 (19.5–26.0)	
25			172 (7.2)	297 (12.4)			(12.9)
26.7	348 (14.5)						
27							(11.5)
28			155 (6.5)	275 (11.5)			(10.9)
29		296 (12.3)					(10.4)
30			149 (6.2)	268 (11.2)			(9.9)
34			139 (5.8)	259 (10.8)			

*See the original paper for possible ± SDs
[a]Marchenko (2001) give a heat constant of 207 ADD and a lower development threshold of 9.0°C

for the most commonly encountered blowflies in forensic entomology at constant temperatures during experimental rearings.

The collection of temperatures recorded at the scene for several days after the body is discovered aims to reconstruct the temperature at the scene of death for the interval between death and discovery of the deceased. Hourly temperatures recorded by a data logger (ideally one that is certified for accuracy or otherwise checked) placed at the crime scene for 5–10 days, can be compared with temperatures measured from the nearest weather station over the same time interval. A regression analysis of such data can help to reconstruct the real temperature regime at the scene. This can be especially important inside buildings, because there can be very big differences between external (weather station) and internal temperatures. Temporal compensation could be required to improve the regression, because changes in internal temperatures commonly lag behind changes in external temperatures by 1–3 h or more, due to the buffering effect of the building.

(A) Isomegalen/isomorphen diagram

The development of insects can easily be visualised in growth curves for various constant temperatures, for example in the isomegalen (introduced by Reiter (1984) [36]) and isomorphen (introduced by Grassberger and Reiter (2001) [18]) diagrams that illustrate morphological (length or stage) changes during development of the fly depending on the time and temperature. If the temperature is roughly constant, as in the case of some corpses found indoors, the age of the maggot could be read off instantly from its length. If temperature is variable, as in the case of corpses found outdoors, an age range can be estimated between the points where the observed morphological change (hatching, pupariation, eclosion or particular size) cuts the graph at the maximum and minimum temperatures recorded.

However, a PMI estimate simply based on larval size can be misleading if account is not taken of the decrease in length preceding pupariation, or of the potential shrinkage effect of certain killing and preservative solutions (see previous section; [1, 39]). It also depends on the conditions in which the measurements of case specimens are carried out, as they must be done similarly to those undertaken to establish the baseline database. For example, if the baseline data was prepared from boiled and fully extended specimens immediately after death, then the case specimens must be prepared in the same way.

In the literature, there are several isomegalen/isomorphen diagrams available for the species of most common forensic interest such as *Calliphora vicina*, *Lucilia sericata*, *Chrysomya albiceps*, *Protophormia terraenovae* and *Liopygia argyrostoma*. For details and related development data, see the original papers [18–21, 36].

Table 3 Development periods in hours and in days (in parentheses) of *Phormia regina* calculated by several authors at different constant temperatures (°C)

T (°C)	Kamal (1958) from oviposition to emergence	Greenberg (1991) from oviposition to emergence	Byrd and Allen (2001) from oviposition to		Anderson (2000) from oviposition to		Marchenko (2001)[a] from oviposition to	
			pupariation	emergence	pupariation	emergence	pupariation	emergence
15			335 (14.7)	458 (19)			(28)	(41.1)
16							(22)	(32.2)
16.1					440.8–525.8 (18.4–21.9)	716.3–863.3 (29.8–36)		
19		(15.6)					(13.3)	(19.5)
20			192 (8)	244 (10.1)			(11.7)	(17.2)
22		336.5 (14.6)					(9.5)	(13.9)
23					217.5–268.3 (9.1–11.2)	369.3–434.8 (15.4–18.1)	(8.7)	(12.7)
25			156 (6.5)	209 (8.7)			(7.4)	(10.9)
26.7	309 (12.9)							
27							(6.5)	(9.5)
29		279 (11.6)					(5.7)	(8.4)
30			148 (6.1)	208 (8.6)			(5.4)	(7.9)
35		(10)	112 (4.6)	148 (6.1)				

*See the original paper for possible ± SDs
[a]Marchenko (2001) has calculated a heat constant of 148 ADD for the development from oviposition to emergence and a lower development threshold of 11.4°C

(B) Accumulated degree hours/days calculations

Because the rate of development of the immature stages depends essentially on the ambient temperature, the age of specimens is positively correlated with the summed heat they accumulate during growth. The thermal history of each specimen can be expressed in ADH or ADD, i.e. temperature (°C) above base temperature multiplied by time (hours or days). The relation between the rate of development and ambient temperature is represented by a curve that is essentially linear in the midrange of a sigmoidal curve, with UDT and LDT above and below which development ceases. The total thermal input required for an insect to develop from the time of oviposition to any stage in development, e.g. the time of adult emergence, can be calculated in terms of ADH or ADD. The use of ADH/ADD is based on the hypothesised

Table 4 Development periods in hours and in days (in parentheses) of *Protophormia terraenovae* calculated by several authors at different constant temperatures (°C)

T (°C)	Kamal (1958) from oviposition to emergence	Greenberg and Tantawi (1993) from oviposition to		Grassberger and Reiter (2002)[a] from oviposition to		Warren and Anderson (2005) from hatching to		Marchenko (2001)[b] from oviposition to	
		pupariation	emergence	pupariation	emergence	pupariation	emergence	pupariation	emergence
12.5		1,454.4 (60.6)	2,176.8 (90.7)						
13						(29)	(52)	(30.8)	(48.3)
15				(22.3)	(37.8)	(22)	(35)	(22.2)	(34.9)
20				(13.1)	(22)	(11)	(20)	(13.1)	(20.6)
23		189.6 (7.9)	333.6 (13.9)					(10.5)	(16.5)
25				(9.6)	(15.8)	(8.5)	(14)	(9.3)	(14.6)
27	301 (12.5)							(8.3)	(13.1)
28						(7.3)	(11.5)	(7.9)	(12.4)
29		150 (6.2)	260.4 (10.9)					(7.5)	(11.8)
30				(6.1)	(11.5)	(6.5)	(10)	(7.2)	(11.3)
32						(7.3)	(11.5)		
35		120 (5)	220.8 (9.2)	(5.2)	(9.2)				

*See the original paper for possible ± SDs
[a]Grassberger and Reiter (2002) have calculated a heat constant of 240.2 ADD and a lower development threshold of 8.95°C
[b]Marchenko (2001) has calculated a heat constant of 251 ADD and a lower development threshold of 7.8°C

linear relationship between temperature and development. However, its use only appears to be valid when experimental temperatures used to generate the ADHs/ADDs are similar to the temperatures encountered in any actual forensic case to which these ADDs/ADHs are applied [4]. In fact, as the temperature range under study increases, the relationship between temperature and development becomes less linear, particularly at the extremes of the optimum range and at low temperatures (Fig. 2, and see [4]). Each developmental stage has its own total temperature requirement and each species requires a specific number of degree-hours/days to complete its development at a defined temperature. Data available in the literature for most common flies of forensic importance show differences of developmental times that can be attributed to variation, not only in extrinsic factors (e.g. experimental method) but also in intrinsic factors (e.g. geographic diversity of species physiology).

Although the ADH/ADD concept can be very useful in estimating the age of larvae in forensic cases, it must be used with appropriate caution because of (1) the inherently large variations in ADH/ADD that have been measured across the temperature range, for example, the differences in ADH measured for *L. sericata* at different temperatures were as great within publications as between publications (Fig. 1), and (2) the variation that has been observed in ADD/ADH at particular temperatures, for example, at low temperatures for *C. vicina* (Fig. 2). The variation seen in these figures could, in part, be due to variation in the base temperatures of different geographical populations of flies and it emphasises the importance of using, where possible, local developmental data to estimate the age of insect evidence.

STD ***Give the minimum duration of development for the oldest stage of insect.***

Arthropod succession patterns

The study of arthropod succession enables scientists to associate each species or group to a well-established decomposition stage [33, 38]. Knowing the chronology of insects colonising carrion in a certain area, analysis of the fauna on a carcass can be used to give a rough approximation of the PMI in the late postmortem period (cadavers in an advanced stage of decay). Forensically useful timetables indicating the relative abundance of different insect groups at different times are available, but much more work needs to be done in this area.

Despite the efforts of researchers to master all the variables affecting the composition of the carrion arthropod community, the PMI assessed with such method is, however, still very approximate: the longer the PMI, the less precise the PMI estimate becomes. When using the entire carrion insect community in forensic investigations, it is mandatory to have some familiarity with site-specific arthropods and a good knowledge of successional patterns based at least on a local experimental study on pig carcasses.

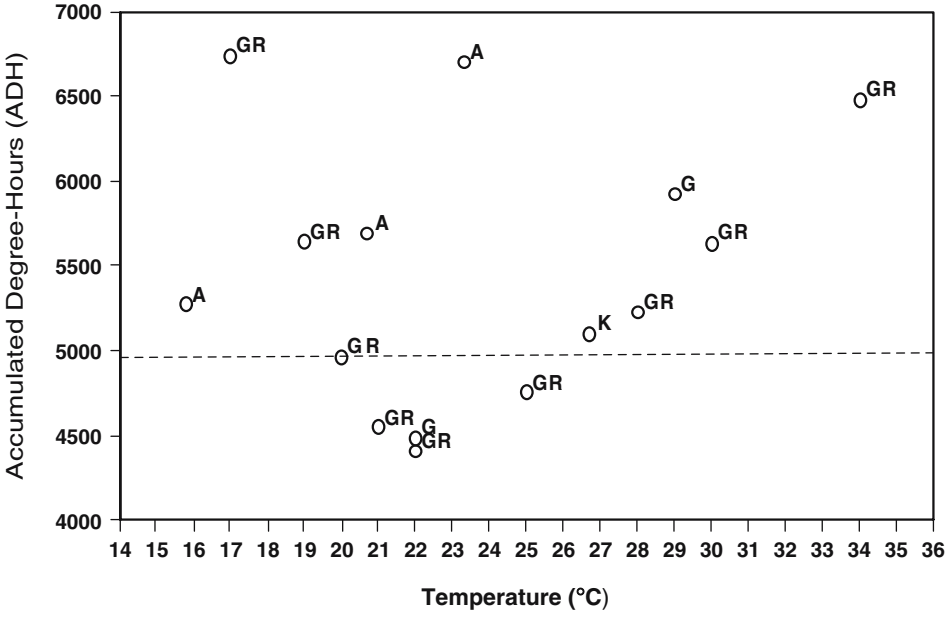

Fig. 1 Compilation of published data showing minimum ADH required from egg-laying to adult emergence of the blowfly *Lucilia sericata* at a range of constant temperatures [adapted from Anderson (2000) [4], *A*; Grassberger and Reiter (2001) [18], *GR*; Greenberg (1991) [22], *G*; Kamal (1958) [29], *K*; and Marchenko (2001) [32], *M*]. ADHs were calculated from published values of hours from egg-laying to emergence, taking a hypothetical base temperature of 9°C [40]. Note that the ADH requirement is very varied (range 4,407–6,736), even within individual publications wherein the only major variable was temperature, with no discernible temperature-related trend overall. All *points* represent observations in the laboratory under constant conditions at the indicated temperature. Marchenko's data (*dashed line*) is presented as a linear regression line derived from a calculated thermal constant, originally developed with the same base temperature of 9°C used here; hence, it is effectively horizontal

We have described, as objectively as possible, different entomological methods to estimate the PMI. This is not an exhaustive review. It has to be considered as an overview of existing methods to estimate the postmortem interval with insects, giving advice on the most important "do's" and "dont's". Such recommendations are not fixed, but will be regularly updated as this discipline evolves.

Final comments on the standards and guidelines for interpretation of insect evidence

This paper provides a framework of internationally accepted standards and approaches for collecting, packaging and transmission of entomological evidence. It will enable the entomologist or the crime scene technician to collect and provide samples to a high quality, which allows for subsequent application of appropriate entomological expertise. Some of the techniques described are basic and none are new to the literature; however, a broad range of professionals, such as crime scene technicians, forensic pathologists, anthropologists, botanists and entomologists, could be involved in the collection of entomological evidence, many of whom might be unfamiliar with the dispersed literature on forensic entomology.

A death investigation usually requires the coordination of a multidisciplinary effort with a number of professionals involved, such as investigators, magistrates and scientists. Only by cooperation between such professionals can a full understanding of the evidence recorded at any crime scene and at the autopsy be obtained [13]. The different institutions to which people belong (e.g. medicolegal institute, biological sciences laboratory, forensic science institute) can lead to differences in how forensic entomology is approached, for example, the people involved, equipment and methodologies.

Of all the professional collaborations needed, a close interaction of the forensic entomologist with the forensic pathologist is, in particular, strongly recommended. The pathologist makes the external and internal examination of the cadaver, analyses the extent of antemortem injuries and postmortem changes, as well as supervises the recovery of physical evidence. Information provided by the forensic pathologist and co-investigators could be fundamental for interpreting insect evidence and determining the period since commencement of insect activity, based on either the composition of cadaver fauna or on the age of developing insects.

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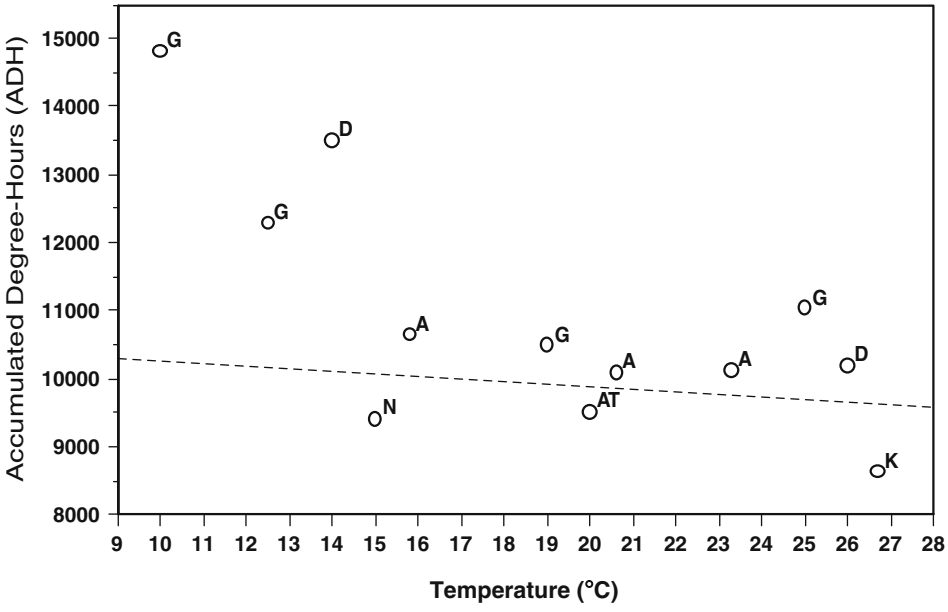


Fig. 2 Compilation of published data showing minimum ADH required from egg-laying to adult emergence of the blowfly *Calliphora vicina* at a range of constant temperatures [adapted from Ames and Turner (2003) [3], *AT*; Anderson (2000) [4], *A*; Donovan et al. (2006) [16], *D*; Greenberg (1991) [22], *G*; Kamal (1958) [29], *K*; Marchenko (2001) [32], *M*; and Nuorteva (1977) [34], *N*]. ADHs were calculated from published values of hours from egg-laying to emergence, taking a hypothetical base temperature of 1°C [16]. Note that the ADH requirement is fairly constant (9,000–11,000 ADH) except at temperatures below about 15°C. Nuorteva's data was based on field studies at the mean temperature indicated. All other *points* represent observations in the laboratory under constant conditions at the indicated temperature. Marchenko's data (*dashed line*) is presented as a linear regression line derived from a calculated thermal constant, originally developed with a base temperature of 2°C. It has a negative slope, rather than being horizontal, because the base temperature used here was 1°C, as for all other data

Appendix

Protocol sheet for the collection of entomological evidence (page 1/3)

Collected by _____ date/time _____ Case N^0/sign _____

Specifications

Age _____ Sex _____ Height _____ Weight _____

Position: buried ☐ (estimated depth: _____) above ground ☐
lying ☐ hanging ☐ (in contact with the ground? ☐
in water ☐
remarks: _____

Clothing: entirely ☐ partial ☐ naked ☐
body covered ☐ with _____
remarks: _____

Degree of decomposition: fresh ☐ early decomposition ☐
advanced decomposition ☐ skeletonization ☐
remarks: _____

Evidence of scavengers *: _____

Wounds *: _____

Scene of death

Outdoor: Forest ☐ Field ☐ Pasture/Grassland ☐ shrubbery ☐
Public park ☐ on grass/soil ☐ on sealed ground ☐

Building: Garage/Storehouse ☐ Barn/Stable ☐ Dwelling house ☐ Cottage ☐
ground (carpet, parquet, etc.): _____
which room: _____ heated: ☐ open window: ☐

Miscellaneous (e.g. car)**:** _____
remarks: _____

Temperatures

ambient 1 (2 m above ground): _____ **ambient 2** (5 cm above ground): _____
Body surface: _____ **If present, larval mass 1***: _____ **larval mass 2***: _____
larval mass 3 *: _____ **Interface between body and ground**: _____ **Soil** (in a depth of about 20 cm): _____ **Water**: _____

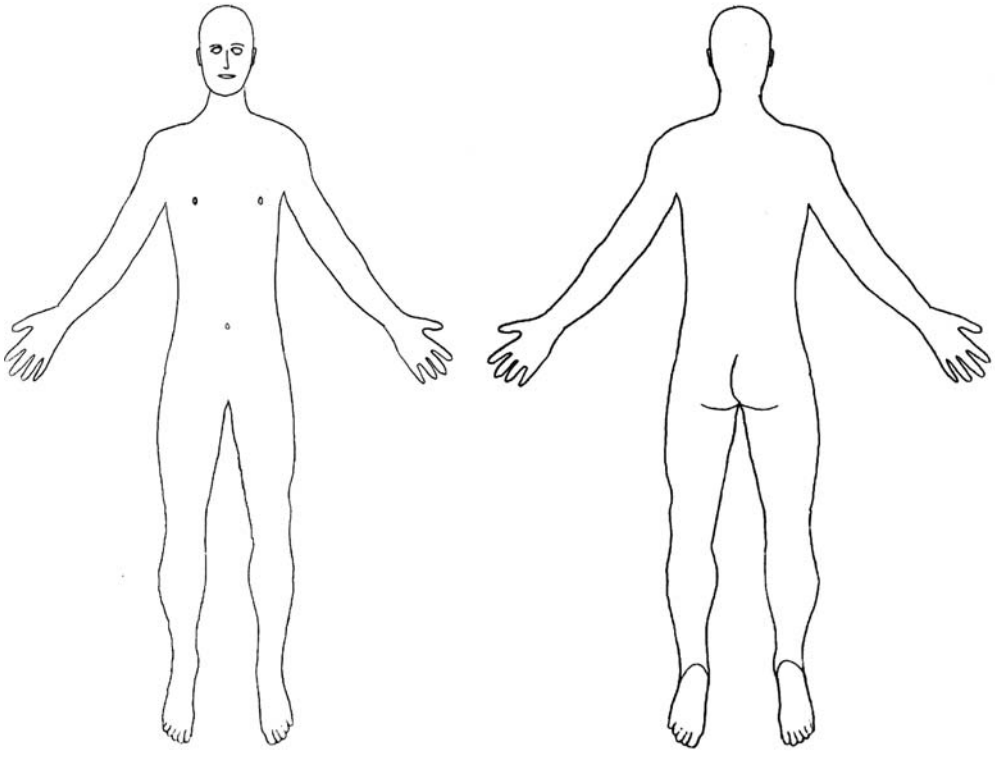
Record air temperature at the scene of death for 5–10 days after the discovery of the body!

* please mark the positions on page 2 of this sheet

Protocol sheet for the collection of entomological evidence (page 2/3)

Collected by _____ date/time _____ Case N^0/sign _____

Please use this sketch for marking
- partial clothing (hatched)
- traces of scavenging (SC ⟶)
- wounds (W⟶)
- larval masses (LM_1, LM_2,. ⟶)
- sample locations (1, 2, 3, …)



Protocol sheet for the collection of entomological evidence (page 3/3)

Collected by _____ date/time _____ Case N°/sign _____

L = Larvae **P** = Pupae
AF = adult flies **AB** = adult beetles
E = Exuviae

Sample N°	Approx. number	Type	pres. / alive [a]	Location on body *
1		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
2		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
3		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
4		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
5		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
6		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
7		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
8		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
9		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
10		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
11		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
12		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
13		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		
14		L ☐ P ☐ AF ☐ AB ☐ Ex ☐		

[a] pres. = killing and preserving; alive = keep them alive for e.g. rearing

* please mark the positions on page 2 of this sheet

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