5

Sampling at the crime scene

The most efficient way to respond to an invitation to attend a crime scene is to have ready a carrying case containing all of the requirements for collecting entomological specimens. This means that there is no great delay in reacting to a request and most contingencies can be addressed. If you are asked to attend the crime scene by the police, you need to collect sufficient samples so that material can be made available, if necessary and requested, for a fellow forensic entomologist to make his/her own assessment for the defence. This is imperative, since if the body is buried or cremated and the relatives wish to view it prior to disposal, they require the body to be insect-free (it may also be that those instructing you in other contexts need to undertake hygiene control activities, which mean that the environment concerned will change; so speed and good sampling techniques are required in this situation).

5.1 Entomological equipment needed to sample from a corpse

The entomological equipment (Figure 5.1) required includes plastic or polycarbonate screw-top sampling jars for both preserved specimens and live cultures, forceps, stepping plates to preserve the scene from contamination, a killing jar containing ethyl acetate, labels, indelible markers with fine points, fine forceps, artists’ paint brushes, an entomological net and killing agents for larvae, such as boiling water, and an insect preservative. A number of preservatives could be used, including 70–80% alcohol, KAAD and Kahle’s solution; each has its benefits.

Kahle’s solution contains both a fungal control and a preservative. It has been used at the University of Lincoln for 5 years and has preserved the samples used in a teaching collection in the same flexible condition as they were when the larvae were first killed. Alcohol has also been used, but this has required that the samples were more frequently curated than when using Kahle’s solution, because of evaporation.
Figure 5.1  The contents of an entomological scene-of-crime case, with equipment

Kahle's solution can also be used to kill larvae if all else fails, although this is not a recommended approach. It is a preservative for dead adult insects, and so provides a means of combining uses and limiting the amount of equipment and chemicals required at the scene. References in the Further Reading section provide details of the effects on size of several preservatives and indicate why it is valuable to kill the larvae using boiling water, or water at a temperature just less than boiling (Adams and Hall, 2003).

Because live specimens must be recovered from the site, it is necessary to bring some food for them. Liver, such as pig's liver, or minced (ground) beef has been found to be the most suitable (although it should be noted that research indicates that larvae show variable growth on different body parts). The food should ideally be at room temperature, not frozen or chilled, when the maggots are placed upon it. For the return journey the cultures should be kept in as low a temperature as possible, ideally below the base temperature of the specimens. A mobile refrigerator for the car or van, or a cool box with artificial ice blocks, would be ideal. A thermometer should be included in the container to ensure that the temperature during transport can be confirmed.

A carrying box, or packaging for the specimens, should be included. The sample jars of preserved and live specimens, from each site on the body, should be retained
Box 5.1 Composition of Kahle’s solution*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% Ethyl alcohol**</td>
<td>30.0 ml</td>
</tr>
<tr>
<td>Formaldehyde*</td>
<td>12.0 ml</td>
</tr>
<tr>
<td>Glacial acetic acid##</td>
<td>4.0 ml</td>
</tr>
<tr>
<td>Water</td>
<td>60.0 ml</td>
</tr>
</tbody>
</table>

*Care should be exercised in the storage of these chemicals.
**Ethyl alcohol is flammable.
*Formaldehyde is toxic.
##Glacial acetic acid is CORROSIVE; the acid should be added slowly to water and NOT the other way round.

together in pairs. Where samples are being taken by a crime scene investigator (scene of crime officer, or SOCO) rather than the forensic entomologist, it is necessary to package the samples and seal them, so that the integrity of the sampling is not at risk. These storage packages can be individual cardboard boxes, which are sealed with both preserved and culture samples from the same site on the body, in the same package. In this instance, the package requires to have holes punched in it and the lids to the culture jars need also to have holes, or a porous covering, which is firmly attached to the top of the container. Larvae are ‘escape artists’ and will push through a top if it is not secured. If this happens, your evidence will have escaped! The French Gendarmerie use polythene bags which are appropriately labelled and sealed as their means of packaging at a crime scene (Figure 5.2). Pin holes are made through the bag to prevent a build-up of carbon dioxide, whilst preventing the larvae from escaping.

In order to kill larvae from each colonization site on the body, they are immersed for 30 seconds in recently boiling water, to fix the larvae at their maximum length. Water can be brought to the crime scene in a thermos flask, or prepared on-site using a small camping stove and kettle (matches or a gas lighter are also required if you are boiling water on site!).

The general habitat at the crime scene should be recorded. This includes: whether the body has been wrapped or covered in some way (Figure 5.3); if indoors, whether the windows are open or closed; the slope of the ground if the crime scene, or where the body was found, is outside; the nature of any vegetation and a general site description, along with associated photographs, should be recorded. The crime scene temperature must also be recorded, along with the degree of light or shading at the scene.

Thermometers should be included in your equipment case. These thermometers should be calibrated so that they read accurately and do not give readings which have to be corrected. For safety reasons, if a digital probe thermometer is not used, it is better to use an alcohol thermometer rather than a mercury thermometer. The
thermostat should be noted on any central heating units which operate indoors and which might dictate the conditions in the building. If at all possible, a weather recorder should also be brought on site, if it is an outdoor location, so that the temperature, light intensity, humidity and wind direction and speed can all be
5.2 The sampling strategy for eggs

Once permission from the senior investigating officer has been obtained, the body should be searched in an orderly sequence. The head region is examined first and then the trunk is searched, moving along towards the legs and toes, which are separated and checked. Any wounds are specifically noted. Once one side has been checked, the body should be turned over and the under-side should be examined. Clothing can be examined cursorily on site. In particular, the pockets, sleeves and clothing folds can be checked at the scene with the agreement of the officer in charge. A more thorough search is possible at the mortuary when the clothes, if present, are stripped from the body. Fly eggs are laid in batches and can be mistaken for everything from yellowish white mould to sawdust, or an encrusting of salt on the body; beetle eggs are often laid individually, so may be easily missed at the crime scene.

Fly eggs are normally laid in or near dark, moist orifices of the body, such as the ears, nose, eye lids, mouth or genitalia. They may also be laid in folds of skin behind the ears, in joint creases, or on clothing which has absorbed body fluid exudates. So it is important that all sides of the body are examined and it may be necessary to attend the post mortem to check further for insects, if the body is fully clothed or has been wrapped in something. The individual clumps of eggs should be picked off and carefully placed in a container without any food. The humidity in the container can be maintained by using a damp paper towel placed in the tube to stop the eggs drying out.

Each sample should be given an item number and the crime scene details. The label should be written in indelible ink (not ball point ink, as this will not survive damp conditions). The label should include the name of the crime scene investigator who collected the sample (Figure 5.4), the officer in charge of the

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Crime Scene No
Officer in charge
Collector
Date
Item No.
Location and description
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Figure 5.4 A label containing information about the scene, date, collector, the crime number and item number should be included inside and outside the collecting jars used at a crime scene
case, the case number, the item number, the date and location on the body from which the sample was taken. This label should be placed on the body of the container, whilst a non-adhesive version is placed inside the container.

Placing this information on a label both within the container and outside it limits the likelihood of losing the information and ending up with a sample of unknown origin. The easiest way of getting a paper label into a container is to roll it round a pencil or paintbrush handle and deposit the roll through the neck of the container, where it unrolls. These data must also be recorded in your scene log.

5.2.1 Larvae

Larvae will be located as the body is searched for eggs. They too tend to be in the orifices, such as the eyes, ears, nose and so on, including any wounds which were made on the body. The larvae should be collected from each site in batches of 20–30 per jar, so that no additional heat or ammonia is generated during transit. More than one collection jar per infestation site may be needed. The first instar is the smallest and most vulnerable of the three larval stages and the larvae, if sampled at this stage, can easily die. It is necessary, therefore, to protect them from drying out when collecting and culturing these from a corpse at a crime scene.

Boiling water is poured into a container such as a styrene cup or a collecting jar to a depth of 3 cm, and larvae which are to be preserved from the specific site are then added. They are left immersed in the water for at least 30 seconds before the contents of the jar are poured through a small sieve and collected in a large waste container. Large bottled water or catering fruit juice containers make excellent waste containers. The contents of the waste container, when full, can be poured down a foul sewer or toilet, away from the crime scene.

Larvae are known, when they reach late second and third instar stages, to mass together. These maggot masses are capable of raising the temperature above ambient and the extra heat can influence the rate of larval development. If a larval mass is noted, it should be photographed and the mass temperature should be taken, prior to the location being sampled. The temperature of every maggot mass should be taken at each site on the body, so that this can be taken into consideration when calculating the crime scene thermal history.

5.2.2 Pupae and puparia

Fly puparia are usually found some distance from the body. The third instar post-feeding larval stages migrate and can be found in soil 3–5 cm below the soil
surface, in pockets, under carpets, in leaf litter or in any nooks and crannies which are available in buildings. If the puparia are still on the body, then either there may have been some restriction to larval migration, or a particular species of insect is indicated. Puparia change colour from white to dark brown over time, so all puparia, of whatever colour, should be recovered.

An organized search strategy should be used to do this. The ideal is to search on a grid of a metre apart over a 36 square metre area surrounding the body, if it is not in a house. This is a slow, time-consuming activity in which the soil should be sampled at the intercepts of the grid, using a trowel to a depth of 10 cm. The soil may need to be sieved over a tray, or it can be hand-searched. As previously indicated, the puparia recovered are placed in a container with a moist paper towel and suitably labelled. They do not require feeding but should be taken back to the laboratory for identification. The puparia should be cultured through to emergence if at all possible, so that species identification can be confirmed. The puparial case should also be retained as additional evidence. Those which do not hatch provide the examples of preserved specimens from the scene.

5.3 Catching adult flying insects at the crime scene

Flying insects present at the scene should be collected first using a net, before hand-collecting any specimens from the body. This is because they are most easily captured using a net and may disappear if disturbed. The net is flicked from behind the insect in an upward sweep, catching it within. Then, with a wrist swing, the net should be folded over at the end to contain the insect. At this point the bag can be grabbed with the other hand (which hand depends on whether you are left- or right-handed) and the insect, in the net base, can be restricted so that a container can be placed over it (Figure 5.5). A firm shake usually keeps the insect in the bottom of the tube for sufficient time to put a lid on top.

These insects can either be kept in individual killing jars, or they can be retained until dead in a single killing jar, as a collection of flying insects from the crime scene. Then they are transferred to individual specimen jars. Since insects are mobile, these insects are representative of the crime scene as a whole. In all cases, accurate labelling and recording is imperative.

If the crime scene is a car, relevant evidence can be obtained by collecting any insects which have been trapped on a radiator grill, bonnet or the windscreen (wind shield) of the vehicle. This may provide details of movement of the body. The temperatures in the car may be important, as inside the vehicle is likely to get quite hot and may affect the speed of the insect development, where flying insects have been able to gain entry and lay eggs.
5.4 Catching adult crawling insects at the crime scene

Insects such as beetles, which are visible on the surface of the body, can be collected by hand-picking and placed in individual, labelled containers. This is a sensible precaution, since beetles may be carnivores and eat any other specimens, thereby destroying your evidence. In an indoor crime scene, it is useful to check the nooks and crannies of the room for crawling insects, as this provides further information about predators and the conditions in which the body has been found. Leaf litter, or ground cover, in an outdoor scene can also be collected at regular points and the contents sieved or again hand-picked. Pitfall traps can be used to catch crawling insects near the body if it is an outdoor crime scene.
Tulgren funnels (Figure 5.6) can be used to recover the soil organisms which are living under the body. Several samples of soil (around 5 g each) are collected. Each is placed in a Tulgren funnel and a light positioned above the sample. As the soil dries out, the organisms are driven down into the container of 70% alcohol below. These can later be identified to give a profile of the specimens below the body and elsewhere at the crime scene.

5.5 Obtaining meteorological data at the crime scene

It is extremely important to determine the temperature at which the insects were growing on the body before it was discovered. The estimates of time since death rely on the figures gleaned at the crime scene and those determined subsequently from other sources.

The body temperature should be determined by placing a thermometer on the body surface. The temperature of the air should be taken at a height of 1.1 metres
(4 feet); this provides a measure of ambient air temperature at a comparable height
to those taken at the meteorological station. Care should be taken to avoid holding
the actual thermometer; use a protector or a rubber band wound round the end. Do
not expose the thermometer to direct sunlight, as this will raise the temperature
and give a false reading – your body may provide some shade. The temperature
directly beneath the body should be taken, followed by the soil temperature. To
take the temperature of the soil, it is better to use a soil thermometer so that there
is little chance of the thermometer breaking as it is forced into the ground.

A copy of a possible protocol for the collection of specimens from the crime
scene is presented in Appendix 1.

5.6 Review technique: investigating the influence of
larval location

5.6.1 Introduction

Thigmotaxis, or the desire for physical contact, is a particular feature of dipteran
larvae and influences their tendency to both form larval masses and also to ‘hug’
the sides of containers. The ‘maggot mass’ can raise the temperature well above
ambient. Catts (1992) recorded a temperature of a ‘maggot mass’ 20°C greater
than the highest ambient temperature at the time. This practical task allows you
to demonstrate this aspect of larval behaviour and to relate numbers of larvae to
temperature (a video clip of maggots demonstrating thigmotaxis is on the website
at: www.lincoln.ac.uk/entomology).

The experiment below also simulates the effect of different numbers of larvae
at different colonization sites on a body at a crime scene, so that you are aware of
why this has to be taken into consideration when deciding the ambient temperature
experienced by the larvae.

5.6.2 Safety instructions (COSHH)

- Fluon can be an irritant if it touches the eyes or skin.
- Wear gloves and a laboratory coat for this activity and observe normal laboratory
etiquette.

5.6.3 Materials

15 Boiling tubes
Alcohol thermometer
5.7 FURTHER READING

Pen or indelible marker
1800 late second instar larvae
Labels
Fluon, 50:50 mixture with distilled or deionized water

5.6.4 Method

1. Take five boiling tubes and place a layer of diluted Fluon around the top of each, to prevent the larvae escaping.

2. Into the first tube, place 40 larvae; into the second, 80 larvae; into the third, 160 larvae; and into the fourth, 320 larvae.

3. Keep the final tube empty. This is your control to determine the ambient temperature.

4. Repeat this distribution of larvae into two lots of five further sets of boiling tubes, so you have three replicates of each number of larvae.

5. Place the boiling tubes away from direct sunlight, so that you have randomly assigned numbers of larvae side by side, in different rows. This ensures that you do not have any bias in your results because of position of the boiling tube.

6. Record the temperatures of each mass of maggots by inserting a thermometer into the mass after 30 minutes of equilibration.

7. Record the ambient air temperature by taking the temperature of the fifth tube at the same time as you sample the larval temperatures.

8. Repeat this temperature assessment after a further period of 30 minutes.

9. Calculate the mean and standard deviation of the temperatures for the populations of maggots for the three replicates of 30 minutes.

10. Present the results of your experiment as a graph of larval numbers (x axis) against temperature (y axis). What did you note about the effect on temperature of the increased larval numbers?

5.7 Further reading


Dadour I. and Cook D. 2005. *Insects and Forensic Entomology: Flies Commonly Associated with Corpses in Western Australia.* University of Western Australia: Perth.

