Dorothy Gennard

FORENSIC ENTOMOLOGY

An Introduction

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Forensic Entomology

Forensic Entomology

An Introduction, Second Edition

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Contents

List of plates ix

List of figures xiii

List of tables xvii

Preface xix

Acknowledgements xxi

1 The scope of forensic entomology 1

1.1 Forensic entomology in urban contexts 1
1.2 Stored product infestation and forensic entomology 2
1.3 Forensic entomology in the medico-legal context 3
1.4 The history of forensic entomology 6
1.5 Professional associations for forensic entomologists 9
1.6 The UK regulator for forensic science 10
1.7 Web addresses of relevant organisations 11

2 Forensic entomology, DNA and entomotoxicology 12

2.1 Preparation of specimens for molecular analysis 15
2.2 Methods of analysis and sources of information 16
2.3 Alternative methods 20
2.4 Validity of methodologies 21
2.5 The use of other molecular means of insect species determination 23
2.6 Insects and entomotoxicology 24
2.7 Forensic applications of arthropod behaviour for chemical analysis 27

3 Insects and decomposition 29

3.1 Indicators of 'time of death' 29
3.2 Stages of decomposition of a body 30
3.3 Volatiles released from the body during decomposition 36
3.4 Decomposition in specific circumstances 38

4 Identifying flies that are important in forensic entomology 42

4.1 What is a fly and how do I spot one? 48
4.2 The fly lifecycle 53
4.3 Forensically important families of flies 60
4.4 Members of other orders that have forensic relevance in aquatic cases 73
4.5 Review technique: larval spiracles or mouthparts – preparation of whole slide mounts 75

5 Key for the identification of European and Mediterranean blowflies (Diptera, Calliphoridae) of medical and veterinary importance – adult flies 77

5.1 Introduction 77
5.2 Key 80

6 Identifying beetles that are important in forensic entomology 82

6.1 What do beetles look like? 82
6.2 The life stages of the beetles 87
6.3 Selected forensically relevant families of beetles 88
6.4 Features used in identifying forensically important beetle families 89
6.5 Identification of beetle families using DNA 97
6.6 Key to selected forensically relevant families in the order Coleoptera 98

7 Sampling at the crime scene 101

7.1 Entomological equipment to sample from a corpse 101
7.2 Catching adult flying insects at the crime scene 104
7.3 The sampling strategy for the body 106
7.4 Sampling at aquatic crime scenes 108
7.5 Obtaining meteorological data at the crime scene 109

8 Rearing insects and other laboratory investigations 111

8.1 Transporting entomological evidence to the laboratory 111
8.2 Laboratory conditions for fly rearing 112
8.3 Methods of maintaining and rearing insects – terrestrial species 113
8.4 Dietary requirements of insects reared in the laboratory 116
8.5 Beetle rearing in the laboratory 117
8.6 Methods of maintaining aquatic species 119

9 Calculating the post mortem interval 121

9.1 Working out the base temperature 123
9.2 Accumulated degree data 124
9.3 Calculation of accumulated degree hours (or days) from crime-scene data 127
9.4 Sources of error 128
9.5 Use of larval growth in length to determine post mortem interval (isomegalen diagrams and isomorphen diagrams) 130
9.6 Calculating the post mortem interval using succession 132

9.7 The effects of hymenopteran parasitoids on post mortem interval determination 137
9.8 Review technique: interpretation of data from a crime scene case study 137
9.9 Further reading 138

10 Ecology of forensically important flies 139

10.1 Ecological relationships of some forensically relevant families 140
10.2 Specific family features 144
10.3 Fly infestation of the living 151
10.4 Flies influencing the crime scene 154

11 The ecology of some forensically relevant beetles 156

11.1 Ecology of carrion beetles (Silphidae) 157
11.2 Ecology of skin, hide, and larder beetles (Dermestidae) 159
11.3 Ecology of clown beetles (Histeridae) 163
11.4 Ecology of chequered or bone beetles (Cleridae) 164
11.5 Ecology of rove beetles (Staphylinidae) 165
11.6 The ecology of dung beetles and related families 166
11.7 Ecology of ground beetles (Carabidae) 168

12 Investigations in an aquatic environment 169

12.1 Decomposition and submergence in water 170
12.2 The nature of the water bodies in which submergence may take place 176
12.3 Methods of establishing time since corpse submergence – indicator species 179
12.4 Attractants to the corpse 182
12.5 Methods of culturing aquatic insects 182
12.6 Algae an alternative source of determining time since submergence 182

13 The forensic entomologist in court 184

13.1 The expert's report 185
13.2 The content of the expert's report 187
13.3 The forensic expert in the courtroom 194
13.4 Communicating entomological facts in court 195
13.5 Physical evidence: its continuity and integrity 195
13.6 The code of practice for experts 196
13.7 Use of single joint experts 198
13.8 Practical assignment – writing an expert report using the post mortem calculations generated from Chapter 9 198
13.9 Further reading on presentation in court 198
13.10 Web site addresses 199

Appendices 201

Glossary 205

References 216

Index 241

List of plates

2.1 Electropherogram
3.1 Decomposition of a human body
3.2 Fresh stage of decomposition
3.3 Bloat
3.4 Active stage of decay
3.5 Advanced stage of decay
3.6 Skeletal remains
3.7 A cadaveric island surrounding the body where vegetation has died away
4.1 A Tabanid larva
4.2 Emerging calliphorid with inflated ptilinum
4.3 Transition from larval stage L2 to larval stage L3
4.4 Puparial colour changes in *Calliphora vomitoria* Linnaeus within the first 25 hours of pupariation
4.5 A pair of Piophilids taken 'in cop'
4.6 Head of *Piophila casei* Linnaeus
4.7 The Chironomidae
5.1A–F General morphology of blowflies. A – habitus, *Chrysomya albiceps*, male; B – head profile, *Calliphora uralensis*; C – thorax, dorsal view, *Lucilia sericata*; D – wing, *Protophormia terraenovae*; E – male terminalia, *Lucilia bufonivora*; F – ovipositior, *Lucilia illustris*.
5.2A–N Details of morphology of blowflies. A – calypters, *Cynomya mortuorum*; B – calypters, *Lucilia caesar*; C – greater ampulla, *Chrysomya albiceps*; D – greater ampulla, *Cochliomyia hominivorax*; E – habitus, dorsal view, *Chrysomya marginalis*; F – head profile, *Chrysomya albiceps*; G – head profile, *Chrysomya megacephala*; H – head profile, *Phormia regina*; I – anterior part of thorax, *Chrysomya albiceps*; J – abdomen, *Chrysomya albiceps*; K – anterior part of thorax, *Chrysomya putoria*; L – abdomen, *Chrysomya putoria*; M – calypters, *Chrysomya bezziana*; N – calypters, *Chrysomya megacephala*.
5.3A–K Details of morphology of blowflies. A – head profile, *Cochliomyia hominivorax*; B – calypters, *Phormia regina*;

C – calypters, *Protophormia terraenovae*; D – calypters, *Calliphora vicina*; E – wing, *Lucilia sericata*; F – head profile, *Cynomya mortuorum*; G – head profile, *Calliphora vicina*; H – head profile, *Calliphora vomitoria*; I – head profile, *Calliphora loewi*; J – basicosta, *Lucilia sericata*; K – basicosta, *Lucilia caesar*.

5.4A–G A – occipital area, *Lucilia sericata*; B – occipital area *Lucilia cuprina*; C – head profile, *Lucilia sericata*; D – head profile, *Lucilia silvarum*; E – head profile, *Lucilia caesar*; F – head profile, *Lucilia illustris*; G – coxopleural streak, *Lucilia caesar*.

5.5A–T Male genitalia of Calliphorinae. *Calliphora loewi*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Calliphora subalpina*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite, lateral view. *Calliphora vicina*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Calliphora vomitoria*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view. *Cynomya mortuorum*: Q – cerci and surstyli, lateral view; R – cerci and surstyli, dorsal view; S – phallus, lateral view; T – pregonite and postgonite, lateral view.

5.6A–P Male genitalia of Chrysomyinae. *Chrysomya albiceps*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Chrysomya bezziana*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite, lateral view. *Chrysomya marginalis*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Chrysomya megacephala*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view.

5.7A–T Male genitalia of Chrysomyinae and Luciliinae. *Chrysomya putoria*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Cochliomyia hominivorax*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite,

lateral view. *Phormia regina*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Protophormia terraenovae*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view. *Lucilia cuprina*: Q – cerci and surstyli, lateral view; R – cerci and surstyli, dorsal view; S – phallus, lateral view; T – pregonite and postgonite, lateral view.

5.8A–V Terminalia of Luciliinae. *Lucilia ampullacea*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Lucilia caesar*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – ovipositor, lateral view; I – pregonite and postgonite, lateral view. *Lucilia illustris*: J – cerci and surstyli, lateral view; K – cerci and surstyli, dorsal view; L – phallus, lateral view; M – ovipositor, lateral view; N – pregonite and postgonite, lateral view. *Lucilia sericata*: O – cerci and surstyli, lateral view; P – cerci and surstyli, dorsal view; Q – phallus, lateral view; R – pregonite and postgonite, lateral view. *Lucilia silvarum*: S – cerci and surstyli, lateral view; T – cerci and surstyli, dorsal view; U – phallus, lateral view; V – pregonite and postgonite, lateral view.

5.9A–O European and Mediterranean blowflies of forensic importance, general appearance (A-C, E, F, I-N phot. by T. Klejdysz). A – *Calliphora loewi*, B – *Calliphora vicina*, C – *Calliphora vomitoria*, D – *Calliphora subalpina*, E – *Cynomya mortuorum*, F – *Chrysomya albiceps*, G – *Chrysomya marginalis*, H – *Chrysomya megacephala*, I – *Phormia regina*, J – *Protophormia terraenovae*, K – *Lucilia ampullacea*, L – *Lucilia caesar*, M – *Lucilia illustris*, N – *Lucilia sericata*, O – *Lucilia silvarum*.

6.1 *Dermestes lardarius* Linnaeus

6.2 *Dermestes maculatus* DeGeer

6.3 A clerid beetle

6.4 *Nicrophorus humator* Gleditsch

12.1 Chironomidae – blood worms

12.2 Caddisfly – cased

12.3 River with bankside vegetation

12.4 Hydrophilid larva

12.5 Hydrophilid beetle

12.6 Dytiscid beetle larva

12.7 *Gammarus* sp., - an example of a shredder
12.8 Culicid pupa
12.9 Rat-tailed larva (Syrphidae)
12.10 Turtle – a scavenger
12.11 Gyrinidae larva (whirligig beetles)

List of figures

1.1	Stored product pests	2
1.2	Insect structural damage to wood	3
1.3	Body in wrappings	4
1.4	Necrophagous insects colonising a body	5
1.5	Predatory beetles will consume eggs and larvae of those flies colonising the body	5
1.6	Omnivores such as wasps will consume both the body and any insects present	6
1.7	Opportunist insects – a spider	6
1.8	An opportunist butterfly attracted to faecal material	7
2.1	Insect mitochondrial genome	13
2.2	Electrophoresis gel for RAPD Analysis of Fly DNA	18
3.1	Fresh stage of decomposition	30
3.2	Bloat	31
3.3	Active decay stage	31
3.4	Advanced decay	32
3.5	Skeletal remains	32
4.1	General morphology of the insect	44
4.2	Structure of the insect leg	44
4.3	Insect wing venation exemplified using the Needham Comstock venation naming system	46
4.4	The insect wing structure illustrating the position of the calypters	47
4.5	Examples of some of the forms of insect antenna	47
4.6	Classification hierarchy	49
4.7	A Nematoceran fly	50
4.8	An example of a member of the Brachycera – a Tabanid	50
4.9	The wing of a member of the infraorder Muscomorpha Aschiza illustrating the long anal cell	51
4.10	The remains of a ptilinum as a ptilinal suture	52
4.11	Occipital dilation present on *Calliphora uralensis* Villeneuve	53
4.12	(a) Copulating blue bottle flies (b) Clump of eggs	54
4.13	The plastron runs the length of the egg	55
4.14	The lifecycle of the blowfly	56
4.15	(a) Electronmicrograph of the tubercles (b) Posterior spiracles	57

4.16	An example of the anterior spiracle on a larva	58
4.17	Electronmicrograph of the puparium with detached operculum	59
4.18	Generalised insect growth curve	59
4.19	The thorax showing the position of the acrostichal bristles	60
4.20	The 'tessselation' (chequer board) effect on the abdomen of flies such as *Calliphora vicina* Robineau-Desvoidy	61
4.21	An example of a larval 'head skeleton' with oral sclerites	62
4.22	*Lucilia richardsi* Collin	63
4.23	A fly showing the characteristic features of the Sarcophagidae	65
4.24	An electronmicrograph of a Piophilid	66
4.25	Hydrotaea larva	69
4.26	A pre-imaginal stage of *Hermetia illucens* Linnaeus	70
4.27	An aquatic larva: a member of the Stratiomyidae	70
4.28	The curved second anal vein characteristic of the Trichoceridae	71
4.29	An example of the features of a Tipulid including the drooping mouthparts	72
6.1	Generalised morphology of a beetle – dorsal view	83
6.2	The structure of the coleopteran thorax	83
6.3	The front legs of a beetle that have been modified for digging (*Geotrupes* sp.)	85
6.4	Examples of the shapes of beetle larvae	86
6.5	The types of pupa and puparia to illustrate the relationship of appendages to the body	86
6.6	Ventral view of the beetle thorax to illustrate the distinction between the Polyphaga (Figure 6.6a) and the Adephaga (Figure 6.6b)	89
6.7	*Nicrophorus humator* Gleditsch	90
6.8	An example of a member of the Staphylinidae	91
6.9	A hister beetle	92
6.10	A trogid beetle	92
6.11	The front coxa of the dermestid projects from the coxal cavity	93
6.12	The lifecycle of *Dermestes maculatus* DeGeer	94
6.13	A clerid beetle	95
6.14	An example of a nitulid beetle	95
6.15	The striations on the elytra of a carabid	96
6.16	Electropherogram profile to allow comparison of the RAPD of a Silphid beetle to that of a calliphorid fly	98
7.1	Entomological crime scene equipment in a carrying case	102
7.2	Sealed, labelled bag containing entomological specimens collected at the crime scene	103
7.3	Retrieving a fly from a net	105
7.4	Label for the inside and outside of the collecting tube	107
8.1	Larvae from a crime scene are ideally reared in a controlled environment cabinet	112

8.2	Aquarium with pump	119
9.1	Insect growth in relation to upper and lower threshold temperatures	122
9.2	Base temperature determination using the linear approximation method	123
9.3	Graph to show the justification for using the accumulation averaged temperatures over time	125
9.4	Regression of crime scene temperature data against the meteorological station temperature to determine a correction factor for the period after the body was found	127
9.5	An insect transporting mites	135
10.1	Electronmicrograph of the head of an adult Piophilid	149
10.2	Piophilid infestation of meat	153
10.3	Fly faeces and vomit contaminate surfaces	154
11.1	Dermestid beetles	159
11.2	Faecal remains (frass) from dermestid beetles	160
11.3	Electronmicrograph of the ventral side of *Dermestes maculatus* DeGeer female and *Dermestes* lardarius Linnaeus male	162
11.4	Geotrupid beetle	167
12.1	Stoneflies	173
12.2	Ephemeroptera	173
12.3	Molluscs feed as scrapers	174
12.4	Nonbiting midges: an example of the Chironomidae	174
12.5	A Caddisfly larva	175
12.6a	Corixid bug	175
12.6b	Notonecta nymph	175
12.7	The riffles and pools of a river	176
12.8	Dytiscid beetle – a predator	177
12.9	*Ascellus sp.* a shredder	178

List of tables

2.1	Examples of primers for cytochrome oxidase	14
3.1	Speed of decomposition of encoffined bodies in graves	36
3.2	Odour profile interpretation of decomposition volatiles during human decomposition recognised using the advanced sensor for detecting clandestine graves (LABRADOR)	40
8.1	Average minimum lifecycle durations of a selection of dipteran species at fixed temperatures	114
9.1	Lower developmental thresholds (base temperatures)	123
9.2	An example of the headings and completed spread sheet to calculate the ADD for *Calliphora vicina* Robineau-Desvoidy	128
9.3	The nature of the Excel spreadsheet	129
9.4	Daily temperature readings from a meteorological station for the period between 7 and 20 April	133
11.1	Beetle succession on decomposing corpses	157
13.1	Contemporaneous notes checklist	185
13.2	Suggested headings for a forensic entomology expert's report	189

Preface

This book is intended as an introduction to forensic entomology, including those aspects that relate to aquatic forensic entomology, in which insects and macroinvertebrates can be used to interpret crime scenes and provide an indication of time since submergence. The book is intended to provide a basic entomological introduction to forensic entomology, using examples from a range of countries. It includes practical activities to enhance understanding of the subject.

The second edition has benefitted greatly from the comments of a number of reviewers to whom I am most grateful. I have tried to respond to their suggestions. Any omissions were due to pressures of space. Forensic entomology has matured greatly as a science and a profession in recent years and owes much to the enthusiasm and energy of those running the professional societies. The work of the taxonomists has also strengthened the identification skills of practitioners and academics alike and their generously shared enthusiasm is of great value.

Alongside this, television series increasingly emphasise the role of insects in solving crime. Hopefully this textbook will therefore also be of interest to a broader audience and trigger an enthusiasm for insects that will encourage 'students' in the broadest sense to explore further both the forensic and entomological literature in this subject area.

Dorothy Gennard

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1

The scope of forensic entomology

Forensic entomology is a branch of forensic science. Forensic entomologists use information about insect lifecycles and behaviour to help interpret evidence in a legal context relating to both humans and wildlife. On occasion, the term 'forensic entomology' is expanded to include other arthropods, mites, spiders, or macro-invertebrates such as freshwater shrimps. The legal contexts in which forensic entomology is of use relate to matters considered in either civil courts or criminal courts. The cases that are heard in civil courts most frequently relate to insect infestation in urban contexts or in relation to stored product pests. Where there is insect infestation of a body, either living or dead, and foul play is thought to have occurred, or a law has been broken, then the case is generally termed a medico-legal case. Such cases can relate to both humans and wildlife.

1.1 Forensic entomology in urban contexts

Cases of infestation of homes or other buildings, such as hospitals, are instances in which forensic entomologists may have a role to play. For example, where structural timber is found to harbour insects such as longhorn beetles (Cerambycidae) an entomologist might be called to assist in determining the cause and source of infestation. Such insects are generally pests of sapwood, but can complete their lifecycle in dry wood that has been harvested. An example of such a beetle is *Eburia quadrigeminata* (Say), the ivory marked beetle, a longhorn beetle, which usually attacks living American oak trees but has been known to survive felling, wood treatment and transformation of the wood into furniture, only to emerge some 10 to 15 years later. In 2007, Cocquempot recorded an instance of this species having been caught when emerging from a bamboo stand in France.

Other examples of the urban application of forensic entomology relate to infestation of food premises and food production sites. For example, the owners of a butcher's shop in London, was closed in January 2010. The Magistrates' Court

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

awarded costs of £560 to the council after meat on sale at the butchers had been found to be infested with maggots and fly eggs.

Poultry production units may similarly be convicted of causing fly infestations that affects residents living nearby. Such was the case in a small Lincolnshire village where, in 2009, the farm owners were fined £20 000 by Skegness Magistrates court, having pleaded guilty to breaching an abatement order, intended to reduce the numbers of flies, which had been put in place in 2008 for a similar misdemeanour.

1.2 Stored product infestation and forensic entomology

In general, only a small number of stored product pest species may be encountered by the forensic entomologist. They include flies, cockroaches, ants, and beetles. The insects that inhabit animal products and their waste include members of families such as larder beetles (Dermestidae), Moth flies (Psychodidae), Scuttle flies (Phoridae), Muscid flies (Muscidae) Blowflies (Calliphoridae) and Flesh flies (Sarcophagidae) such as *Sarcophaga carnaria* Linnaeus, ants (Formicidae) such as the Pharaoh ant (*Monomorium pharaonis* Linnaeus), or the Copra beetle (*Necrobia rufipes* DeGeer). These may also be cited in medico-legal cases (Figures 1.1 and 1.2).

The following are examples of phytophagous insects, which may infest food, resulting in forensic entomologists contributing to court cases.

Biscuit beetles (*Stegobium paniceum* Linnaeus) will infest not only food items such as flour bread and biscuits but also wool, hair and leather material. The saw-toothed grain beetle (*Oryzaephilus surinamensis* Linnaeus) and the Indian meal

Figure 1.1 Stored product pests

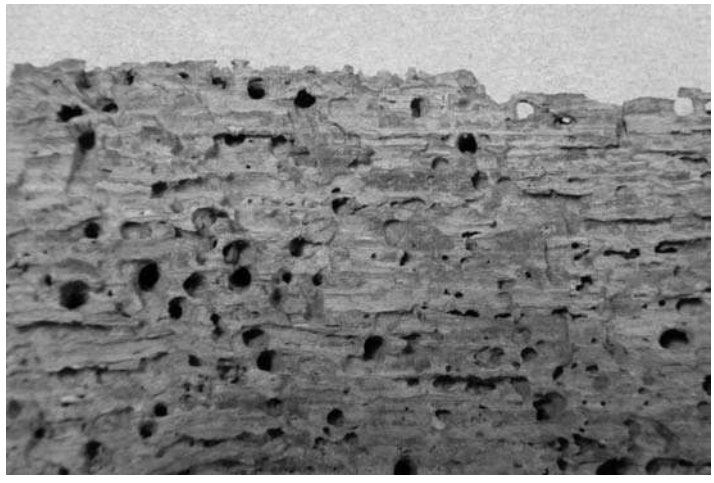

Figure 1.2 Insect structural damage to wood

moth (*Plodia interpunctella* Hubner) both infest dried fruit, breakfast cereals and pasta.The Indian meal moth is also known to consume dried dog food and fish food products. The rust-red flour beetle (*Tribolium castaneum* Herbst) and the confused flour beetle (*Tribolium confusum* Jacquelin du Val), infest grain and flour. The fruit fly (*Drosophila melanogaster* Meigen) will infest any fermenting fruit or vegetable, including tomatoes, onions, and bananas. It can also inhabit compost heaps and piles of rotting garden waste and so could be the subject of urban forensic entomology cases.

Because food-processing plants have difficulty in reducing insect infestation levels to zero, a legal tolerance level is specified in many countries, contravention of which can lead to prosecution. For example, the American Food and Drug Administration considers that, in canned citrus juice, a maximum of five or more eggs of Drosophila or other insects per 250 ml is allowable. The presence of one maggot per 250 ml of canned citrus juice is also considered acceptable (AOAC 970.72).

In the majority of instances in stored product and urban forensic entomology, the main focus of the contribution is confirmation of the identity of the insect species and interpreting its biology in the particular context in question. In such instances this aspect of forensic entomology is confirmatory and relates to the work of the Environmental Health Department or the Office of Trading Standards.

1.3 Forensic entomology in the medico-legal context

Insects have a role in crime scene investigations on both land and in water (Anderson, 1995; Erzinçlioğlu, 2000; Keiper and Casamatta, 2001; Hobischak and Anderson,

Figure 1.3 Body in wrappings

2002; Oliveira-Costa and de Mello-Patiu, 2004: Moretti, Bonato and Godoy, 2011). The majority of medico-legal cases where entomological evidence is used are the result of illegal activities that take place on land and are discovered within a short time of being committed. In France for example, 70% of cadavers are found outdoors and, of these, 60% are discovered within less than one month (Gaudry *et al*., 2004).

All insects could be of potential relevance to a medico-legal question, however a number of species from several families are found more often than others. The insects of particular relevance to forensic entomological investigations include blow flies, flesh flies, cheese skippers, hide and skin beetles, rove beetles and clown beetles. In some of these families only the juvenile stages are carrion feeders and consume dead bodies. In others both the juvenile stages and the adults will feed on the body (are necrophages). Yet other families of insects are attracted to the body solely because

Figure 1.4 Necrophagous insects colonising a body

they feed on the necrophagous insects that are present. Forensically relevant insects can be grouped into four categories based on feeding relationship. These are:

- Necrophages, which feed only on the decomposing tissue of the body or body parts – for example blowflies, hide beetles and clown beetles (Figures 1.4 and 1.5).
- Predators (and parasites) of the necrophages – for example rove beetles and ground beetles.

Figure 1.5 Predatory beetles will consume eggs and larvae of those flies colonising the body

Figure 1.6 Omnivores such as wasps will consume both the body and any insects present

- Omnivores that consume both the live insects inhabiting the corpse and the dead flesh – for example ants (Formicidae), and wasps (Figure1.6).
- Opportunist (adventive) species, which arrive because the corpse is a part of their local environment – for example mites, hoverflies, butterflies and, on occasion, spiders (Figures 1.7 and 1.8).

On occasion waste material or faecal material may be the attraction (Figure 1.8). The roles of specific species which have these feeding strategies will be considered in later chapters.

1.4 The history of forensic entomology

Insects are known to have been used in the detection of crimes over a long period of time and a number of researchers have written about the history of forensic entomology (Benecke, 2001; Greenberg and Kunich, 2002). The Chinese used the presence of flies and other insects as part of their crime-scene investigative armoury and instances of their use are recorded as early as the mid-tenth century (Cheng, 1890, cited in Greenberg and Kunich, 2002).

Such was the importance of insects in crime-scene investigation that in 1235, a training manual on investigating death, *Washing Away of Wrongs,* was written by Sung Tz'u. In this early medico-legal book it is recorded that attention paid by a number of blowflies to a particular sickle caused a murderer to confess to murdering a fellow Chinese farm worker with that sickle.

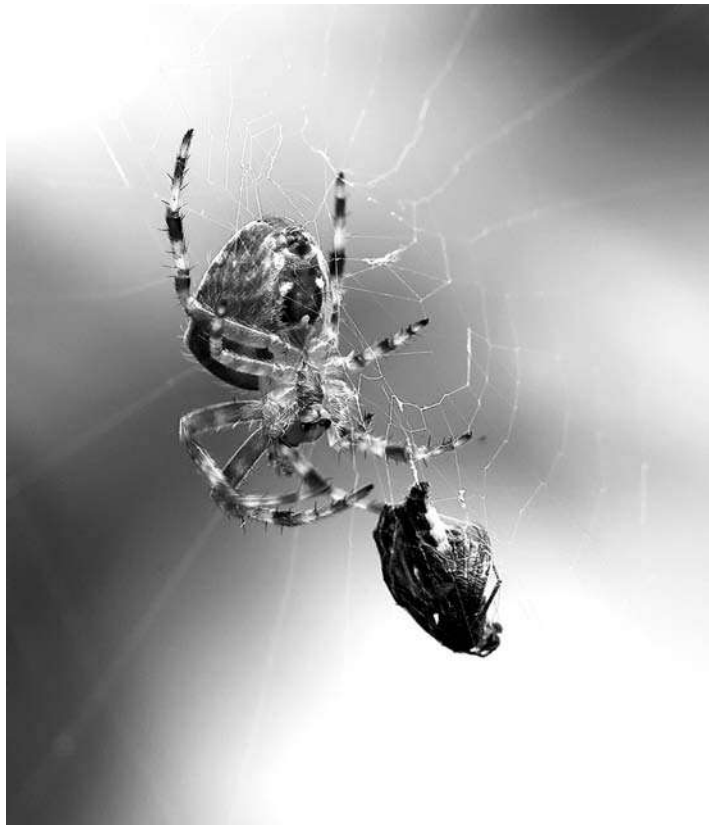

Figure 1.7 Opportunist insects – a spider. *Source:* Reproduced by permission of Mr. Ian Ward

Between the thirteenth and nineteenth centuries a number of developments in biology laid the foundation for forensic entomology to become a branch of scientific study. The two most notable were, perhaps, experiments by Redi (1668), an Italian who, using the flesh of a number of different animal species, demonstrated that larvae developed from eggs laid by flies, and the work by Linnaeus (1735) developing a system of classification. In so doing, Linnaeus provided a means of insect identification (including identifying such forensically important flies as *Calliphora vomitoria* Linnaeus). These developments formed foundations from which determination of the length of the stages in the insect's lifecycle could be worked out and the indicators of time since death could be developed.

A particularly significant legal case, which helped establish forensic entomology as a recognised tool for investigating crime scenes, was that of a murdered newborn baby. In 1850 a baby's mummified body, encased in a chimney, was revealed behind a mantelpiece in a boarding house when, during renovation work, Dr Marcel Bergeret carried out an autopsy on the body and discovered larvae of a flesh fly, *Sarcophaga carnaria* and some moths. He concluded that the baby's body had been sealed into the

Figure 1.8 An opportunist butterfly attracted to faecal material. *Source:* Reproduced by permission of Mr. Ian Ward

chimney in 1848 and that the moths had gained access in 1849. As a result of this estimation of the time since death, occupiers of the house previous to 1848 were accused and the current occupiers exonerated (Bergeret, 1855).

The next significant point in the history of forensic entomology resulted from observations and conclusions made by Mégnin (1894). He related eight stages of human decomposition to the succession of insects colonising the body after death. He published his findings in *La faune des cadavres: Application de l'entomologie à la médicine légale*. These stages of decomposition were subsequently shown to vary in speed and to depend upon environmental conditions, including temperature and, for example, size of the corpse and whether or not the corpse was clothed. The similarity in overall decomposition sequence and the role of insect assemblages in decomposition has been demonstrated for a number of animal species.

Knowledge about insect succession and the periods of insect activity on a corpse has become the basis for forensic entomologists' estimations of time since death, although this is acknowledged as being based on assumptions of time of colonisation relative to the point of death. Research continues to be required in order to establish the accuracy levels of estimates of time since death and to interpret variation in different biotopes (Tomberlin *et al*., 2011).

In the twentieth century, insects were shown to be of value in court cases involving insect colonisation of body parts recovered from water and not just for entire corpses found on land. On 29 September 1935 several body parts, later identified as

originating from two females, were recovered from a river near Moffatt in Scotland. The identities of the deceased were Isabella Kerr, the wife of a Dr Ruxton, and Mary Rogerson, the family's 'nanny'. The presence of third instar larvae of the blowfly *Calliphora vicina* Robineau-Desvoidy indicated that the eggs had been laid prior to the bodies being dumped in the river. This information, combined with other evidence, resulted in the conviction of Dr Buck Ruxton, for the murder of his wife and Mary Rogerson.

The level of acceptance of forensic entomology by the courts has depended upon the results of scientific study. This has been carried out since the early twentieth century, both by academics and practitioners working alongside the police and legal authorities. As a result the subject base has been refined and protocols and rigorous forensic procedures have been developed to raise its level of esteem. However, there remain several areas for which accurate information and levels of error remain undetermined. These aspects of uncertainty with respect to forensic entomology will be addressed with focused research. The requirement for this to happen quickly is dictated both by the professional aspirations of the forensic entomology community and also as a result of reviews and legislation.

In the USA the report concerning the whole of forensic science produced by the National Research Council, was significant. The council recommended that an independent federal organisation – the National Institute for Forensic Science, be set up in order to establish mandatory standards for laboratories, the promotion of scholarly, peer-reviewed research and the establishment and reinforcement of methods of best practice and the use of standardised protocols. A similar approach was taken in the United Kingdom and in 2007 the Office of the Forensic Science Regulator was set up to help establish and maintain standards in forensic science in general. These national organisations influence the work and aspirations of the professional associations who respond to guidance that they provide.

Forensic entomologists in a number of countries have set up professional organisations to provide a forum for the exchange of ideas and experience and to develop and maintain professional standards in forensic entomology. These organisations include the North American Forensic Entomology Association and the European Association for Forensic Entomology (EAFE).

1.5 Professional associations for forensic entomologists

The nature and aspirations of two major professional associations for forensic entomologists are described below.

1.5.1 North American Forensic Entomology Association (NAFEA)

This organisation is a charitable, non-profit-making educational organisation for the promotion of good practice and research in forensic entomology. It

had its first annual meeting in 2003 and seeks to collaborate with other international societies to enhance the moral, ethical and scientific base of forensic entomology. It currently has over 60 members. The strength of the organisation is its inclusivity. To quote its web site (www.nafea.net/, accessed 26 October 2011):

> NAFEA is an organization for anyone interested in the application of forensic entomology to civil or criminal matters of law, research on arthropods of forensic importance, or carrion ecology.

From a student perspective it is also a valuable source of support, and conference funding may be available to student members. The organisation seeks to promote good practice and the presentation of scientific research, casework, and cooperative ideas on forensic entomology. As such, it is a forum through which research in forensic entomology can receive peer-review and new developments in approach can be discussed.

1.5.2 European Association for Forensic Entomology (EAFE)

The European Association for Forensic Entomology (EAFE) was founded in 2002. The Association was launched in France and has a number of aims:

- To seek a common protocol for forensic entomology case investigation.
- To foster high standards of competency in specimen collection and analysis.
- To create a solid scientific basis so that forensic entomology can be a valid analytical tool.

In 2006, EAFE produced a protocol of good practice in order to ensure that the methods used in forensic entomology investigations at a crime scene could be standardised and good forensic entomological practice could be developed by following standard operating procedures. Its annual meetings also provide an opportunity for dialogue, discussion and collaboration.

1.6 The UK regulator for forensic science

The regulation and maintenance of standards for forensic expert witnesses is currently voluntary and based on the membership of such organisations as the Academy of Experts or the Institute of Expert Witnesses. In the UK in 2007, the Office of the Forensic Science Regulator was set up by the Home Secretary to operate on behalf of the criminal justice system.

The purpose of the Office of the Regulator is primarily to i) determine new and improved quality standards for organisations and if necessary to take the lead in their development; ii) advise and guide organisations undertaking forensic analysis to ensure that they can show compliance with the generally accepted standards that may be required by the courts; iii) ensure sure that that there are appropriate arrangements for quality assurance and standards monitoring; iv) ensure that there are procedures in place for the determination competence of the individual forensic scientist.

The Regulator is supported in this role by a Forensic Science Advisory Council (FSAC). Amongst other things this committee is responsible for offering advice on accreditation and procedures for validating and approving new technologies. They also have the responsibility 'for tasking and overseeing the work of Expert Working Groups established to advise on or develop quality standards . . .'

Currently, forensic organisations are required to be accredited through the United Kingdom Accreditation Service (UKAS) in order to conduct their work and to observe ISO9000 guidelines. These organisations are required to quality control their work and a number of options including participation in blind trials have been proposed.

The accreditation of individuals is under discussion because it is the individual and not the organisation that appears as a witness and is responsible to the court. At present forensic entomologists are not included on the list of expertise that is being considered. Those forensic disciplines that are on the list include more laboratory-based experts such as toxicologists, fingerprint officers, and document examiners. Membership of professional organisations, for the forensic entomologist, therefore remains an important means of standardising operating procedures and ensuring and also demonstrating that good practice is maintained.

1.7 Web addresses of relevant organisations

European Association for Forensic Entomology (EAFE): www.eafe.org (accessed 26 October 2011).

North American Forensic Entomology Association (NAFEA): www.nafea.net (accessed 26 October 2011).

The Forensic Science Regulator
http://www.homeoffice.gov.uk/agencies-public-bodies/fsr/ (accessed 26 October 2011).

2

Forensic entomology, DNA and entomotoxicology

Molecular identification of insects feeding on corpses can be an important technique in forensic entomology, particularly if indeterminate larval species are recovered at a crime scene. Analysis is frequently carried out by molecular biologists, although the answers are interpreted by entomologists. In casework, the life stages collected from a corpse are reared to the adult stage in order to identify the species using morphology. This is a slow process so using molecular methods, alongside morphological identification may, on occasion, be a more rapid and accurate way of providing the basis for determining the PMI.

Many forensically relevant molecular techniques were originally developed to investigate insect phylogeny and particular genetic profiles have been constructed for individual species. An example of the combined use of molecular and morphological techniques is provided by Pai *et al*. (2007) who used them to identify larvae and determine the PMI for a murdered Taiwanese girl whose burned body was recovered from a sugarcane field. In combination the techniques confirmed that the colonising fly was *Chrysomya megacephala* Fabricius.

Potential molecular biology identification methods range from using chromosome C-banding (Angus, Kemeny and Wood, 2004), for example, on the one hand, to using genomic material on the other. Genetic material can be harvested from both the nucleus and from mitochondria. Mitochondrial DNA (mtDNA) is the more frequent source of genetic information, not least because more DNA is available.

Mitochondria are haploid structures with genetic material solely from maternal origins. No recombination occurs in its manufacture. The mitochondrial genome contains around 16 000 base pairs of double-strand DNA (Lessinger *et al*., 2000) and is a stable source of genetic information. Cells contain a large number of mitochondria and much is known about insect systematics as a result of their use in phylogenetic studies. Hence mtDNA is a ready source of information for use in forensic contexts (Figure 2.1).

In mitochondria a stage of respiration called oxidative phosphorylation takes place, generating adenosine triphosphate (ATP) using enzyme complexes called cytochromes. These enzyme complexes include cytochrome c oxidase (Complex IV),

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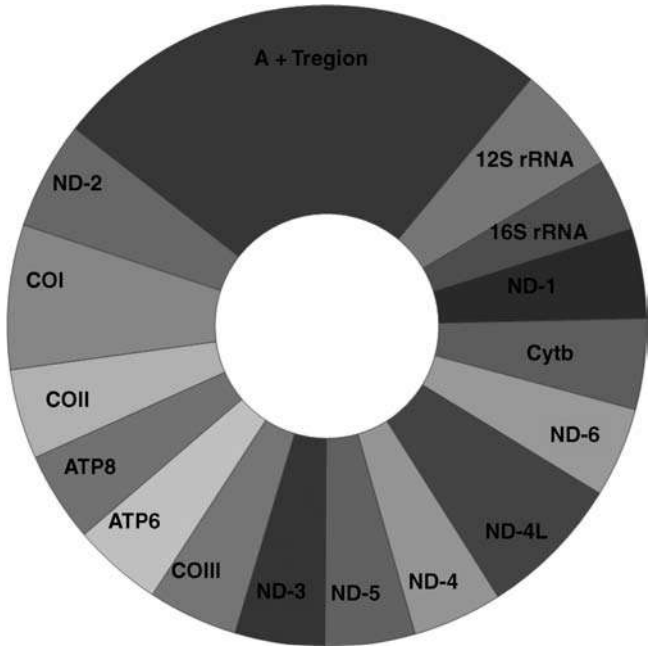

Figure 2.1 Insect mitochondrial genome. *Source:* Professor Lin Field, The Royal Entomological Society of London for permission to reproduce the Mitochondrial genome from Antenna (presented in this book as Figure 2.1)

which is found in the mitochondrial inner membrane. Cytochrome c oxidase is the third and final electron transfer chain enzyme complex involved in oxidative phosphorylation. It is made up of three subunits; the genes of two of which are useful for molecular investigation. The mitochondrial genome comprises approximately 37 genes (22 for transfer RNA, two for ribosomal RNA, and 13 for peptides). Amongst the 37 genes are those for the two subunits of cytochrome c oxidase, subunits I and II (COI and COII). Molecular biologists originally chose COI to investigate genetic profiles, because it is the biggest of the three mitochondrially encoded cytochrome oxidase subunits and the protein sequence combines both variable and highly conserved regions (Saraste, 1990; Gennis, 1992; Beard, Hamm and Collins, 1993; Morlais and Severson, 2002, quoting Clary and Wolstenholme, 1985). This allows refinement of information so that the degree of geographic variation may also be interpreted.

Species identification is based on sequences of nucleotides. These sequences are termed loci and are made up of strings of nucleotide base pairs-adenine (A), thymine (T), cytosine (C) and guanine (G). The non-coding region of insect mtDNA is called the control region or the A-T region. This region is made up of a large number of adenine and thymine nucleotides and controls mitochondrial DNA replication and RNA transcription (Avise *et al*., 1987). To describe the sequence of base pairs so that an individual 'signature' or **haplotype** can be specified for a particular species, a

nucleotide position numbering system is used. This follows that described for the fruit fly *Drosophila yakuba* (Burla) (GenBank accession Number NC-001322).

The base pairs sections can be very short. Where the sections are made up of fewer than 1000 base pairs, it is necessary to artificially increase or '**amplify**' the length of DNA before it can be interpreted. The process used to do this is called the **polymerase chain reaction** (PCR). To replicate the required sections of the DNA sample, previously generated regions are joined at known sites on the DNA, to enable it to be copied. These artificially generated sections are called **primers**. Specific primers are generated for particular insect families, for example the Calliphoridae (Table 2.1). This means that the amplified DNA product is from a known site, so the nucleotides and their position on the DNA molecule can be interpreted.

Fortunately some species 'signatures' are based on quite short sections (loci), often of fewer than 350 significant base pairs. This means that, although the DNA chain degrades over time, specimens that have been stored for a long time or which have dried out, can still be reliably identified. Mitochondrial DNA (mtDNA) is extremely useful; for the most part, it is resistant to degradation. Its use can provide fly species identification within a day.

Details of primer sequences specifically complementary to calliphorid mtDNA have the reference numbers L14945–7. They can be accessed from GenBank (Malgorn and Coquoz, 1999). This information can then be used to request prepared primers from biotechnology companies. These enzymes (primers) are robust at a range of temperatures, can be used with various buffers and are moderately inexpensive. They are designed with different degrees of specificity allowing amplification of, for example, only insect DNA or more selective species-specific products.

Once extracted, the mtDNA sequences for the protein-coding regions are compared with known species 'signatures' in a database using computer software.

Table 2.1 Examples of primers for cytochrome oxidase

Primer Title	Composition	Reference Source
CO – I2f	5′-CAG CTA CTT TAT GAG CTT TAG G-3′	Vincent, Vian and Carlotti 2000
CO – I 3r	5′-CAT TTC AAG C/TTG TGT AAG CATC-3′	Vincent, Vian and Carlotti 2000
TY – J – 1460	TAC AAT TTA TCG CCT AAA CTT CAG CC	Wells and Sperling 2001
C1 – N – 1687	CAA TTT CAA ATC CTC CAA TTA T	Wells and Sperling 2001
C1 – J – 2319	TAG CTA TTG GAC/TTA TTA GG	Wells and Sperling 2001
C1 - N - 2514	AAC TCC AGT TAA TCC TCC TAC	Wells and Sperling 2001
C1 – J – 2495	CAG CTA CTT TAT GAG CTT TAGG	Also used by Harvey, Dadour and Gaudierie 2003
C1 – N – 2800	CAT TTC AAGT/CTG TGT AAG CATC	Also used by Harvey, Dadour and Gaudierie 2003

GenBank is an example of a database of genetic profiles which is ratified and publicly available. Software such as Blast Search (www.ncbi.nlm.gov) is used to search GenBank. Some degree of concern has been expressed about the validity of the information held in GenBank but over time the quality is improving (this aspect is discussed later under Validity of Methodologies).

2.1 Preparation of specimens for molecular analysis

In all instances, specimens for molecular analysis should be killed and stored appropriately and any possibility of contamination minimised. The chemicals and/or the extraction method chosen can influence the outcome of the analysis, although there is some disagreement on good practice (Fukatsu, 1999; Dean and Ballard, 2001). For example, Dillon, Austin and Bartowsky (1996) considered that using ethyl acetate as a killing agent could reduce the amount of DNA extracted. Logan (1999) found that genomic DNA was adequately recovered from insect specimens that had been preserved in acetone. Espeland *et al.* (2010) expressed concern about the effects of the insecticide, Dichlorvos, on recovery of nuclear DNA, noting that COI amplification was prevented after 229 days.

The initial means of evidence storage is also important. Ideally, storage of samples in 95% alcohol at the crime scene, or by freezing, ensures reliable recovery of the genetic information. Storing specimens in 99% alcohol provided fragments of up to 1400 base pairs according to Sperling, Anderson and Hickey (1994). In contrast, flies stored dry, or preserved in 75% ethanol, provided DNA fragments reduced to up to 350 base pairs. If neither freezing nor alcohol use is possible then the specimens should be kept on ice in a cool box, or refrigerator, until they are received at the laboratory. Contamination by organisms residing on the external surface of the maggot must be removed prior to starting to extract. A 20% solution of bleach is effect for this purpose and does not interfere with the results of molecular analysis (Wells, 2002).

A further precaution against contamination is to analyse the genetic material from the head or thorax of an adult fly, or the mid-section of a larva. This allows, as is necessary in all forensic work, the retention of voucher specimens in the form of the remaining body parts. Where possible in forensic work, the post-feeding stage should be used, or the larvae should be starved so that their gut is empty of food. This ensures that only the DNA from that particular individual is investigated and that contamination by gut contents does not occur.

Where possible, insect specimens chosen for DNA extraction should be taken from live cultures and killed by freezing. Freezing adult flies immediately at $-70\,^{\circ}C$ ensures that the DNA does not degrade as rapidly as it might if other preservation methods are used. However work by Lonsdale, Dixon and Gennard (2004) indicates that the length of time in frozen storage will affect the degree of degradation of DNA molecule if storage time is longer than one year.

If the crop content is required for analysis, the larval outer coat should be treated with 20% bleach solution (Linville and Wells, 2002) and the crop excised from the larval body. This makes interpretation of the analytical results easier. The rest of the larval specimen is also available for more traditional analysis or for preservation. At this point a preservative such as Kahle's solution can be used for specimen storage.

2.1.1 DNA extraction

There are several methods for extracting DNA and the preferred choice of extraction chemicals varies between laboratories. DNA extraction using Qiagen tissue kits such as DNeasy® Tissue Kit or Chelex® can be helpful because the pre-prepared extraction chemicals ensure standardisation of the technique. More frequently in insect molecular science phenol-chloroform extraction is used. However, Junqueira, Lessinger and Azendo-Espin (2002) concluded that DNAzol® was the most effective chemical for extracting DNA, compared to extraction using either Chelex® or the phenol/chloroform method, particularly if there was a fear that the DNA could be damaged. More recently, genomic DNA has been extracted using automated processes such as the BioRobot EZ1 workstation and EZ1 DNA Forensic Cards (Qiagen) (Cainé *et al*., 2006).

2.1.2 DNA concentration

The polymerase chain reaction (PCR) splits the double-stranded DNA by heating, and replicates it to increase the amount of genetic material available. Artificially generated single DNA strands kick start or 'prime' the DNA synthesis. The primers are designed to position either side of a chosen section of genetic coding. They bind to complimentary sequences and using them the DNA polymerase enzyme manufactures a new strand. This new strand forms the basis for repetition of the process; more DNA is generated over a number of cycles until there is sufficient material for extraction and realisation of the DNA. At this point, depending on the original source of the DNA, several further methods of analysis can be used to determine the genetic signature.

2.2 Methods of analysis and sources of information

Examples of these methods include **restriction fragment length polymorphism** (RFLP), **random amplified polymorphic DNA** (RAPD), analysing genes for particular enzymes such as **NADH**, **RNA** analysis including sites related to **ITS**, ribosomal, (for example 28S RNA), as well as **pyrosequencing** amongst others. These will each be discussed under separate headings.

The use of PCR itself as a means of analysis has also been explored, particularly for determining the age of fly pupae from inside the puparia since this is a life stage the duration of which is not easily determined. Zehner, Mosch and Amendt (2010) used differential display PCR to examine changes in gene expression as the pupa developed inside the puparium. They found that only in later stages of development was there a significant difference and considered the procedure had some potential, but required further work.

2.2.1 Restriction fragment length polymorphism (RFLP)

This type of analysis has been used to analyse the degree of variation in populations of a particular species. It provides information relevant to the interpretation of forensic samples from data provided from a wide context. For example nuclear DNA PCR-RFLP has been used to examine variation in populations of the secondary screwworm *Cochliomyia macellaria* (Fabricius) in Uruguay, as a means of determining the identity of a particular specimen.

In forensic cases, PCR- RFLP methods have been successfully used to assist in species identification to make a post mortem interval determination. Schroeder *et al.* (2003) carried out RFLP analysis, using a modification of the method described by Sperling, Anderson and Hickey (1994) for analysing mitochondrial DNA. They separated species of *Calliphora vicina*, *Calliphora vomitoria,* and *Lucilia sericata* Meigen using a 349 bp section of the mtDNA using subunit I (COI), the cytochrome oxidase subunit II gene (COII) and the tRNA-leucine gene. From these specific regions they clearly distinguished between the three most common corpse-infesting fly species in the vicinity of Hamburg, Germany.

PCR-RFLP has also been used to identify some Australian chironomid fly larvae that are often difficult to identify (Carew, Pettigrove and Hoffmann, 2003). An identification key has been built using the RFLP profiles of the species of non-biting midge (Chironomidae) present in the water body. This method was validated using specimens from both wetlands and streams and has application in aquatic forensic entomology.

2.2.2 Random Amplified Polymorphic DNA (RAPD)

This method uses non-specific primers and the PCR products come from many areas of the DNA of the specimens. Primer 5 and REP 1R are often used for forensic case work (Benecke, 1998). Based on a 5′-3′ sequence the RAPD primers are:

REP 1R* XIIIACGTCGICATCAGGC
Primer 5 XAACGCGCAAC

(*From a primer description provided by Pharmacia Biotech, estimating the post-mortem interval of pupae and referenced in Benecke, 1998.)

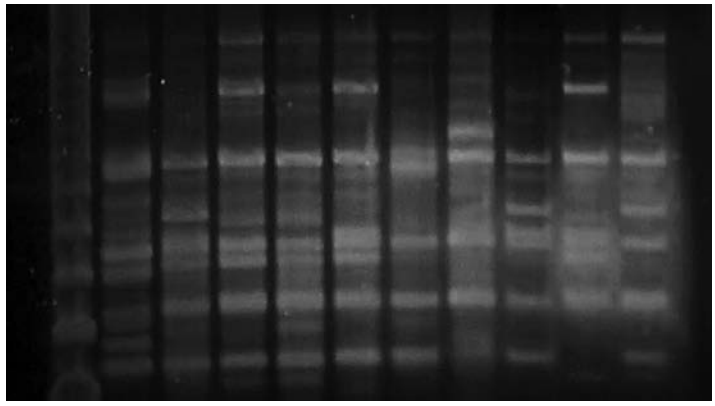

Figure 2.2 Electrophoresis gel for RAPD Analysis of Fly DNA

The separation of the PCR-products is carried out using gel electrophoresis. The results are read as peaks to give a peak profile, or 'signature' (see colour section). Only peaks of a maximum intensity of at least 50% are used to generate the profiles of individual fly species. If the numbers of strong peaks are small, further primers are needed to generate a trustworthy profile. If the peak tops split on the display, the sequencer view using the silver stain-like banding pattern should be used. False positives may be generated if a strict methodology is not adhered to. For example samples from the same DNA source should never be loaded next to each other on the electrophoresis gel to avoid this happening (Figure 2.2).

Stevens and Wall (1995) used RAPD analysis to compare the degree of geographic variation between *Lucilia sericata* populations at farms near Weybridge in Surrey and those in the Bristol area. Control specimens were provided by a laboratory culture of *Lucilia sericata* from the University of Bristol. Their results confirmed the value of RAPD in separating closely related *Lucilia sericata* specimens.

Small intra-specific differences in the sequences of individual larvae from the same species do occur, although the overall 'signature' is constant. For example Wells *et al*. (2001) recorded a substitution of adenine at position 2058 (with reference to the *Drosophila yakuba* numbering system). Because the resulting amino acid sequence is unchanged, this is called a 'silent substitution' and considered typical of a cytochrome oxidase subunit b (COI) haplotype variation. (Where there is more than one haplotype, because of mutation in individual members of a particular species, the condition is called **heteroplasmy**.)

Using RAPD analysis for species identification can be disadvantageous as there is no standardisation in different species signatures; there are no recognised databases against which to compare RAPD profiles of insect species; no statistical data is available in order to exclude chance when interpreting the results. For these reasons, RAPD analysis is not a technique of choice in forensic analysis.

2.2.3 RNA

RNA is found in three forms, **transfer RNA** (tRNA), **messenger RNA** (mRNA), and ribosomal RNA (rRNA). Of these investigating the sequences coding for mRNA and rRNA have been found to be of value in molecular forensic entomology. Ribosomal RNA is used in insect systematics more frequently than in forensic entomology.

Analysing RNA reveals the genes that are 'switched on' in the insect tissue at the point at which the analysis is undertaken. Both nuclear DNA and mitochondrial DNA have associated RNA genes. Nuclear RNA, in particular, is present in sufficient quantity to provide an easy means of replication and analysis from which to determine the species identity of a specimen.

Messenger RNA gene concentrations change as a function of time. Tarone, Jennings and Foran (2007), using *Lucilia sericata,* showed that the concentration of two mRNA genes changed between pre, and post-feeding stages. Therefore this distinction can be used in order to confirm the developmental state of a specimen which is not morphologically distinct in the third instar. In turn, this aids determination of the time since death and enhances the value of using the mRNA genes.

Transfer RNA (tRNA)

On occasion COI and COII are used in association with tRNA-leucine genes to obtain a profile from which to confirm insect identity. Mazzanti *et al*. (2010) used the approach to identify species using empty puparial cases, although they also warn of the problems that degradation of DNA may cause – the amount of DNA available can be seriously reduced in degraded puparial cases.

Ribosomal RNA (rRNA)

Three of the four forms of cytoplasmic rRNA come from the genes located on the nuclear organiser sites of the chromosomes. The ascription of the number to the cytoplasmic rRNA is based on their coefficient of sedimentation i.e. *5S, 5.8S, 16S, 18S,* and 28S. Ribosomal RNA described as 16S can accumulate mutations faster than the nucleus. It has been used to explore the distinction between Ophyra species and Fannia species with some success.

Stevens and Wall (2001) showed that, based on the variation in 28S rRNA gene sequences of 13 specimens, the Calliphorinae and Luciliinae were, without doubt, two distinct clades. Both can be reliably separated from *Protophormia terraenovae* and the Chrysomyinae using this gene sequence. In the process of the investigation they concluded from analysis of sub regions np: 511–710 and np: 1521–1830 that 39 characters provide phylogenetically informative sites from the 28S subunit.

2.2.4 Internal transcribed spacer region (ITS)

The internal transcribed spacer (ITS) region is a non-coding region of the genome.

The second ribosomal internal transcribed spacer has been successfully used to distinguish Chrysomya species by Nelson, Wallman and Dowton (2008), so ITS has a potential value in forensic entomology. It is used in association with techniques such as RFLP for exploration purposes. It is also used in association with analysis of other gene subunits to distinguish between closely allied species.

Chen and Shih (2003) confirmed that the partial gene of subunit cytochrome oxidase and the first internal transcribed spacer ITSI region of ribosomal RNA (rRNA) could successfully distinguish between *Chrysomya megacephala, Chrysomya pinguis* Walker, and *Chrysomya rufifacies.* The internal transcriber space regions, *ITS1* and *ITS2*, are valuable for distinguishing a number of species but not for *Cochliomyia macellaria.*

GenBank contains a number of ITS sequences for calliphorid species but there are only a small number of case studies in the literature.

2.2.5 Nicotinamide adenine dinucleotide dehydrogenase (ND4)

Genes for the NADH Dehydrogenase subunit 4 of ribosomal RNA, along with the internal transcribed spacer, has been found to be a useful means of distinguishing intraspecies variation. For example, in the blackfly (*Simulium ochraceum* Walker), four NADH alleles were identified from populations in Mexico (Rodriguez-Pérez *et al.*, 2006), which revealed both intrapopulation and interpopulation differences.

Tan *et al*. (2010) emphasised the value of incorporating a large number of species into an analysis when preparing a database of species. He considered that using COI and COII, as well as tRNA-leucine alleles facilitated the distinction between members of a difficult species. This they did for the classification and characterisation of members of the Malaysian Sarcophagidae; a family that is under-represented in terms of the genetic signatures available in the database for use in forensic circumstances.

2.3 Alternative methods

2.3.1 Pyrosequencing

This method is a real-time method of exploring a short nucleotide string of base pairs; for example a 100 nucleotide base section of DNA. It is based on the release of pyrophosphate during DNA sequencing. The visible light generated is proportional to the number of incorporated nucleotides. Each nucleotide is copied into a

synthesised string, identified, and recorded in real time. Because the identities of the nucleotides that are added are known, the sequence of unknown nucleotides can be determined. The technique is potentially valuable for identifying heteroplasy in species as well as for confirming species identity.

Salk, Sanchez, Pierce *et al*. (2006) enabled the direct amplification of single-stranded DNA for pyrosequencing by developing an automated Linear-after-the Exponential PCR (LATE-PCR) method. He and his co-workers were able to sequence a region containing only 191 base pairs using the method. Pyrosequencing advantages are its accuracy, flexibility and that it is a process which can be readily automated, eliminating the need for primers and gel electrophoresis. However pyrosequencing is not currently standard practice in forensic entomology although some attempts have been made to increase the genetic information available on Sarcophagids through its use. For example, a genomic analysis of *Sarcophaga crassipalpis* Macquart using parallel pyrosequencing provided a library containing 9000 transcripts, which were then classified into 14 major subcategories relating to biological processes (Hahn, Ragland, Shoemaker and Delinger, 2009).

2.4 Validity of methodologies

The validity of assessment using mtDNA has been explored by Wells and Williams (2007). They confirmed their confidence in the method using flies from the Chrysomyinae and tested a number of sequences using mtDNA. Wells and Williams confirmed the identity of all of the specimens they investigated. However, from a forensic perspective, the validity of each method requires that there is a reasonable chance that the larval specimens collected are from insects that are likely to be found in the area concerned and that the profiles are already present on the database. The effects of global warming and the resultant species migration may be the cause of identification which gives unexpected results where species are not normally found in a specific location. More work is needed to build up the database, ensure that the information available is accurate and to confirm the repeatability of the molecular methods used.

Current databases such as GenBank are considered to contain a number of misidentifications and a biased sample range (Mitchell, 2008), so caution has to be exercised in interpreting the results using the available databases. Meir *et al.* (2006), for example, revealed that the COI sequences in GenBank could not be used to distinguish 30% of the dipteran species they examined. However, Nelson, Wallman and Dowton (2007) and Meiklejohn *et al*. (2011) have shown that the approach works satisfactorily for the Australian Chrysomya species and Australian Sarcophagidae respectively. A further example of the need for the sequences used for comparison to be valid is provided by Lessard, Wallman and Dowton (2010) who confirmed the single taxon status of *Chrysomya rufifacies* Macquart when reassessment of the original COII sequences revealed that they originated from specimens of

Lucilia porphyrina (Walker) and not *Chrysomya rufifaces*. Quality assurance procedures are in place to assist in reducing these problems.

Another area of concern about the validity of the conclusions that can be drawn is that of the effects of the variation revealed both phenotypically and as a result of genetic investigation within a particular species. The implications of this need to be taken into consideration when evaluating data to decide the species identity of members of a particular population of insects collected from a body or crime scene.

Analysis of **single nucleotide polymorphisms** (SNPs) have shown them to be found at a frequency of at least 7.2 SNPs per 10 kb in one species (Hahn et al., 2009) and microsatellite (repeated regions of noncoding base pair sequences) analysis may be able to provide details of such variation. These latter sets of repeat base pairs arise because of mutation, independent variation in chromosomes and as a result of recombination. They are therefore distinct to the individual rather than the species. The implication of this variation and its effect, if any, on the development of forensically relevant species of insects is as yet unclear – a further aspect needing study. Such examples indicate the value of the close working partnership that is required between the entomologist and those involved in molecular biology.

2.4.1 DNA barcoding

The current concern regarding biodiversity and genomics has resulted in the establishment of a Consortium of the Barcode of Life (CBol) in 2004 (Ratnasingham and Herbert, 2007). The objective of this consortium is to develop a genetic barcode for every eukaryotic organism. The proposal is that a database and work platform utilising a specific 658-bp section of the cytochrome oxidase I gene (COI) be generated for use in identification. The COI region is used as the standard for exploring the identity of a species and has, for example, been provided for *Sarcophaga (sub genus Robineauella) caerulescens* Zetterstedt, which will be of future benefit for confirming the identity of a species newly found on indoor corpses in Finland (Pohjoismäki *et al*., 2010). The consortium also propose a large-scale approach to the molecular identification of a large number of species using standardised protocols, voucher specimens so that there is the possibility of reconfirming the identity of a species, morphological methods, and the development of a large database that is actively maintained. Such proposals are very attractive for the forensic world, including the area of biosecurity.

From such an approach, determining the genetic makeup of the fly would be readily achieved and forensic entomologists would be able to interrogate the database to confirm a specimen's identity. Barcoding for Life (BOLD-IDS) is an online tool that accepts a sequence of DNA from the barcode region and provides a taxonomic ascription, down to species if it is possible. The database allows this to be undertaken using overview sequences. The use of high through-flow protocols and the examination of a large number of samples could also result in a reduction of the cost of such analysis which would be favoured by the forensic science end users.

The measure of the percentage divergence of the specimen profiles has been considered a numerical means of bar code evaluation of the security of the species identification of the specimen (Meiklejohn, Wallman and Dowton, 2011); a further aspect increasingly considered by the courts as necessary in presenting evidence.

Some degree of caution, however, needs to be expressed regarding the approach and the system needs to be policed in order to maintain quality. The presence of endoparasites and micro-organisms in specimens could cause some degree of difficulty in identification.

2.5 The use of other molecular means of insect species determination

DNA is not the only molecular method which has been used to characterise flies. Chemicals, including hydrocarbons and allozymes, have also been used for this purpose. For example, by extracting 22 cuticular hydrocarbons using GCMS, Byrne *et al*. (1995) discriminated between both the sexes of *Phormia regina* Meigen and the three different geographic locations they sampled.

2.5.1 Allozymes

Allozymes are enzymes which, because of genetic mutation, vary within individual species. Heterogeneity in enzymes can be investigated by using electrophoretic techniques such as **iso-electric focussing**; isolating the proteins as bands on polyacrylamide gels and visualising them by staining. Examples of the enzymes that have been explored for enzyme heterogeneity include hexokinase and glycerol dehydrogenase. Szalanski (1995) provided the descriptions of components for a range of stains for these and other enzymes arising out of his research on the genetic characterisation and population genetics of *Stomoxys calcitrans* the stable fly.

Allozymes have been used in southern Australia to distinguish between four calliphorid species of forensic significance (Wallman and Adams, 2000); *Calliphora dubia* Macquart, *Calliphora stygia* Fabricius, *Calliphora hilli hilli* Paton, and *Calliphora vicina.* Using 42 allozymes, Wallman and Adams were able to show a clear distinction between species, both for third larval instars and adults.

The main advantage of this method is its speed; results can be generated in three hours, which is more rapid than is possible for DNA analysis. The method is also considered cost effective and to have a high level of reliability. It also has the potential to be a field technique, unlike the standard DNA methodologies. On occasion the banding patterns produced do not develop strongly but Loxdale and Lushai (1998) suggest that this can be overcome by applying the sample accurately to the gel plate, investigating the buffers so that the most effective is used, and keeping the reaction cool whilst electrophoresis is being carried out.

Alternative chemicals have been investigated to identify the age of individual species. A number of researchers have used **pteridines** (Thomas and Chen, 1989) and **lipofuscins** for this purpose. Thomas and Chen found that pteridines were useful for aging adult screwworms, *Cochliomyia hominivorax* Coquerel. Robson and Crozier were less successful in using this chemical for aging a species of the Formicidae (ants). They did, however, demonstrate a relationship between lipofuscin concentration and the age of the spiny weaver ant (*Polyrhachis sexpinosa* Latrielle) (Robson and Crozier, 2009). A similar relationship between age and concentration of lipofuscin has been found in the honey bee *Apis mellifera* Linnaeus, suggesting that these chemicals may, for individual species, assist in determining the duration of PMI.

Further studies need to be undertaken in order to develop reliable diagnostic markers for forensically important fly species. The limits of variability also need to be confirmed before this technique is routinely encountered in the court room.

Overall, though, using standardised methods that are validated is the preferred approach in forensic science. For this reason, the use of mtDNA is likely to become the recognised standard approach for species confirmation in the near future.

2.6 Insects and entomotoxicology

At a crime scene, insects, particularly larvae, can be valuable as a source of information about the poisoning and/or drug consumption of a victim. This is especially true where the body is so badly decomposed that it is not possible to take samples of blood, urine, or the stomach contents. Immature insect stages, as well as adults, may retain or accumulate foreign chemicals present in their food – the corpse. However, the time period over which specific chemicals last in a cadaver will vary, dependent on both the conditions under which the body is kept and the toxicant itself. For example, Grellner and Glenewinkel (1997), in their actual and desk-based study of exhumations, found indications of diazepam for a period of five years; mercury for one month (based on the literature), morphine up to 1.1 years (based on the literature), whilst amitriptyline was still present in the cadaver after 5.5 years. If these chemicals remain in a body for such a long time it is important to determine what their effect is likely to be on insect development so that this can be taken into account when estimating time since death. Equally the ability of the insects to retain indications of such drugs means that they, themselves, can be a valuable source of information about the past behaviour of the deceased.

It has been known that heavy metals could be extracted from cadavers by using the larval stages of insects that feed on corpses for a number of years. Arsenic has been recovered from the larvae of the Piophilidae (cheese skippers), Psychodidae (moth flies) and Fanniidae (Leclercq and Brahy, 1985). Other heavy metals have also been recovered using insects. For example mercury has been extracted from calliphorid larvae, puparia, and adults feeding on fish which contained methylated-mercury (Nuorteva and Nuorteva, 1982).

Poisons besides heavy metals have been recovered from corpse tissue at both medicinal and recreational concentrations. In many instances, larvae are the most useful source of information about the drug consumption of the deceased. A comparison of the relationship between the recovery of drugs such as oxazepam and triazolam (benzodiazepines), alimemazine and clomipramine (tricyclic antidepressants) and phenobarbital (barbiturate) from calliphorid larvae and the organs of a two month-old corpse was undertaken using liquid chromatography (Kintz *et al*., 1990). The larvae provided a more sensitive means of determining the nature of the drug than using tissue from the corpse.

Wood *et al.* (2003) developed a rapid, sensitive method for the quantitation of benzodiazepines from insects. They confirmed that *Calliphora vicina* larvae and puparia are a suitable source of toxicological information. Sadler *et al*. (1995) pointed out that *Calliphora vicina* post-feeding stages analysis provided estimates of concentration of drugs like temazepam and amitriptyline, which were lower than the original experimental dose. Gola and Lukose (2007) also recovered diazepine, prednisolone, and phenobarbitone from larvae, puparia, and adult blowflies. So, depending upon the drug, all stages of metamorphosis may potentially be used for entomotoxicology.

However the quantities of drugs recovered may not be strictly related to the levels present in the body. In laboratory experiments Introna *et al.* (1990) showed a significant correlation ($r = 0.790$) using regression analysis, between morphine concentrations recovered from larvae and that present in the intoxicated liver upon which the larvae had been fed. However, Hédouin *et al*. (1999, 2001) could not demonstrate a similar relationship in experiments using rabbits intoxicated with the drug when alive. They recovered amounts of morphine 30 to 100 times lower than the concentrations recovered from rabbit tissue.

The majority of experimental work related to the influence of drug intoxication of tissue on larval growth has been carried out using visceral organs such as liver as a food source, rather than muscle, a more likely diet of corpse-dwelling insects. Nolte, Pinder and Lord (1992) investigated larvae feeding on the corpse of a 29-year-old drug addict who was considered to have died five months prior to the recovery of the body. Using gas chromatography and gas chromatography–mass spectroscopy, cocaine and its dominant metabolite benzoylecognine were detected in both the larvae and the decomposing skeletal muscle. However, it was not possible to determine the concentration of the drug. A similar situation was experienced by Definis-Gojanović *et al.* (2007) who recovered amphetamine from the body of a suicide victim in the early stages of decomposition. The person was estimated to have been dead for approximately one month. In this instance the presence of amphetamine was unequivocally confirmed but the levels could not be related to the concentrations found in either the blood or liver tissue.

The effects of drugs on larval life-cycle duration must be taken into account when estimating time since death. Sarcophagid species larvae of *Boettcherisca peregrina* Robineau–Desvoidy fed on drug-intoxicated tissue containing cocaine and its metabolite at both the median lethal dose and twice the median lethal dose, had an accelerated development time due to a reduced time in the larval stages, early

pupation and adult eclosion (Goff, Omori and Goodbrod, 1989). On heroin the speed of growth of their larvae increased by up to 29 hours (Goff *et al.*, 1991). In contrast, Arnaldos *et al.* (2005), investigating the effects of heroin on the duration of individual life stages of a different flesh fly, *Sarcophaga* (*Curranea*) *tibialis* Macquart, showed that the life stages were extended considerably compared to those of larvae fed on tissue that were not drug intoxicated.

The effect of the antidepressant amitriptyline has similarly been shown to increase development time in other species of flesh fly (Sacrophagidae). Hydrocortisone similarly increased the time in the larval stages of *Sarcophaga tibialis* – their dose-dependent effects were nonlinear (Musvasva *et al.*, 2001). (This chemical is often prescribed for the treatment of allergy and inflammatory conditions such as rheumatism. It can be applied topically and as such would be in direct contact with any eggs laid on the skin of a treated corpse or available to larvae consuming the flesh.) Overall, however, the length of time from egg eclosion to adult emergence for *Sarcophaga tibialis* was not dissimilar to that of the controls, suggesting that it has no lasting effect on the determination of the post mortem interval if the insects have been present on the body over a long period.

Bourel *et al.* (1999) demonstrated that morphine reduced the speed of development in *Lucilia sericata* and that if this was not taken into consideration then the time since death could be underestimated by 24 hours. Oliveira *et al.* (2009) similarly revealed that if *Chrysomya megacephala* larvae were exposed to the drug active ingredient butylscopolamine bromide, then with increasing concentrations so the development time increased. Therefore the effect of a particular drug or its metabolites (or both) on speed of insect development appears to be species specific, due to variation the in tolerance levels for particular drugs. This depends on whether the chemical is sequestered in or excreted by the insect.

Bourel *et al.* (2001), using radioimmunoassay, identified a concentration relationship between recovery of morphine and the original amount in the host tissue. They confirmed that *Lucilia sericata* larvae were capable of excreting morphine during the post-feeding stage and that tissue with concentrations of 100 to 1000 mg Kg^{-1} were the most reliably determined because false positives were also recorded at some concentrations. Equally other drugs have little or no effect on development insect time. Work by Kaneshrajah and Turner (2004) and Estrada *et al.* (2009) have shown that consumption of medicinal drugs such as aspirin do not alter the speed of larval development.

Flies are not the only source of toxicological information: many of the beetles that feed directly upon a corpse can also be a valuable source of forensic evidence about the drug consumption of the decedent. For example, Miller *et al.* (1994) examined the mummified female and confirmed that the shed dermestid larval skins (exuviae) and dermestid faecal material on and around the body contained the drug amitriptyline. The researchers were able to recover more amitriptyline from skull fragments and stomach contents of the mummified remains of the person who was thought to have died two years previously. This confirmed the value of dermestid remains as a suitable source of entomotoxicological evidence.

Predators feeding on insects consuming a corpse can be a useful source of information about drug presence. Beetles that are predators of blowfly larvae can bio-accumulate drugs and heavy metals. Therefore these predators, recovered from the later stages of succession, particularly from badly decomposed bodies, can be an effective means of drug detection (Introna, Campobasso and Goff, 2001). They may also provide an explanation for variation in the time of death established by other forensic analytical techniques. A staphylinid, *Creophilus maxillosus* Linnaeus, a predator of dipteran larvae feeding on mercury contaminated fish, was also found to be a source of mercury, which it had bioaccumulated having consumed the maggots feeding on the mercury source.

In some instances the poisons concerned are designed for application to other animals and are not intended for human consumption. Agricultural chemicals such as insecticides are a potential cause of both intentional and unintentional poisoning. The organophosphate Malathion, present in corpses, has, for example, been identified and successfully recovered using *Chrysomya* larvae. Gunatilake and Goff (1989) isolated this from both *Chrysomya megacephala* and *Chrysomya rufifacies* larvae. Using pooling they detected the insecticide in a suicide victim at a concentration of $2050\,\mu g\,g^{-1}$ of larvae. Recognition of the presence of chemicals such as Malathion is important as Yan-wei *et al.* (2010) confirmed. These co-workers showed that Malathion increased *Chrysomya megacephala* larval development time but not the puparial duration and that the larval length and puparial weight were increased. Similarly Gola and Lukose (2007) revealed that the insecticide dichlorodiphenyltrichloroethane (DDT) could be recovered from the larval stages of blowflies but could not identify it in the adult stages. This is also true of humans where DDT has been recovered from human tissue as it was stored in body fat and also in breast milk (Jaga and Dharmani, 2003). Many Africans and Asians have higher concentrations present in their bodies than do the Europeans.

Insects collected from drug hauls can be useful as an indicator of plant origin. For example, beetles on cannabis leaves, impounded from a ship in a New Zealand harbour, were examined by entomologists. The beetles identified were Carabidae, Bruchidae and Tenebrionidae from species which were not native to either the ship's original port or those it had visited. The beetles came from a region between the Andaman Sea and Thailand. Based upon the geographic distribution of the insects, it was concluded that the drugs originated from the Tenasserim region. When this entomological evidence was revealed one of the two suspects confessed to illegal drug smuggling (Crosby *et al.*, 1986).

2.7 Forensic applications of arthropod behaviour for chemical analysis

Arthropods, including spiders, can be of value as a source of detection and some species can be trained to indicate the presence of drugs and explosives by expressing

particular behaviour patterns. Insects will demonstrate particular behaviour patterns in response to chemical stimuli such as carbon dioxide, lactic acid, or the odour from nectar or other substances to which they have been trained to respond. For example, honey bees have been trained to indicate the presence of explosives at places like airports, or train and bus stations and moths are being trained to indicate the location of landmines (King *et al*., 2004). Bees are able to detect explosive substances despite their being masked by materials such as oil, dead bodies, and other odours (Ornes, 2006). The period over which an individual honey bee can be used to detect TNT, according to research by the Stealthy Insect Sensor project, tends to be around two days; training though, is achieved in an extremely short period.

Similarly, parasitic wasps (*Microplitis croceipes* Cresson) have been trained, using associative learning, to indicate the presence of explosives, food toxins or dead bodies (Tomberlin, Rains and Sanford, 2008). Cruz (2006) explored the effects on blowfly larval development of their consuming trinitrotoluene (TNT) contaminated flesh and the possibility of transfer of explosives as a result of ingestion by insects. He showed that the TNT could be recovered from second and third instar larvae which had been fed upon liver laced with explosives. This researcher confirmed the presence of the explosive using scanning electron microscopy and energy dispersive spectroscopy **(SEM/EDAX)**.

The forensic value of insects and other arthropods as a source of evidence or evidence interpretation for a court is therefore wide. They can be integrated with a variety of technologies and used for a variety of forensic purposes in contexts which range from security to toxicology and time since death determination. Currently new areas of chemistry incorporating nanotechnology such as nanoparticles (Bhattacharyya *et al*., 2010) are being developed, which may have a role to play in forensic entomology.

3

Insects and decomposition

The rate of decomposition of the body, and the interpretation of the post mortem interval, are influenced by the arthropods, including insects, which visit and colonise the body.

3.1 Indicators of 'time of death'

The determination of time since death has, historically, been based upon the condition of the corpse itself and such physical features as fall in body temperature. Once the body has been dead for over 72 hours there is less medical information available with which to correlate post mortem interval. Forensic entomology can provide a measure of the minimum post mortem interval, based upon the lifecycle stages of particular fly species recovered from the corpse, or from the succession of insects present on the body. This estimate can be given over a period of hours, weeks, or years once the normal pathological features are no longer usable to determine time since death.

The point at which the fly first laid its eggs on the body is considered to coincide with the point of death and its end is considered to be the discovery of the body. The life stage and name of the oldest colonising species infesting the body is used as a marker of this initial colonisation. The summation of the time in the individual life stages taken to reach the insect life stage recovered from the body, in relation to its particular stage of decay, gives a good estimation of the probable length of time the person has been dead. Indeed this may be the best estimate of the time since death that is available.

Decomposition can be influenced by the age of the person, their physical build, and also their previous activities. Bodies that are obese will decompose more rapidly because bacteria have access to tissue with a good supply of water. People who have a low level of muscle tissue or who are starving will have a rapid onset of active decay and a faster rate of decomposition. Active decay is much slower in elderly people and in babies; so overall decomposition will be correspondingly slow.

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

3.2 Stages of decomposition of a body

There are three recognisable decomposition processes through which a body passes as it decays. These are autolysis, putrefaction, and skeletal bone decomposition (diagenesis). During autolysis, a process of natural breakdown, the cells of the body are digested by enzymes including lipases, proteases and carbohydrases. This process can be most rapid in organs such as the brain and liver (Vass, 2001). A 'soup' of nutrients is released, which forms a food source for bacteria.

Putrefaction is the breakdown of fat and muscle tissues by members of such anaerobic bacterial genera as *Clostridium* and *Bacteroides*. Anaerobic fermentation generates propionic and butyric acids. The production of fermentation gases results in an increase in the size of the body due to bloating. Eventually the skin can contain the gases no longer and liquid leaks from any gaps in the skin, including the oral and nasal and anal orifices. As a result there is an increase in the amount of body tissue available for insect consumption. A greater exposure to the air encourages an increase in the populations of aerobic species of micro-organisms associated with the body, which also speeds up decomposition.

When the soft tissue is removed, skeletal material – organic and inorganic remains – are further broken down by environmental conditions and are finally reduced to components of the soil (Figures 3.1–3.5) (see also coloured section). Carter, Yellowlees and Tibbett (2007) consider that each square kilometre of the landmass receives 5000 kg of cadaveric material annually. The decomposition of the body provides a nutrient-rich island around the body in which there is an increase in

Figure 3.1 Fresh stage of decomposition

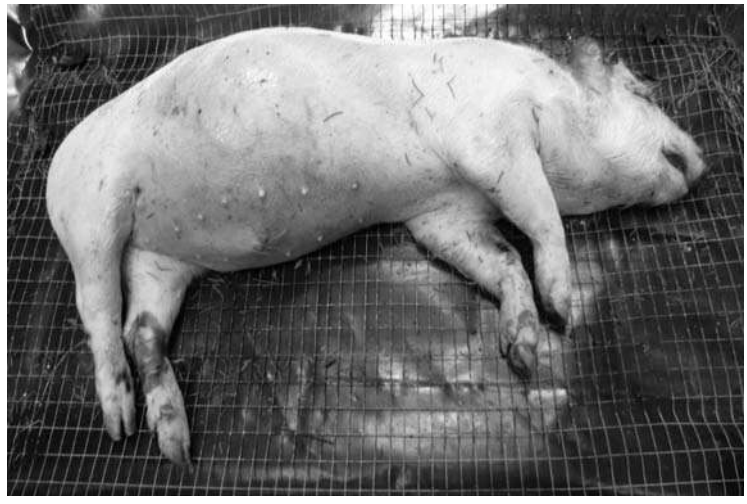

Figure 3.2 Bloat

soil carbon, nutrients, and pH (see colour section). According to Vass *et al*. (1992) a 68 kg human in advanced decomposition, will enrich each gram of the top three to five centimetres of soil by 300 µg potassium, 50 µg calcium and 10 µg magnesium. The island is visually distinct because of a change of vegetation in the area.

The rate of decomposition is temperature dependent. A formula has been proposed by forensic pathologists to estimate the time of body decomposition to skeletisation,

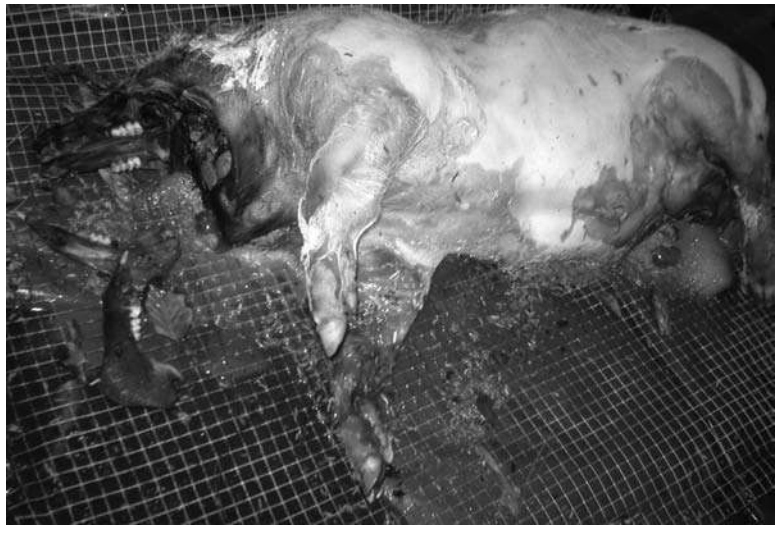

Figure 3.3 Active decay stage

Figure 3.4 Advanced decay

in relation to temperature (Vass, 2001). The formula is:

$$Y = 1285/X$$

where Y is the number of days to mummification or skeletonisation and X is the average temperature on the day that the body was found (Vass *et al*., 1992).

Figure 3.5 Skeletal remains

Box 3.1 Diagenesis, mummification and adipocere

Besides skeletonisation, with the resultant change in the bone structure (Diagenesis), two other outcomes of the decomposition process may occur. These are mummification and the generation of 'grave wax' or adipocere.

Diagenesis

When the body reaches the skeletal stage, changes to the bone called diagenesis occur. Diagenesis is defined, in chemical terms, according to the *Collins Dictionary of the English Language* (Hanks, 1984), as recrystallisation of a solid to form large crystal grains from smaller ones. The changes in the bone structure depend upon the breakdown of the soft tissue. This is affected by the nature of the death and subsequent treatment of the body, including the type of environment in which the body is buried.

Investigating bone can tell us about the latter stages of post mortem change because a number of features can be quantified. The length of the post mortem interval can be estimated if the bone histology is investigated under the microscope – the degree of bone porosity is determined; the carbonate and protein content of the bone are calculated; the crystalline nature and content of the bone mineral made of calcium fluorophosphate or calcium chlorophosphate (apatite), is examined and the components that have leached out of or into the bone are determined.

Insect attack, both before the body is buried and after, has a role to play in causing change to the environment and hence bone diagenesis.

Adipocere

If the body is in an environment that combines high humidity with high temperatures the subcutaneous body fat of the face, buttocks (breasts in the female) and the extremities become hydrolysed. Fatty acids are released. These form food for bacteria, which can speed up the rate at which adipocere is made. For example *Clostridium* bacteria will convert oleic acid (a fatty acid) into hydroxystearic acid and oxostearic acid.

Two types of adipocere are found, depending on whether the fatty acids combine with sodium or with potassium. If sodium from the breakdown of intercellular fluid is bound to the fatty acids the adipocere is hard and curly. Where the cell membranes break down and potassium is released a softer adipocere results, which is often termed 'pasty'. An indication of submergence in cold water is the uniform cover of adipocere over the body (Spitz, Spitz and Fisher, 2006).

Mummification

If water is removed from skin and tissue, the tissue becomes desiccated and mummification will occur. This happens particularly where a body is kept in an environment with a dry heat and little humidity and where the airflow is good. Chimneys are examples of good locations with these features. In mummified corpses in temperate conditions the extremities become shrivelled and the skin tends to be firm but wrinkled and to have a brown colouration. The internal organs such as the brain will decompose during mummification.

3.2.1 Body decomposition and the sequence of insect colonisation on land

The body can be allocated to one of five recognisable post mortem conditions, which can be linked to the eight waves of arthropod colonisation proposed by Mégnin (1894). The transition from one stage to the next is not distinct and Gaudry (2002), on the basis of 400 cases, considers Mégnin's first two waves to be one.

Although no stage has a fixed duration, each stage can be associated with a particular assemblage of insects. The profiles of insects would appear to be universal, although the majority of research on this aspect has, until recently, been undertaken primarily in North America (Hough 1897; Easton and Smith, 1970; Rodriguez and Bass, 1983; Catts and Haskell, 1990; Mann, Bass and Meadows, 1990; Goff, 1993; Dillon and Anderson, 1996; VanLaerhoven and Anderson, 1999; Okiwelu, Ikpamii and Umeozor, 2008; Byrd and Castner, 2010; Tüzün, Dabri and Yuksel, 2010; Valdes-Perezgasga *et al*., 2010).

These post mortem transitions on land are discussed in this chapter; the sequence of decomposition in aquatic environments is discussed in Chapter 12.The stages of decomposition of corpses left on the soil surface are:

- Stage 1. *Fresh stage.* This stage starts from the moment of death to the first signs of bloating of the body. The first organisms to arrive are the blowflies (the Calliphoridae). In Britain these are usually *Calliphora vicina* or *Calliphora vomitoria*, or in early spring they may be *Protophormia* (=*Phormia*) *terraenovae* Robineau-Desvoidy (Nuorteva, 1987; Erzinçlioĝlu, 1996).
- Stage 2. *Bloated Stage.* Breakdown of the body continues because of bacterial activity, or putrefaction. This is perhaps the easiest stage to distinguish. Gases causing the corpse to bloat are generated by anaerobic bacteria metabolising nutrients. The whole body swells, starting with the abdomen, and becomes stretched like an inflated air-balloon (Figure 3.2). At this stage more and more blowflies are attracted to the body, responding to the odour, including that of the breakdown gases. Vass *et al*., (1992, 2004) studied the odours emanating from buried bodies and those dead bodies resting on the surface. Their work provides clarification of the identity of some of these gases and allows interpretation of the observations of Mégnin (1887, 1894); Hough (1897); Smith (1986); Matuszewski *et al*. (2008, 2010) and others. At bloat, rove beetles (Staphylinidae) may be attracted to the body. These and other predators can affect the interpretation of the range of insects and insect-life stages present as they feed on larvae, or remove puparia (Smith, 1986).
- Stage 3. *Active decay stage.* At this stage the skin of the corpse is split in places and starts to slough off the body. Sloughing allows decomposition gases to escape. The body gradually deflates as putrefaction continues (Figure 3.3). In the later stages of putrefaction, fermentation occurs and butenoic and caseic acids are generated, amongst others. This is followed by a period of advanced putrefaction, which includes ammoniacal fermentation of the body, to which a different cohort of insects

is attracted. These include the silphid beetle *Nicrophorus humator*, (Gleditsch), the histerids *Hister cadaverinus* Hoffmann and *Saprinus rotundatus* Kugelann, and the muscid fly *Hydrotaea capensis* Wiedeman (=*Ophyra cadaverina* Curtis).

- Stage 4. *Post-decay stage.* In the later stages of decay all that remains of the body is skin, cartilage, and bones with some remnants of flesh, including the intestines (Figure 3.4). Any remaining body tissue can be dried. The biggest indicator of this stage is an increase in the presence of beetles and a reduction in the dominance of the flies (Diptera) on the body.
- Stage 5. *Skeletonisation.* At this stage the body is only hair and bones (Figure 3.5). No obvious groups of insects are associated with this stage, although beetles of the family Nitidulidae can, in some instances, be found. The body has clearly reached its final stage of decomposition. Any further breakdown is best described in terms of the decay of individual components of the body, such as the bones of the feet and legs, the skull and the ribs.

3.2.2 Decomposition of buried bodies

Buried corpses may require investigation from one of two situations: the result of either criminal activity, where the body is covered over or placed in a hole dug in the soil to conceal it from view, or legitimate burial of the deceased in a coffin in a grave where exhumation is requested. Research has been undertaken on both types of burial. Breitmeier *et al.* (2005) explored the stages of decomposition of legitimately buried corpses and found that evidence of decomposition was divided into four stages. These were from early decomposition to skeletisation of most or all of the body. Environmental conditions such as how deep the body was buried, the condition of the ground in which the body was buried, and the season of burial all played a large role in dictating the speed of body decomposition (Table 3.1).

Gunn and Bird (2011) consider that in general, in the UK, a body that is buried to conceal a crime (a clandestine burial) tends, on average, to be covered by an average of 0.4 m of soil. In the USA the depth of soil was considered to be slightly greater at an average depth of 0.56 m.

Wilson *et al.* (2007) investigated the decomposition of buried corpses using pigs (*Sus scrofa* Linnaeus), which were buried directly into the ground – an

Table 3.1 Speed of decomposition of encoffined bodies in graves

Decomposition stage	Average time	Range
1. Early decomposition	22 days	5–58 days
2. Moderate decomposition	93 days	8–8.7 months
3. Advanced decomposition including minor skeletalisation	2.8 years	5.7 months–10 years
4. Skeletalisation (complete or predominant)	12.7 years	8.4–16.8 years

Source: After Breitmeier *et al.* (2005)

approach reminiscent of the type of burial characteristic in criminal activity and also the approach taken for mass graves. In their research, water-table movements, changes in soil temperature and moisture levels strongly influenced the rate of decomposition. Interestingly, decomposition rates between one corpse and the next at a particular site also varied. The rate of decomposition in general, however, was determined by the season the body (pig) was buried and soil temperature, irrespective of location; be it pastures deciduous woodland or moorland. This was also the experience of Turner and Wiltshire (1999) who showed that burial of pigs in winter in cold, oxygen-limited conditions in heavy, base-poor clay soil resulted in good corpse preservation.

Gaudry *et al.* (2006) revealed that depth of burial affected the colonisation of buried bodies by insects. In this instance, the carcasses chosen were sheep (*Ovis aries* Linnaeus). The main colonising insects were flies from the families of the House flies (Muscidae), lesser house flies (Fannidae) and hump-backed flies (Phoridae). Muscidae were found on corpses buried at 10, 30, or 90 cm below the soil surface. However the attractiveness of the corpse to this family diminished with depth. Colonisation lasted between 60–120 days post-burial at 30 cm; in contrast colonisation continued over six months where the bodies were covered by only 10 cm of soil. In contrast to the situation where bodies were retained on the soil surface, no species of the Calliphoridae were found on any of the buried sheep.

Gunn and Bird (2011) found a similar relationship with depth and colonisation but noted that the eggs of both *Lucilia sericata* and *Calliphora vomitoria* could hatch beneath the soil surface. Their results linked efficiency of colonisation of remains by *Calliphora vicina* and *Calliphora vomitoria* with loose soil which covered remains to a depth of 5 cm. *Calliphora vomitoria* was unable to colonise remains at any greater depth. Therefore Gunn and Bird conclude that if there is a large number of larvae of either *Calliphora vicina* or *Calliphora vomitoria* present on a body buried at a depth of 5 cm or greater, then there is some possibility that the body was exposed to the elements above ground prior to its subsequently being covered over with soil. This is in contrast to *Lucilia sericata,* which could colonise decomposing remains at a depth of at least 10 cm. Muscid flies such as *Muscina stabulans* are far more successful and can colonise a body to a depth of 40 cm at least. Soil conditions therefore have a role to play in colonisation of buried bodies and may dictate the species successfully colonising the body. Spilza, Voss and Pape (2010), for example, showed that Sarcophagidae were more common colonisers of buried bodies in habitats that were dry; the soil loose and dry. In such circumstances they were able to colonise bodies buried to a depth of around half a metre.

3.3 Volatiles released from the body during decomposition

Statheropoulos *et al.* (2007) investigated the volatiles released from a human body in early decay four days after death. Thirty volatiles were identified comprising

hydrocarbons, alcohol, aldehydes, ketones, sulphide compounds, and benzene derivatives. Only 12 substances were recovered immediately after the four day-old body was examined directly after being in a refrigerator for 24 hours. These substances were octane, trimethyl decane, ethanol, 2-propanone, 2-butanone, dimethyl disulphide, dimethyl trisulphide, methyl ethyl disulphide, methyl benzene, *o*-xylene, *m*-xylene, and ρ-xylene.

At active decay, simple volatiles such as hydrogen sulphide, sulphur dioxide, carbon dioxide, methane, ammonia, hydrogen, and carbon dioxide are released. Protein and fat decomposition produce glycerols and phenols and proteins are also broken down by bacteria into fatty acids (Vass, 2001).

A large number of odours are released from the decomposing human corpse over the whole sequence of decomposition. Vass *et al.* (2008) recorded 478 volatiles of which 30 were considered to be key indicators of human decomposition. From the surface of the soil above bodies buried for 12 months, Vass *et al*. (2004) recorded cyclic and non-cyclic hydrocarbons, sulphur compounds, acids, esters, oxygen compounds, and halogens. Volatile chemicals recorded from decomposing humans irrespective of whether or not they were buried were: toluene, 1,1,2-trichloro-1,2,2-trifluoro ethane, hexane, undecane, alpha, alpha, dimethyl benzenemethanol, dimethyl disulphide, dimethyl trisulphide, decanal, carbon disulphide, nonanal, 1,4-dimethyl benzene, benzene, ethyl benzene, 1-ethyl, 2-methyl benzene, dichlorodifluoromethane, 1,2-benzenedicarboxylic acid, diethyl ester, methenamine, carbon tetrachloride (Vass *et al.*, 2008). LeBlanc and Logan (2010) found that two of these volatiles, which were derived from the unburied corpse of a pig, dimethyl disulphide and dimethyl trisulphide, stimulated an electrophysiological response from *Calliphora vomitoria,* but she failed to gain a response from the fly to benzene and hexane.

Upon death, the body can emit body fluids soon after the muscles of sphincters cease to remain constricted, which attracts insects and in particular the Muscidae. The decomposition compounds originating from urine contain attractants such as 1-octen-3-ol, 6-methyl-5-hepten-2-one and (z)-3-hexen-1-ol which draw muscids *Hydrotaea irritans* Linnaeus, *Musca autumnalis* DeGeer and *Stomyx calcitrans* Linnaeus (Birkett *et al*., 2004). These chemicals draw the species to the body and its waste material, adding to the assemblage of early colonising insects that arrive at the recently dead body.

Decomposition odour differences have also been found to distinguish between the decomposing corpses of different species. For example the emission of 1,1-dimethylcyclohexane distinguishes dead rat from the corpses of dog, human, cat, bird or rabbit. The volatile phenanthrene distinguished humans from the other species (Verplaetse *et al*., 2007).

The decomposing body will respond to different environmental conditions. Under buried conditions where moisture is present and the pH is quite high, fatty regions of a body such as the cheeks, thighs and buttocks, can undergo **saponification** with the formation of 'grave wax' or adipocere. The nature of adipocere, whether it is hard and crumbly or more like a paste, can indicate source of material and the speed of decomposition.

According to Vass (2001), if the fatty acids are bound with sodium from the fluid from between the cells (interstitial fluid), a hard, crumbly adipocere will be produced. The pasty form of adipocere is the result of binding of fatty acids with potassium, available where cell membranes have decomposed. The nature of these fatty acids varies with body and location. Conditions in the moorland and deciduous woodlands of the Pennine uplands of the UK resulted in a wide range of different fatty acids being recovered from pigs buried for 12 months.

The average relative percentages of fatty acids recovered from pigs buried in moorland and deciduous woodland soils respectively were: myristic acid (6.5% and 13%); palmitic acid (38.5% and 40%); palmitoleic acid (5.5% and 7.5%); stearic acid (15.5% and 4.5%); oleic acid (27.5% and 27%); triacyl-glycerol (17.5% and 32%) (Wilson *et al.*, 2007). In general the presence of adipocere protects the body from decomposition through insect attack and acts as a preservative.

3.4 Decomposition in specific circumstances

Frequently bodies are recovered from inside vehicles or other confined spaces such conditions may influence the speed of development of the insects.

3.4.1 Decomposition inside vehicles

Decomposition of bodies recovered from inside vehicles is influenced by the conditions within the vehicle, where the vehicles are located and how accessible the interior is. Many people intending suicide – one of the main reasons why bodies are found in cars – choose to drive to secluded spots in order to avoid rapid detection. Despite the site being in shadow or exposed, the sequence of body decomposition is not altered although, because of the increased heat inside a vehicle, the decomposition rate may be faster because of the speed of development of both bloat and the dry stage of decomposition (Voss, Forbes and Dadour, 2008).

It might be expected that the effect of being contained in a vehicle would influence the sequence of insect succession on a body. This is because the speed at which insects both find the body and oviposit is likely to be delayed through restriction of access. Voss, Forbes and Dadour (2008) found that insects did not detect the presence of a body in a car for a period of between 16–18 hours longer than they detected an exposed body in the same location. This applied to the speed of colonisation of both Calliphoridae and beetles. The beetles were delayed in colonising the body until a later stage of decomposition (active decay rather than bloat) when the body was within a vehicle. The location and conditions within the vehicle are also important. Campobasso, Di Vella and Introna (2001), citing unpublished work by Meek, recorded a delay of three days for flies laying eggs on bodies confined in a car boot and of around seven days where the body was confined in a burned-out car.

3.4.2 Decomposition of burned bodies

Bodies are burned for a number of reasons. They may be burned through accident in cases of fire setting or the body may be burned in the context of a suspicious death, as a means of disposal. Avila and Goff (1998) compared the colonisation of insects on burned bodies in a dry habitat compared to that in a rain forest. In both instances there was no difference in the colonisers in either condition but oviposition by most Calliphoridae occurred one day earlier on the burnt carcass than the control carcass in dry conditions in Hawaii and four days earlier in the rain forest for all of the colonising species.

Hart, Hall and Whitaker (2011) outlined the value of insects in determining post mortem interval in burned bodies. They cite the case of a suicide for which determination of time since death was difficult using pathology. Fly larvae of which had colonised the body after it had been burned were used to confirm time since death. They indicated that the body had been in the disused ammunition bunker for at least six days prior to its discovery. Colonisation of a body may therefore occur after it has been burned.

Work by Chin *et al.* (2008a) indicated that the degree of burning is significant. Partial burning of the pig carcass did not prevent it from bloating. Campobasso, Di Vella and Introna (2001) point out that burnt tissue provides a poor source of protein for developing larvae as well as a physically difficult medium upon which to lay eggs. They concluded that it is the internal organs that provide the attractive odour for diptera and that these are exposed by burning if the skin splits.

Anderson (2005) carried out a series of very elegant experiments to explore the effect of a subsequent fire on larval survival. She showed that on occasion, if the body is burned after flies have colonised it, the maggots may survive by moving into the centre of the corpse. She also points out that it is possible to use dead maggots if the body has been colonised initially and then burned. The maggots which fail to survive can be used to determine the post mortem interval provided that the rapidity with which the fire was extinguished, and the date and time that the fire was extinguished are known. This can then be added to the estimated PMI in order to determine a minimum time since death.

3.4.3 Decomposition of hanging bodies

The speed of decomposition of a hanging body is influenced by its being suspended above the ground. The body is no longer easily accessible by soil-inhabiting insects, nor does it benefit from the increased moisture available from the soil as it decomposes. However bacteria may be encouraged to increase because the asphyxia caused by hanging may result in the blood carrying bacteria to a number of regions of the body in the process of dying. This increase can speed up the initial stages of decomposition.

There is less predation by maggots in these circumstances, so decomposition is slowed down and the time to reach the dry stage is extended. However, as Chin *et al*. (2010) point out, the lifecycle of the colonising insects is not affected. Gennard and Robson (2007)] showed that the location in which the body was hanged also influenced the speed of colonisation by flies and hence the post mortem interval.

3.4.4 The effect of wrapping bodies

Whether or not the body is easily located and eggs are readily laid on the corpse by flies, is dictated by how easy it is for the odours to percolate from the body and the flies to be attracted to the corpse. The presence of clothes and also wrappings such as blankets, polythene bags, and carpets will all deter the flies from gaining access to the body to lay eggs because it restricts the emission of decomposition odours. This will limit the interpretation of the minimum time since death because the period of time between death and oviposition cannot be established accurately. However the degree of tightness of wrapping will dictate whether or not this is true, as Kelly, van der Linde and Anderson (2009) showed that where the clothes were loosely attached there was no reduction in speed of colonisation compared to bodies without clothes.

The presence of clothes on the body will also slow down the speed at which the body cools. However this will also mean that tissue breakdown is likely to start more rapidly and hence the succession of insects attracted to the body may also speed up. In addition once the insects gain access to the body clothing and other body wrappings, these provide shelter and maintain the environmental conditions generated by the insects. Açikgöz, Yüksel and Açikgöz (2005) showed that larvae on unwrapped corpses required more time to reach a similar size to those larvae on wrapped corpses. They urged caution in such circumstances where the post mortem interval is determined using larval length.

The influence of body fluids on maggot survival may be large where the wrappings (and body fluids) combine to limit gas exchange. Kelly, van der Linde and Anderson (2009) concluded that a buildup of toxic waste gases or a reduction in the availability of oxygen could cause high larval mortality. This, however, did not affect development in those larvae that were not surrounded by tight bindings. Leakage of body fluids, even where the access is limiting to insects, may also result in attraction of insects to the wrapped copse and the stimulation of egg laying. Muscid flies, for example, may be attracted by blood and stimulated to oviposit on blood-soaked soil. Sufficient nutrients may be provided by this fluid to support larval development (Gunn and Bird, 2011).

3.4.5 The effects of cold environments

The effects of conditions of prolonged darkness and cold temperatures on insect development should also be considered when interpreting the post mortem interval.

Table 3.2 Odour profile interpretation of decomposition volatiles during human decomposition recognised using the advanced sensor for detecting clandestine graves (LABRADOR)

Percentage decomposition of the buried body	ADD	Number of odour sensors triggered
5–8%	113	7
15–20%	216	9
45%	362	8
65%	412	11
85–90%	1020	5

Source: Dr Arpad Vass and co-workers for permission to quote information from their unpublished report Award Number 2007-DN-R.104 (presented in this book as Table 3.2).

Bodies have been recovered from beneath snow and yet have been infested by insects (Wyss *et al.*, 2003a). They have also been recovered from conditions of apparent total darkness in caves in Switzerland where the temperatures have been around 5 °C and yet the bodies have been infested with insects (Faucherre, Cherix and Wyss, 1999).

The environmental conditions and the stage and nature of decomposition have an effect on determining time since death. Facets of both the condition of the corpse and also the habitats and environmental conditions in which the body is located should all be taken into consideration when determining time since death and the sequence of decomposition of the corpse or using the corpse for other purposes such as the recovery of genetic or toxicological information. The significance of unseen environmental conditions in influencing post mortem analysis and interpretation is exemplified by the work of Zhong *et al.* (2002) who showed that very low magnetic fields have an effect upon messenger RNA and its transcription of cytochrome oxidase. They suggest that there is a biological effect from low-level magnetic fields, even where exposure is for a period as short as 20 minutes, which may be an important consideration in interpreting molecular analysis of corpse tissue.

Consideration of the overall decomposition process in the context of the environment will allow rapid interpretation of time since death. To this end Vass, Thompson and Wise (2010) have designed an advanced sensor for determining the location of buried bodies using a partial human decomposition odour profile (LABRADOR). This will allow linkage to the decomposition stages of a body – based on the different odours present. For example they concluded that at 113 accumulated degree days (ADD) there was 5–8% decomposition of a buried body based upon the presence of seven odours (Table 3.2). At an ADD of 362 there was a 45% level of decomposition and the body could be identified by an odour profile based on eight odours. Such research is also of relevance to the forensic entomologist in refining the cues to which the insects respond when they colonise bodies left on the soil surface of a crime scene.

4

Identifying flies that are important in forensic entomology

In order to interpret a crime scene it is important to know which insect species have colonised the body and something about their habits and environmental requirements. To identify the species you will need to know what the parts of an insect are called, so that you can use the scientific keys written by taxonomists to identify the insects.

Insects are invertebrates. They are classified within the Arthopoda but differ from other arthropods because they have a number of differing features. Insects have a hardened body case (an exoskeleton) which is split into three distinct regions. These sections are called:

- the **head**;
- the **thorax**;
- and the **abdomen**.

The sections have three dimensions, a top (**dorsum**), an underneath (**sternum**) and sides, each of which is a called a **pleuron**. The thorax is split into three segments, which may or may not be clearly defined, depending upon the species. Starting from the head and working backwards along the thorax, these segments are called the **prothorax**, the **mesothorax** and the **metathorax** (Figure 4.1).

All insects have six legs (three pairs of jointed legs). These too are made up of sections – starting at the point nearest to the body (the proximal region) we have sections called the **coxa** (plural coxae), followed by the trochanter (a small section), the **femur**, **tibia** and the **tarsus** (plural tarsi) (Figure 4.2). The numbers of tarsal segments (**tarsomeres**) may vary, but usually there are five per leg. The legs are located on the thorax. One pair of legs is found on each of the thoracic segments – i.e. one pair is on the prothorax, the second pair is on the mesothorax and the third pair of legs is attached at the metathorax.

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Box 4.1 Arthropoda

Members of the phylum Arthropoda are significant in forensic entomology because a large proportion of the identified living Arthropoda are insects. Arthropods have an open circulatory system referred to as a coelom, and an exoskeleton made up of amongst other things a polysaccharide called chitin and protein. The arthropod body supports jointed appendages.

The Arthropoda classification for organisms which are currently living is divided into a number of lineages. The division into a particular lineage is based on both morphology and on molecular features – a combination of phenotypic and genotypic similarities.

For those invertebrate organisms which are not currently extinct there are currently considered to be four lineages, defined by a number of specific features or lifestyle aspects:

- *Chelicerata* – based on the presence of feeding appendages modified into a claw or claws; the presence of a combined head and thorax the cephalothorax, and a posterior abdomen. Members of this subphylum do not have antennae but do have simple eyes. This subphylum includes members that are of obvious forensic relevance: the ticks, mites, spiders and scorpions. Mites are significant in forensic entomology as indicators of the stage of decomposition.
- *Myriapoda* – these are long segmented organisms. Of major interest within the subphylum are the millipedes and the centipedes. Millipedes have two pairs of legs per apparent segment (each segment is the product of the fusion of two segments each with a pair of legs). Millipedes are plant feeders and, although they may be found in association with a body, they are merely using it as shelter rather than having a direct forensic influence. Centipedes have only one pair of legs per segment and are able to catch prey and immobilise it by using front claws that deliver a poison.
- *Hexapoda* – all six-footed organisms but primarily the insects. There are many insects of great forensic significance.
- *Crustacea* – crabs, lobsters, shrimps (freshwater and marine) and barnacles, which may have some forensic relevance in connection with submerged bodies. This subphylum also includes small plankton – copepods and the woodlice, which are of less forensic relevance. Crustacea have walking legs and abdominal appendages. They also have a number of pairs of appendages (three or more) modified into mouthparts including a pair of mandibles. Crustacea have two pairs of antenna, unlike insects, which have only one. (Barnacles are included in the subphylum because barnacle larvae are similar to those of the other aquatic crustacean organisms.)

Having determined the subphylum, class and order to which the organisms of interest belong, the original binomial naming system becomes of relevance. The names are found in insect keys as an end product of identification. The keys are used in order to specify which species are present upon a body. Time since death can be determined from this.

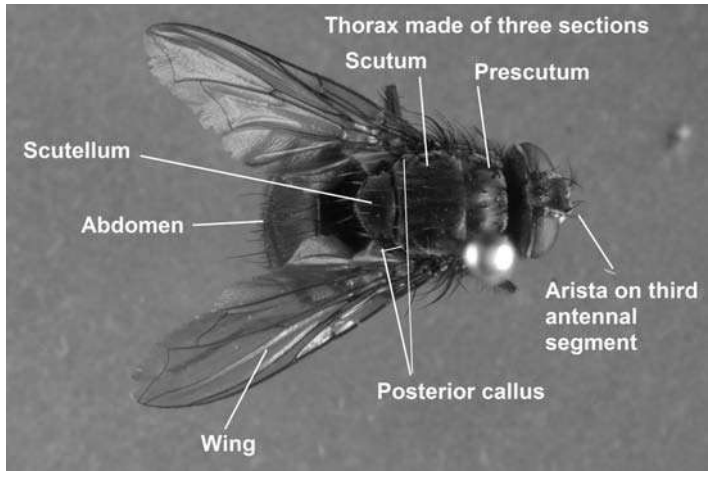


Figure 4.1 General morphology of the insect

Insects have two pairs of membranous wings (one pair of which may be modified). The first pair of membranous wings is attached to the mesothorax. The second pair of wings (or a modification of the second pair) is attached at the metathorax – the final segment of the thorax. The membranous wings are supported by veins. These veins have been named in order to assist in identifying the species of insect. They are counted from the first vein i.e. the first vein running along the wing edge. (The system of naming described here is based on work by two scientists called Needham and Comstock and is called the Needham Comstock System (Figure 4.3).

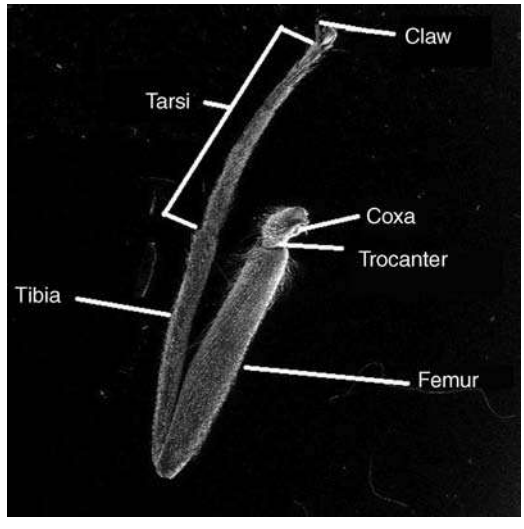
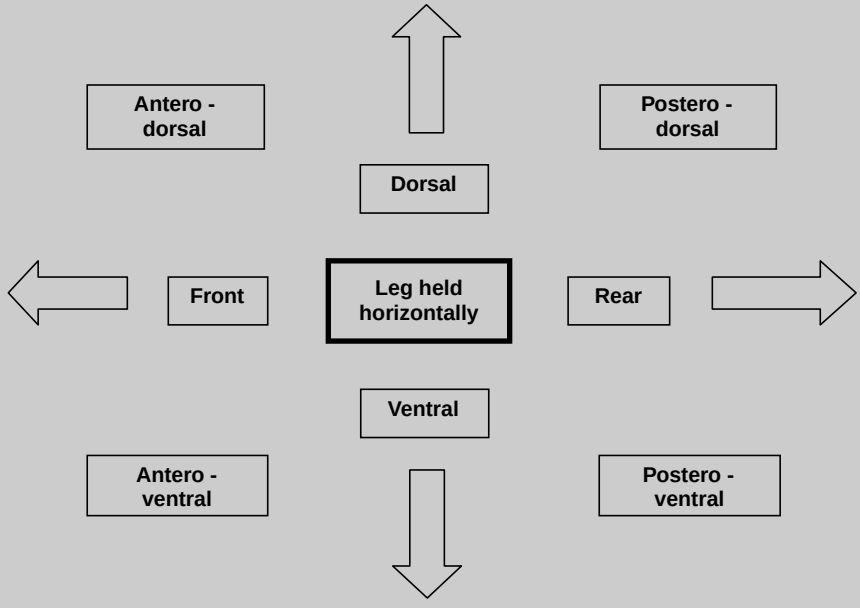


Figure 4.2 Structure of the insect leg

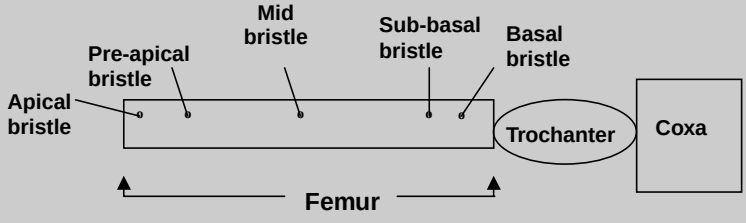
Box 4.2 How to name Leg Bristles

Bristles can be distributed down the femur at set points. A bristle near the joint of the femur with the trochanter is called a basal bristle. The bristle near the joint with the tibia is called an apical bristle. The bristle just before the joint with the tibia is the pre apical bristle. The one just before the joint with the coxa is a sub-basal bristle.



Orientations of bristles on the insect leg

In the early twentieth century, a system was proposed that helps us to decide the names of the other bristles (Grimshaw, 1917, 1934). The method requires that you think of the bristle position in terms of a cross-section through the leg as if it is stretched out horizontally from the body. (The next figure indicates the positions of bristles when the leg is held horizontally.) The positions of the bristles are thought of as positions on a clock face. At 12 o'clock the bristle would be a **dorsal** bristle; at 6 o'clock it would be a **ventral** bristle. At 10 o'clock it would be called an **antero-dorsal** bristle whilst at 2 o'clock it would be called a **postero-dorsal** bristle.



Examples of names of bristles on the femur

(*Continued*)

Moving round the clock face, at the 3 o'clock position, as the leg is held horizontally from the body, the bristle is a **posterior** bristle. At the 9 o'clock position the bristle is the **anterior** bristle and at the 7 o'clock position the bristle is called the **antero-ventral** bristle and at the 5 o'clock position it would be called the **postero-ventral** bristle. In identification keys, having a bristle present on any of the three pairs of legs constitutes presence of a bristle. It does not necessarily need to be present on all three pairs of legs.

The first of the wing veins, **vein 1**, is called the **costa**. This is a thick, hardened vein and gives the wing some rigidity for flying. The second vein, **vein 2**, is called the stem vein or **sub costa**. The third vein at the proximal (body) point of attachment, **vein 3**, is called the **radius**. The fourth long vein, **vein 4**, is the **media** (or medial) vein. This can be split into four veins as it passes to the wing edge. The fifth vein, **vein 5**, is called the **cubitus** vein and in some insect species also splits.

There are in addition, several **cross veins.** These wing cross veins are named on the basis of the veins between which they pass. The **c** and **sc** vein runs between the costa and subcosta. The '**r**' veins run between the splits in the radial vein. The **r – m** vein runs between the radial and the median veins and the **m – cu** veins run between the media and the cubitus veins. An example of the structure of the Calliphorid wing is found in Figure 4.4. The vein numbers are hard to work out from first principles and diagrams for the group should always be consulted. One book may not be consistent with another so it is necessary to use an insect key with diagrams with which you are comfortable.

Insects also have a pair of segmented structures on their heads, which are sense organs (anterio-dorsally positioned). These are the **antennae**, although they are

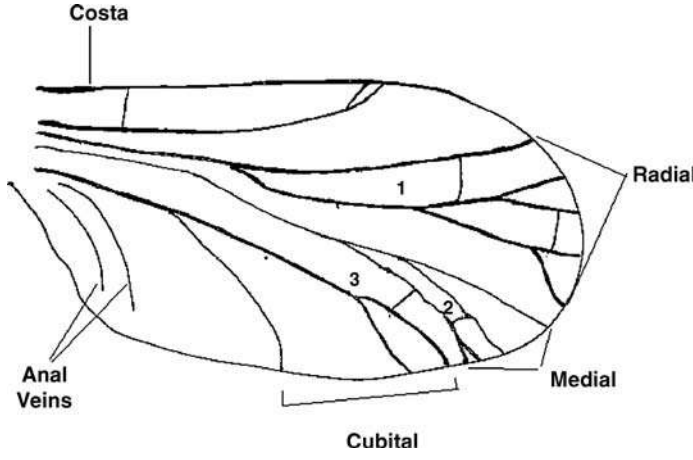


Figure 4.3 Insect wing venation exemplified using the Needham Comstock venation naming system

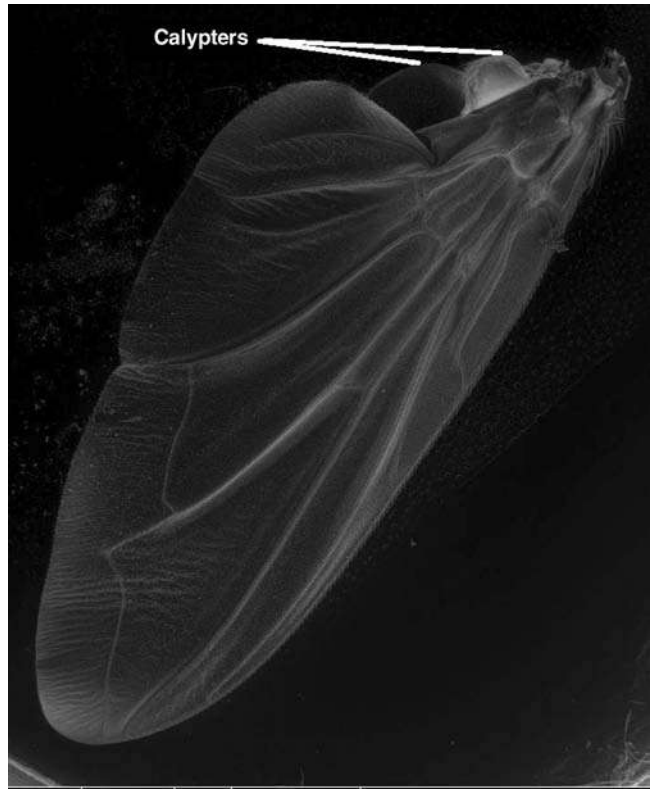


Figure 4.4 The insect wing structure illustrating the position of the calypters

commonly called 'feelers'. There are many different forms of antennae and their shapes can assist in identification (Figure 4.5). Antennae provide the insect with a means of gaining both chemical (contact chemoreceptors) and mechanical information (mechanoreceptors over a distance) from its surroundings.

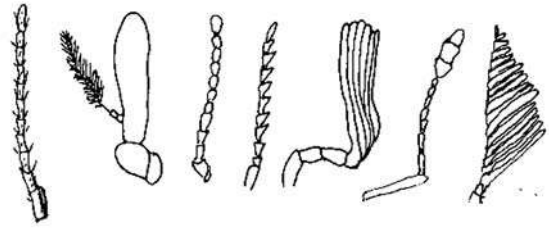


Figure 4.5 Examples of some of the forms of insect antenna

Box 4.3 Insect antenna

The insect antenna is made up of three discernible sections:

- Section 1 – the scape. This is the part nearest to the head. It can be moved using what could be termed a 'ball-and-socket' joint.
- Section 2 – the pedicel. This is a small segment. The pedicel carries a special a region called Johnston's organ which informs the insect about changes in its position. Johnston's organ is stimulated when the flagellum moves and also provides information about aspects such as air currents or the presence of solid objects as well as about information on position changes for the insect.
- Section 3 – the flagellum. This consists of a variable number of segments depending upon the insect species. They also are of variable shapes and this feature is used as a means of identification in identification keys. For example, some may be elbowed; some may end in a club and others may be simple repeated segments of similar dimension.

If the antenna has expanded terminal segments that look swollen and make a club on top of much more slender segments nearer to the pedicel, the flagellum appears to be in two parts. The name of the slender region, which can resemble a stem, is the funicle. The name of the swollen terminal region is the head.

Antennae are primarily sense organs providing information in terms of odour and touch for the insect. Peglike or conelike structures, which are chemoreceptors, are found projecting from pits on the antennae of several diptera including members of the Muscidae. In the Coleoptera a different shape of chemoreceptor sensilla have been noted, which are contained in pits on the antennal surface and do not project above the cuticle surface.

Within the phylum Arthropoda (or jointed limbs phylum), insects are incorporated within the Hexapoda and are divided into a large number of groups called orders. Each **order** is divided into a number of families. Each **family** is made up of a number of **genera** (singular genus) and each genus has one or more **species** (Figure 4.6). The named groups, at each level of this hierarchy, are called **taxa** (singular taxon).

One of the orders of insects which are forensically relevant is the order Diptera – the true or two-winged flies. These are discussed in the following sections. Three other insect orders are discussed at the end of the chapter as these can have forensic relevance in aquatic crime scenes.

4.1 What is a fly and how do I spot one?

True flies are easily distinguished from other insects by having two fully developed, usually obvious, front wings but with each of its two back wings (the second pair of

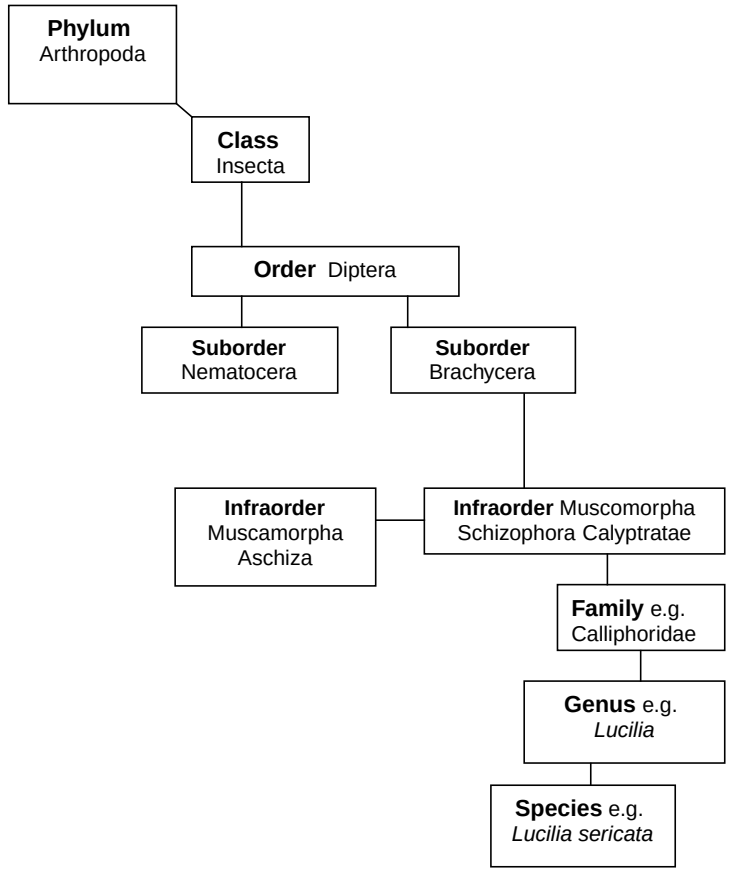


Figure 4.6 Classification hierarchy

wings) modified into balancers, called **halteres**. These structures resemble tiny drum sticks attached to the metathorax.

There has been a considerable change in fly taxonomy recently and the agreed groupings of flies arise from modern developments in taxonomy including molecular studies. (In older forensic textbooks the taxonomy by Kloet and Hincks (1976) has been used. In this classification the diptera were divided into three suborders, with the third, the Cyclorrhapha subdivided into the Aschiza, Schizophora-Acalyptratae and the Schizophora-Calyptratae.)

Currently, the phylogenetic classifications between the **suborders** and the families are mainly a response to practical considerations, so that now, rather than three suborders within the diptera, there are two suborders.

The first of these suborders, the **Nematocera** or Thread horns, contains the crane flies (Figure 4.7). These insects have a long slender body, long antennae with more than six segments, and a complex wing venation. The Nematocera have mouthparts that are 'droopy' (pendulous).

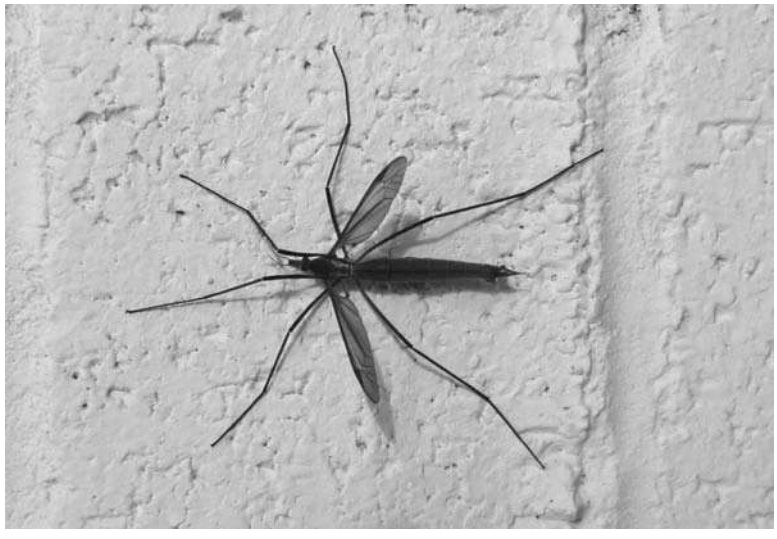


Figure 4.7 A Nematoceran fly

The larvae of this suborder have a structurally distinct head (exserted head) with horizontal biting mouthparts. The pupa is not encased and so morphological structures such as wing buds are visible.

The winter gnats (Trichoceridae) are an example of a nematoceran family that has forensically relevant members. They have been used to determine the post mortem interval in the winter having colonised a body found on the soil surface when many other insects are no longer available.

The second suborder is called the **Brachycera** or Short horns. These are much more robust flies and are often called the higher flies (Figure 4.8 and see also the

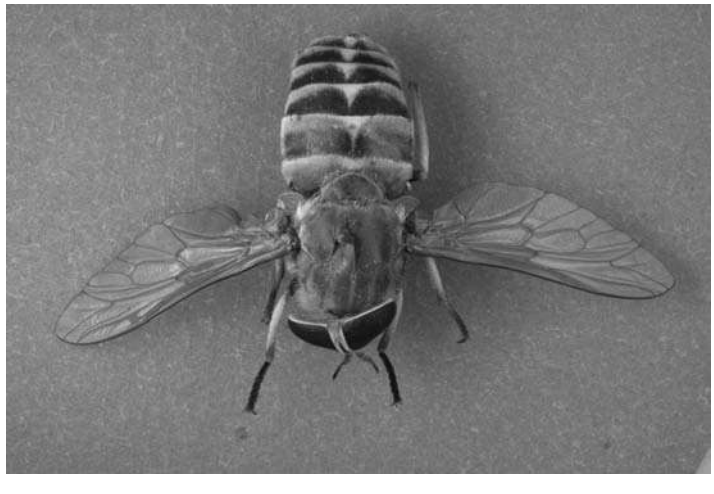


Figure 4.8 An example of a member of the Brachycera – a Tabanid

colour section). They have shorter antennae than the Nematocera, with eight or fewer segments. Their wing venation is less complex and their mandibles are divided. The male genitalia are separated into two parts. The larva has an elongated head, which is combined within the first segment of the thorax (prothorax), and their pupae grow inside a case, which is the hardened remains of the last larval (third instar) coat.

The Brachycera are split into groups (**infraorders**). The groups which are called the Tabanomorpha and Asilomorpha also made up the Brachycera under the old classification system.

A third group (infraorder), the Muscomorpha, is most important forensically. These are predominantly the Cyclorrhapha of the old classification system. This group has antennae with a bristle and have three larval stages in which the morphological distinction into head and body of the larva is absent.

This group is subdivided into:

1. The Muscomorpha Aschiza. In this division the depression and suture over the antenna is either absent or very indistinct. Some wing veins close off a wing compartment ('cell') called the **anal** cell (Figure 4.9). This is very long and closes or almost closes, at a point at least more than half to two thirds the way to the edge of the wing. The Phoridae are of forensic note in the Muscomorpha Aschiza division. Usually though, the anal wing cell in insects of forensic importance, is short or even not there at all. This is a feature of the Muscomorpha Schizophora, which includes the blowflies, cheese skippers and the flesh flies. This group is divided into two, depending upon whether or not there are flaps or **calypters** (Figure 4.4) at the base of the wing with the lower one joining the wing to the thorax.

Figure 4.9 The wing of a member of the infraorder Muscomorpha Aschiza illustrating the long anal cell

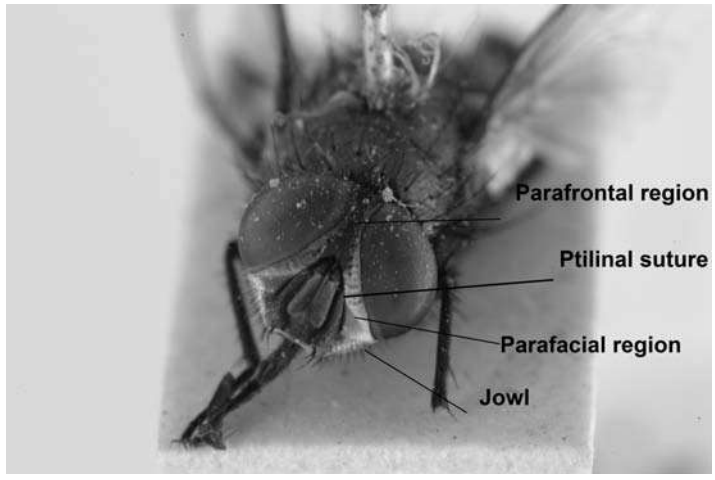


Figure 4.10 The remains of a ptilinum as a ptilinal suture

2. The Muscomorpha Schizophora Acalyptratae. These flies emerge from the puparial case by using an 'air bag' or **ptilinum**. This deflates and draws back below the eyes and above the antennae. Its presence is indicated by a depression like a crease or furrow just below the eyes called a **ptilinal suture** (Figure 4.10; see also the colour section). Flies in this group do not have a thorax with sutures that completely divide the prothorax. The halteres are exposed and the antennae do not have a slit in segment three. Such flies are termed **acalypterate**. Of forensic note are the Piophilidae, Sphaeroceridae, and Sepsidae.
3. The Muscophora Schizophora Calyptratae. A division is made depending upon whether the halteres are exposed or covered. The opaque flaps concealing the halteres are termed the **squamae** or calypters. (Working from the body outwards the proximal (near) flap is termed the lower thoracic calypter (squama) and the distal (far) flap is called the upper thoracic calypter (squama).

Flies that have covered halteres also have a complete line, or suture, across the prothorax and thoracic protrusions sticking out from the middle of the thorax. These prominent protrusions are called **posterior callus**. Such fly species may also have a slit or cleft in the second antennal segment (working outwards from the head). According to Watson and Dallwitz (2003) however, this is considered an unreliable feature for identification purposes. These flies also have a ptilinum.

On the top or dorsal side of the antenna there is a feathery protuberance that stands out from the antennal segment. This protuberance is called **arista**. Those flies with flaps covering the halteres and the features described above are termed **calyptrate**. Of particular forensic importance in this grouping are the families Calliphoridae, Sarcophagidae, Fannidae, and Muscidae.

Figure 4.11 Occipital dilation present on *Calliphora uralensis* Villeneuve

4.1.1 How to sex flies

The adult head has two large **compoundeyes**. Those of the female are more widely spaced than those of the male blowfly, as you look at them head on from the front. Another feature which is important is **occipital dilation** of the eyes. To see if this feature is present, look at the fly head from the side view. It is possible to see if the eye is expanded. This occipital dilation is used to distinguish some species (Figure 4.11).

The region below the eye, as you look at the fly face side on (lateral view), is called the jowl. In *Calliphora vomitoria,* a species which is of forensic importance, the lower part of the jowls and around the sides of the mouth have a mass of golden coloured hairs on it. The jowl itself is coloured black. In contrast, in *Calliphora vicina* the top two-thirds of each jowl is a reddish orange colour.

The families and the identification characteristics of some of the important species are described in the next section. However, to ensure that you have correctly identified a specimen you should use keys, and confirm your identification using a collection of named species and the diagnostic and differential description in the identification handbook. Or you should ask a taxonomist to check your identification so that you are absolutely certain of the name of the species. On this identification hangs the determination of the post mortem interval and your evidence for the court.

4.2 The fly lifecycle

Flies complete a number of stages in their lifecycle; the egg stage, larval stage, pupal stage and the adult stage. The lifecycle shows complete metamorphosis (Figure 4.14). This means that the different lifecycle stages look dissimilar.

We term this a **holometabolous** lifecycle. The cycle starts when the adult female lays eggs.

4.2.1 The egg stage

Most flies mate lay eggs **(oviposit)** in batches. The clumps of eggs are laid on the corpse in places that provide protection), moisture and food (Figure 4.12). In general the number of eggs laid is around 150–200 although the numbers can vary depending on the nutrient source available to the fly.

The blowfly egg is usually very shiny and white, ranging in size from around 0.9 mm to over 1.50 mm long and 0.3–0.4 mm wide (Rognes, 1991). The outer, textured coating of the egg is termed the **chorion**. This sculpturing, which may, for example, be reticulate or spotty, can be used to identify different species of fly. The end of the egg has a pore called a **micropyle**. This is the route by which the sperm gain entry to fertilise the egg.

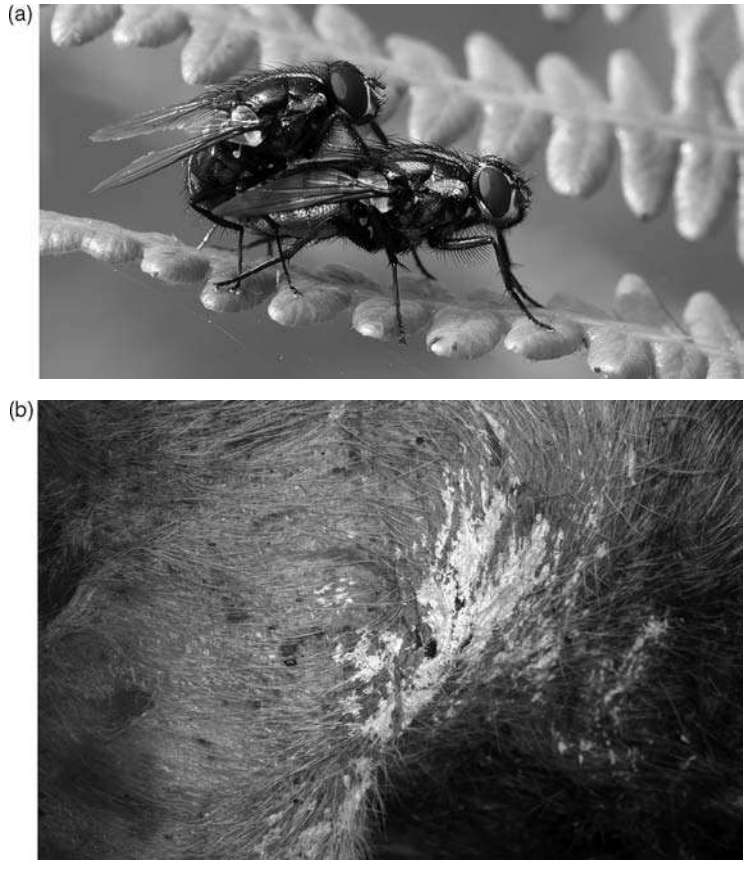


Figure 4.12 (a) Copulating blue bottle flies (b) Clump of eggs

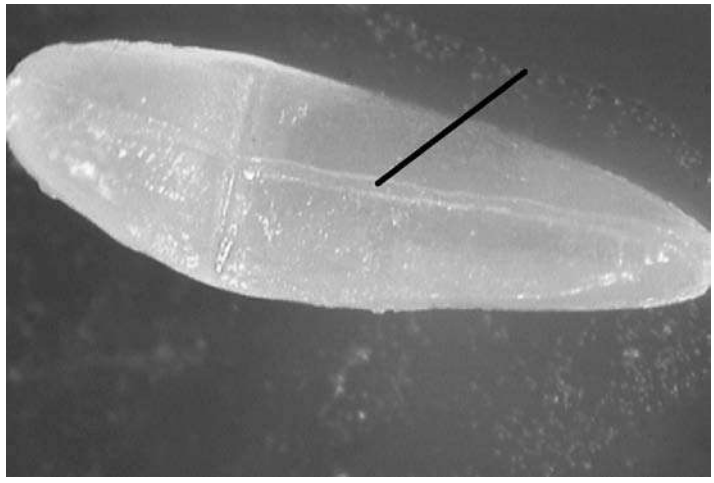


Figure 4.13 The plastron runs the length of the egg

A furrow called the **plastron** runs the length of the egg along one side (Figure 4.13). This acts as a means of trapping air should the egg become covered by water droplets or be drowned in water, and so aids continued respiration. The emergence of the first instar larva from the egg is called **eclosion,** although the term is used to describe any form of hatching.

Not all flies lay eggs some deposit the larvae on to the corpse. Examples of such behaviour is seen in Sarcophagidae – the flesh flies and in species of Calliphorid such as *Calliphora dubia*, *Calliphora stygia*, and *Calliphora hilli* will lay either eggs or larvae (are **ovoviviparous**) whilst C*alliphora augur* larviposits (is **viviparous**). Erzinçlioğlu (1996) commented that *Calliphora vicina* may lay live larvae where fertilisation had taken place without a suitable oviposition site being immediately available.

If an electron microscope is available investigating the surface of the egg may be a means of making identification to at least genus, of the fly species which has colonised the body (Greenberg and Singh, 1995).

4.2.2 The larval stage

The larva has 12 segments and a pointed anterior end, all that remains of the head capsule found in other insect larvae, with a black structure comprising mandibles and related sclerites and ending in mouth hooks (the cephalopharyngeal skeleton) (Figure 4.21). The posterior end is blunt and has two brown circular areas on the final segment. These are the posterior spiracles.

In the fly there are three larval stages or instars. A particular larval stage out of the three is described by referring to it as LI, LII, or LIII. The specific life stage of the larva can be identified by the number of slits present in each posterior spiracle. In

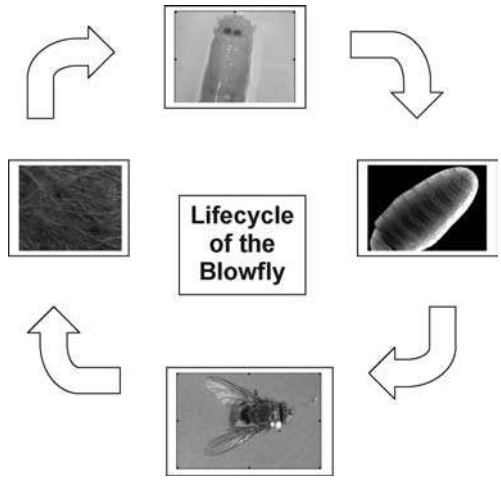


Figure 4.14 The lifecycle of the blowfly

the first instar, one slit is present; in the second instar, two slits are present; in the third instar, three slits are present. In blowflies there is normally a difference in size of larvae in the three larval stages. The first instar tends to be less than 2 mm in length, whereas the second instar is between 2 mm and 9 mm long. The third instar can be between 9 and 22 mm long. However, size is a relatively unreliable measure of age because it depends upon the amount and quality of food available – although a body may be considered to be an abundant source of food.

Projections called **tubercles** surround the edge of the posterior segment of the larva. The spiracles are located on the horizontal face of this final posterior segment. The distance between tubercles plays a role in the identification of larval species. For example, Smith (1986) points out that in larvae of *Lucilia sericata,* the inner tubercles (those at 12 o'clock), are separated from each other by a distance roughly equal to the distance between the inner and the median tubercles (those at 10 o'clock and 2 o'clock respectively) (Figure 4.15a and Figure 4.15b).

Sticking out from the third anterior segment (second thoracic segment) of the larva is an anterior spiracle, which looks like a hand with fingers projecting from it (Figure 4.16). The morphology of this spiracle can also be used as a means of identification in some species.

Larvae in the third instar are the largest. Half way through this stage they stop feeding and become migratory, seeking a place for **pupariation** (the final developmental stage of metamorphosis into the adult stage). This is called the larval postfeeding stage. Larvae move away from the body, towards dark and somewhat cooler areas. In the postfeeding stage the contents of the crop begin to reduce, until finally there is no obvious dark line of crop material visible through the white larval cuticle. Cragg (1955) suggests that the postfeeding larvae may move up to 6.4 metres from the carcass. On concrete floors, such as might be found in buildings, post feeding larvae have been known to migrate for up to 30 m from the body

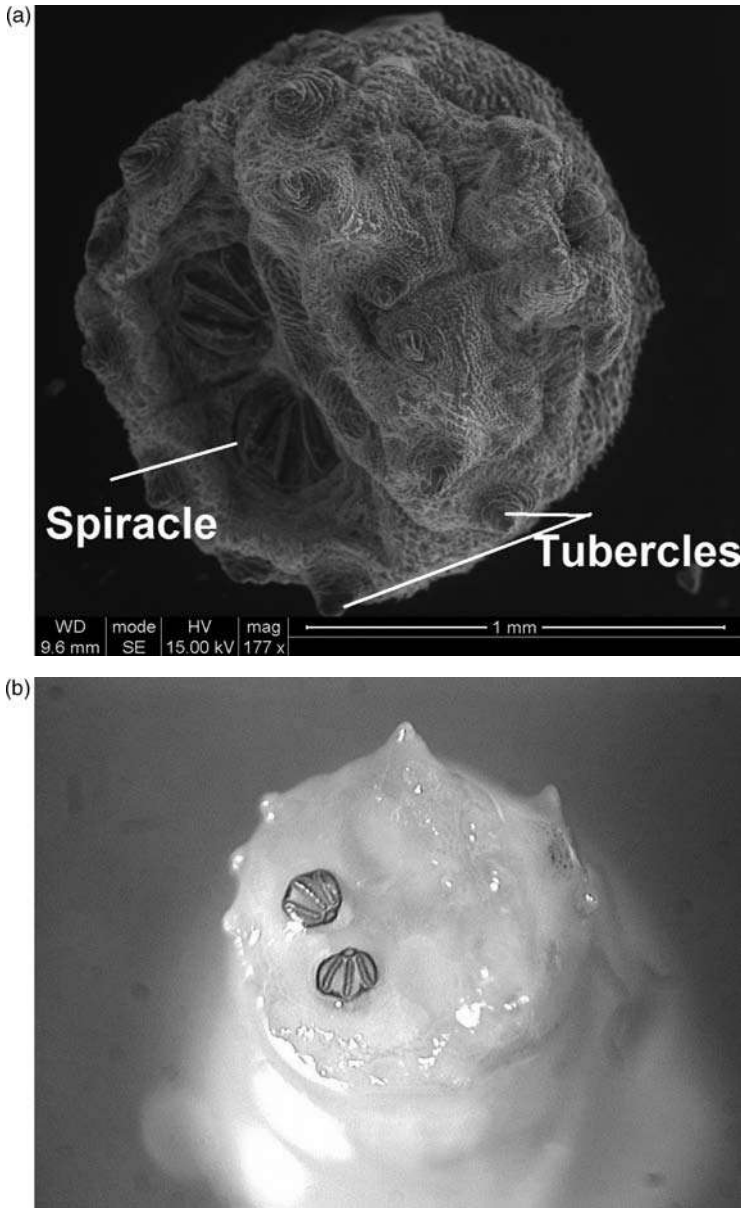


Figure 4.15 (a) Electronmicrograph of the tubercles (a) Posterior spiracles

(Green, 1951). Usually the postfeeding larva attempts to bury itself in soil, or some other dark location. They may be found by searching in the first two or three centimetres depth of soil at outdoor crime scenes. This tendency to migrate is not true for all species, some, for example *Protophormia terraenovae,* have been found to pupate on the corpse (Erzinçlioğlu, 1996).

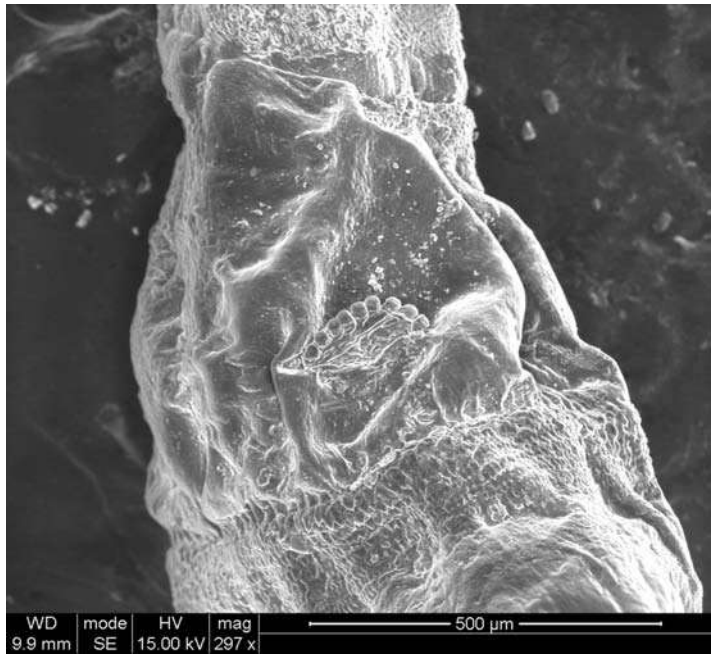


Figure 4.16 An example of the anterior spiracle on a larva

4.2.3 The puparial stage

The puparial case changes colour over time, becoming an oval object resembling an uncut cigar, coloured somewhere between reddish brown, and a dark mahogany brown or black (see colour section).

This case maintains all of the features of the third instar. So there is some possibility of identifying this stage to species, using keys for the identification of third instar dipteran larvae. Some attempts have been made to try and relate state of colouration development of the puparium to post mortem interval, but to date the methods have not shown great accuracy beyond the first 24 hours (Greenberg, 1991).

Emergence of the adult, at the end of the lifecycle, is achieved by its pushing the cap (**operculum)** (Figure 4.17), off the puparium, using a blood-inflated region on its head, called a ptilinum. This is like an 'airbag' that projects from the anterior dorsal region of the head as the fly emerges (see colour section). It later sinks back into the facial structure generating the crease, or ptilinal suture, just above the antennae. The mouth hooks (cephalopharyngeal skeleton) remain inside the broken puparial case and can be used to confirm identification if you can find them.

The adult pushes out of the puparial case and up through the soil responding, according to Fraenkel (1935), to light. The fly 'dries out' and eventually its wings expand and the greyish coloured fly becomes recognisably pigmented as, for example, a bluebottle or greenbottle.

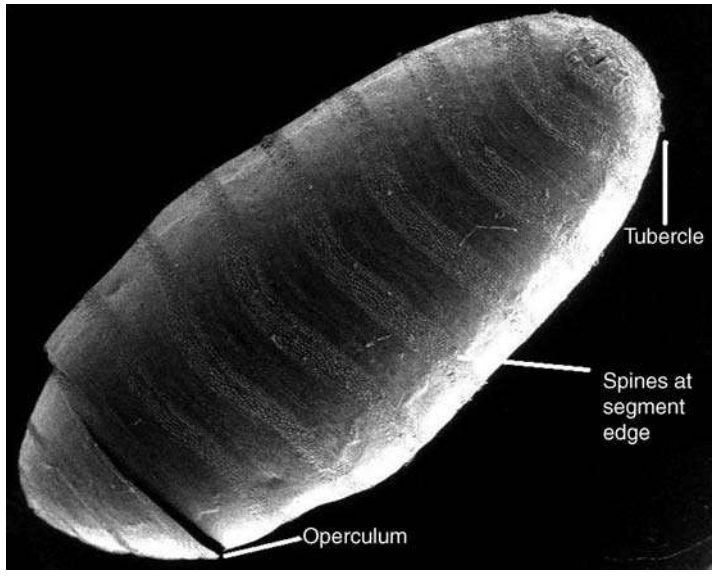


Figure 4.17 Electronmicrograph of the puparium with detached operculum

The speed of insect development is determined by temperature (Figure 4.18). A number of researchers in a number of countries have determined the duration of the life stages of locally relevant species of fly, at particular temperatures. These include Kamal (1958); Greenberg (1991); Reiter (1984); Anderson (2000); Grassberger and Reiter (2002) and other researchers. This information forms the experimental measure for post mortem interval because from it the energy budget for development (the accumulated degree hours) can be determined. It is important to use the sum of the duration of each of the individual life stages and to work with both the average and the maximum and minimum durations, at the specific experimental temperatures used. Further discussion of the method for calculating the post mortem interval is found in Chapter 9.

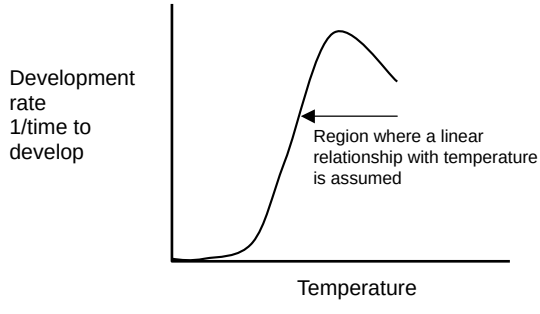


Figure 4.18 Generalised insect growth curve

4.3 Forensically important families of flies

4.3.1 Calliphoridae

The particular species of fly that are forensically important will differ from location to location. In Europe including Britain, the first three species listed below are common initial colonisers of corpses that are not buried or in some way 'modified'. Schroeder *et al*. (2003) consider, for example, that *Calliphora vicina*, *Calliphora vomitoria* and *Lucilia sericata* are the most common species recovered from corpses in Germany.

Calliphora vicina (Robineau-Desvoidy)

This is a large blowfly, which is between 9 and 11 mm in length. (It is also recorded in the older literature as *Calliphora erythrocephala* Meigen.) The front thoracic spiracle is orange in colour (Smith, 1986). The head is black on top and the front half of the cheek (bucca) is reddish orange. The lower region of the face is black. There are black hairs on the jowls irrespective of the jowl colour. The thorax is black and the top of the thorax (the dorsum) is covered with a dense greyish shine (**pubescence**). There are four pairs of strong bristles in a row in the centre of the thorax. These are called the **acrostichal bristles** (Figure 4.19). Like other blowfly species, this species also has a fan of bristles, the **hypopleural bristles**, on a plate above the coxa of each hind (third) leg, near the **posterior spiracle**. Look for this spiracle and you will spot them.

The abdomen is blue with a silvery chequerboard effect (**tessellation**) (Figure 4.20). The basicosta on the wing is yellowish in colour although this can fade to a yellowish brown colour.

Figure 4.19 The thorax showing the position of the acrostichal bristles

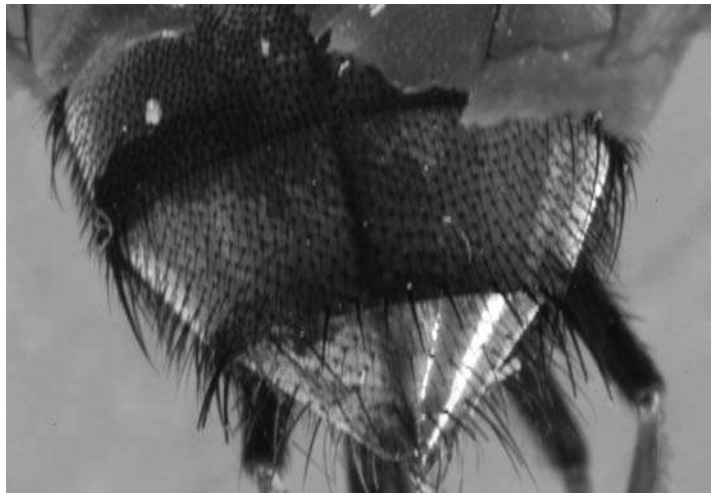


Figure 4.20 The 'tessselation' (chequer board) effect on the abdomen of flies such as *Calliphora vicina* Robineau Desvoidy

Calliphora vomitoria (Linnaeus)

These are also large bluish-coloured blowflies. The species has a longer lifecycle than the previous species and is found more often frequenting rural environments. The hairs on the jowls and the colour of the basicosta help distinguish *Calliphora vomitoria*. The basicosta is black in colour (see colour section) as opposed to the orange in *Calliphora vicina* and the hairs on the base region of the jowls and around the side of the mouth are gingery orange. The spiracle at the front (anterior) of the thorax is brownish in colour in *Calliphora vomitoria*.

Where both *Calliphora vicina* and *Calliphora vomitoria* are found together as third instar larvae they can be separated, according to Smith (1986), by the width of their posterior spiracles. He indicates that in *Calliphora vicina* the spiracles are between 0.23 and 0.28 mm wide. The spiracles in this species are smaller than in *Calliphora vomitoria* and are separated by the same, or a bigger, distance than the width of a single spiracle. In *Calliphora vomitoria* the spiracles are larger, being in the region of 0.33 to 0.38 mm. Its spiracles are separated by less than the diameter of an individual spiracle.

Lucilia sericata Meigen

This is commonly called a greenbottle because all the flies in this genus are a metallic green colour. In North America, *Lucilia sericata* is called *Phaenicia sericata*. *Lucilia* species are distinguished from other blowflies by having a ridge just above the squama, the rear wing flap (hence the suprasquamal ridge), which has tufts of hair on it. *Lucilia sericata* has a yellow-coloured basicosta.

One of the differences between the larvae of *Calliphora* and *Lucilia sericata* is that the oral sclerite in the head skeleton (**cephalopharyngeal** skeleton) is transparent and so

seems to be absent in larvae of *Lucilia sericata*. The identity of *Lucilia sericata* larvae can also be confirmed by looking at the rim of the final posterior segment of the larva.

The tubercles are named from the top (12 noon position), the inner, median and outer (lower) tubercles. If the distance between the two inner tubercles is the same as the distance between the inner and the median tubercle, then this species can be identified as *Lucilia sericata*. This feature is characteristic of the third instar larvae. Erzinçlioĝlu (1987) found that around the posterior spiracles in first and second instar larvae of *Calliphora* and *Lucilia* there was a circle of hairs. In *Calliphora* sp. these hairs would be visible under low power, being very well developed in *Calliphora vomitoria*, but would not be visible under low power in *Lucilia* species.

Lucilia illustris Meigen

The **basicosta** (plate on the base of the costa) is blackish or brown in colour in this species and the arista on the antenna has up to 10 hairs on its underside. There are no bristles on the sides of the abdominal segments in the males of this greenbottle species, according to Erzinçlioğlu (1996). The males can be distinguished from *Lucilia caesar* Linnaeus males, by the presence of curved surstyli (exterior structures of the genitalia) (see colour section). This fly has been found to be of value as a post mortem indicator in a murder in Washington State (Lord *et al*., 1986).

Lucilia caesar Linnaeus

These flies are similar to *Lucilia illustris* in that they share a dark coloured basicosta. In males the sides of the second abdominal segment lacks bristles, when you look at

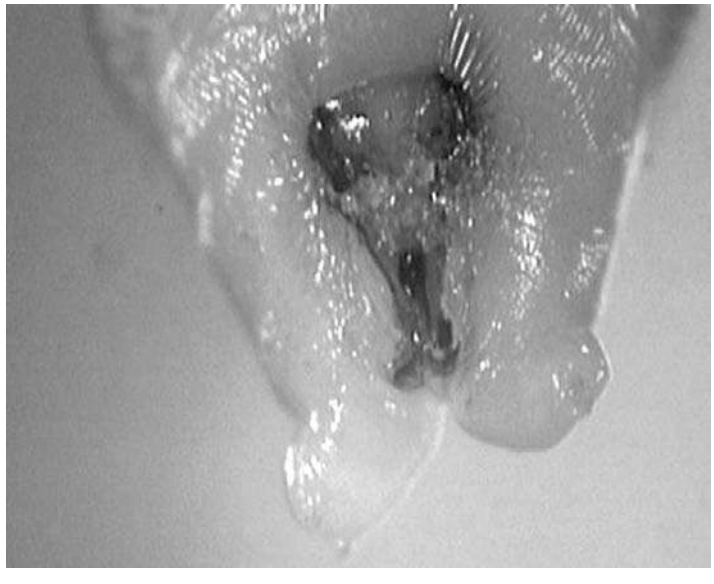


Figure 4.21 An example of a larval 'head skeleton' with oral sclerites

Figure 4.22 *Lucilia richardsi* Collin

the fly from the dorsal view (i.e. from the top). They can also be distinguished by having surstyli with a straight projection – i.e. fork (see colour section) (Erzinçlioğlu, 1996).

Lucilia richardsi Collin

In this fly the basicosta is white, or yellowish. The spacing of the eyes in the male assists in distinguishing this species from adult *Lucilia sericata* (Figure 4.22). The distance between the eyes in males is not more than the width of the third antennal segment (Erzinçlioğlu, 1996). The abdominal sternites are hairy in both males and females (Greenberg and Kunich, 2002). Smith (1986) indicates that the tibia of the middle leg has two anterior bristles, which also distinguish this species from *Lucilia sericata,* which has only one bristle (the leg articulates in the posteroventral plane – i.e. backwards and downwards).

Protophormia terraenovae Robineau-Desvoidy

This species is between 8 to 12 mm long. The fly has a greenish blue abdomen, black-coloured legs, and a dark calypter with hairs that are dark (see colour section). According to Smith (1986) this species is widely distributed and found in waste land and marshy areas in Britain. The fly overwinters as an adult and so may be amongst the first flies to colonise a body in spring. Its puparia may be recovered from the body rather than at some distance from it according to Busvine (1980) in Smith (1986). Tantawi and Greenberg (1993) provide information about the lengths of the life stages of *Protophormia terraenovae* at 12.5 °C, 23 °C, 29 °C and 35 °C.

Phormia regina Meigen

This is a smaller fly than those previously described and is a **Nearctic** and **Palaearctic** species. It is between 7 and 9 mm long and has a green or greeny olive-coloured body. Its head is large proportional to the body and is black in colour. A distinguishing feature in this species is the anterior spiracle on the thorax which has obvious orange hair. In contrast to that in *Protophormia terraenovae,* the calypter is white, with white hairs on its surface. *Phormia regina* is commonly known as the black blowfly.

Cynomya mortuorum Linnaeus

This is a metallic blue-green blowfly, which is about the same size as *Calliphora* species. Its face and jowls are yellow to bright orange (see colour section). It is infrequently found in the south of England and MacLeod and Donnelly (1956) note that it favours cool uplands.

Chrysomya species – Chrysomya rufifacies Macquart

These are large, blue- or green-coloured flies. *Chrysomya rufifacies* are most commonly found in the Orient, Australasia and the neotropics. *Chrysomya rufifacies* is metallic bluish, or green in colour. The adults are between 6 and 12 mm long, with at least the front part of the cheeks on the head being yellow or orange in colour (Smith, 1986). *Chrysomya rufifacies* is one of the initial colonisers of corpses in Hawaii (Goff, 2000). Its larvae have spines on the sides of their tubercles.

Chrysomya rufifacies is often accompanied by *Chrysomya megacephala* Fabricius, which is of a similar size. In contrast to *Chrysomya rufifacies,* its anterior spiracle is orange to black-brown in colour, rather than being white to pale yellow. The front part of the cheeks (bucca) in this species is yellowish or orange. *Chrysomya megacephala* has also been identified from corpses along with *Cochliomyia macellaria* Weidemann, a native American species in Brazil (Oliveira-Costa and de Mello-Patiu, 2004).

Chrysomya albiceps Wiedemann is a third species that is found at crime scenes. It has a yellowish or white thoracic spiracle, its abdomen has dark bands across it and its legs are dark. Larvae of *Chrysomya rufifacies* and *Chrysomya albiceps* are hard to distinguish visually. However, Wells and Sperling (1999) demonstrated that the two species could be distinguished by using mitochondrial DNA.

4.3.2 Sarcophagidae

The common name for this family of flies is the flesh fly. There are three subfamilies and recently (Spilza, Voss and Pape, 2010) a potential forensic indicator was recorded from a subfamily (Miltogramminae) other than the Sarcophaginae. Spilza, Voss and Pape describe the members of the Miltogramminae as of comparable size

Figure 4.23 A fly showing the characteristic features of the Sarcophagidae

to house flies, with a grey abdomen with either black spots or dark abdominal banding. A Nearctic and a Palaearctic species have been recorded as able to develop on buried corpses.

The flesh flies most recognisably considered as corpse colonisers are large and greyish in colour and have a thorax with three stripes down it and a chequerboard (tessellated) abdomen, which is silvery grey (Figure 4.23). In volume 12 of the *Checklist of Insects of the British Isles* (new series) part 1: Diptera (Chandler, 1998), it is noted that there has been a division of the old genus *Sarcophaga* into a number of subgenera, resulting in a number of name changes from the early. It may be helpful to consider this in your reading of the earlier scientific papers.

Colyer and Hammond (1951) considered Sarcophagidae a difficult group from which to distinguish species with any degree of certainty, unless adult specimens are captured whilst mating ('in cop'). (Using the identity of the male, it is easier to confirm the identity of the female species.) Help should be sought from taxonomists if these are the only family recovered from the body.

Sarcophagid larvae are characterised by having a barrel-like shape with their posterior spiracles sunk into a hollow. The edge of the posterior segment has a large number of tubercles. This makes this family easy to distinguish at the larval stage. Some success has also been made in identification to species of larvae of the Sarcophagidae, using molecular methods (Zehner *et al*., 2004).

4.3.3 Sepsidae

Sepsid flies are small and a shiny black colour. They have a head which looks spherical with bulging (convex) eyes and an abdominal constriction (an apparent waist!). The legs of male sepsids have spines and are elongated, which makes the legs look deformed. The costa on the wing is unbroken. This family is characterised by its habit of wing waving. There may be swarms of these flies at the crime scene, depending on its location. Pont and Meier (2002) have revised the European Sepsidae and provide further details of their distribution as well as details of characteristics for identification.

4.3.4 Piophilidae

The Piophilidae are small, shiny, black flies (Figure 4.24; see also colour section). They are between 2.5 and 4.5 mm in length. The costal vein of the wing appears broken at one point in this family. One of the most researched members of the Piophilidae is *Piophila casei* Linnaeus, which is a pest on food products such as cheese.

Piophila casei Linnaeus

This fly is usually found on the corpse at the end of active decay and the start of the dry stages (Byrd and Castner, 2010). It is a small black fly, 2.5–4 mm in length and is

Figure 4.24 An electronmicrograph of a Piophilid. *Source:* Reproduced by permission of Dr Marta Salona

commonly called the cheese skipper. It has prominent cheeks, which are more than half the eye height (see colour section). These species of flies have a yellow colour on their legs, antennae and on the jowls of their faces. Their ocellar bristles are found opposite the simple eye (front ocellus), which are small and widely spaced.

Piophilid larvae are similar to sepsid larvae, although the posterior larval region is narrower in the Piophilidae. The behaviour of *Piophila casei* larvae makes them easy to identify, as it is particularly characteristic. If disturbed, larvae bend round to grasp two small papillae on the posterior segment, with their mouth hooks. They suddenly release the papillae and the larvae unexpectedly 'jump' up to 15 cm into the air.

Other species of Piophilidae may also be present on corpses. For example *Stearibia* (=*Piophila) nigriceps* Meigen was recorded by Oldroyd (1964) as feeding on a human corpse. Therefore do not make an assumption about the name of the species of piophilid that you have recovered from the body.

4.3.5 Phoridae

These flies are small, often minute flies. They have a humped back and can be greyish brown, or bluish in colour. The forehead (**frons**) is usually wide, and has bristles which are very robust and upward curving. On the antenna the third segment is large, although in this family the arista can be found either dorsally or apically (frontward). Phorid wings are characteristic; with veins one to three appearing very pronounced and crushed together. The wing costa also has a spine at its proximal end, nearest the body of the fly.

On a corpse, Phoridae can be identified by the fact that they are active flies, capable of running and jumping, and this gives them their common name of scuttle flies. Dewaele and LeClerq (2002) define their flight period as April to November.

4.3.6 Muscidae

Muscid flies are frequently greyish in colour and are characterised by having lines running down the length of their thorax and no hypopleural bristles. Wing veins 6 and 7 are short and do not move towards each other (as they do in *Fannia* spp.). Their calypters are of roughly the same size, or the lower calypters may be bigger (Unwin, 1981). The eggs can be differentiated into two groups based on their morphology and the strength of projections, hatching pleats down the sides of the furrow or plastron – the Musca and Phaonia types.

Larvae undergo three larval stages and the puparium is typically barrel-shaped. The larvae and indeed the puparia of the Muscidae by the shape of their posterior spiracle slits which range from straight through sinuate ('s-shaped') to bowed. For example *Musca domestica* are recognisable by the wiggly 'S-shaped' slits on the posterior spiracles.

Muscid flies, such as *Musca domestica,* a common member of the Muscidae, will visit a body soon after death, attracted by any exudates rather than the corpse itself. *Musca domestica* is a greyish fly, about 6 to 7 mm in length. It is characterised by four narrow black stripes along its thorax and a greyish or yellowish abdomen. A sharp-angled wing vein is found at vein 4 (Smith, 1986).

Musca autumnalis De Geer is also recorded as visiting corpses (Smith, 1986) but can also have forensic importance as a nuisance fly. It is commonly called the 'face fly' and the male is easily identified by its bright yellow abdomen with a black stripe up the middle. According to Smith (1986), female *Musca autumnalis* are very similar to female *Musca domestica,* but can be distinguished by a smaller frontal stripe. In female *Musca autumnalis* this stripe is less than twice the width of the eye orbit, whilst in *Musca domestica* it is three to four times as wide. *Musca autumnalis* is rare in the north of England up to the borders of Scotland and is not found in Ireland.

Other Muscid flies of significance include Hydrotaea species. These are dark brown coloured flies that are able to hatch from the egg as a second instar although those of the *Hydrotaea dentipes* group hatch as first instars (Ferrar, 1987). The larvae (Figure 4.25) are often distinguished on the basis of the cephalopharyngeal skeleton and the puparia on the basis of the length of the pupal horn.

4.3.7 Fannidae

These flies are commonly called latrine flies. They are distinguished from the muscid flies by the much greater curve on the axillary vein – vein 7. This is the vein which is

Figure 4.25 Hydrotaea larva. *Source:* Reproduced by permission of Dr Marta Salona

nearest to the upper calypter and the species also lacks the sharp angle on vein 4, which reaches to the wing margin. *Fannia canicularis* Linnaeus, the lesser house fly, is common in houses as it is attracted to light. Smith (1986) comments that this is the more common house fly until July. Benecke and Lessig (2001) recorded *Fannia canicularis* in a case of child neglect in central Germany. They suggested that urine and faeces attracted the adult flies, resulting in larval infestation of the child's genitalia.

4.3.8 Sphaeroceridae

Sphaeroceridae are known as small dung flies. They are a dull-coloured fly of between 1.5 to 5 mm. Their antennae are three segmented with an arista. **Vibrissae** (bristles) are present on the sides of the mouth. The wing costa has two breaks and vein 6 is present, but does not extend to meet the wing margin.

Sphaeroceridae filter feed on bacteria. Only a few species in this family are recorded from dead bodies. They have been noted from the fresh, bloat and advanced stages of decay (the fifth wave of insects) at between 4–8 months after death. Grassberger and Frank (2004a) recorded them on dressed pig corpses placed in an urban garden in Vienna between May and November, 2001. Ammonia is an attractant for the dung- breeding flies. Hence voiding of urine early in decomposition can attract members of this family, as much as ammonia release during later decomposition.

4.3.9 Stratiomyidae

Members of this family are termed soldier flies and some species are distributed across all geographic zones. They range in size from 3 to 20 mm. The flies are recognisable by appearing slightly flattened and having wings that fold one over the other so that the costal margins of the wings are in parallel. The costal vein does not run round the entire wing margin but stops at the wing tip. There is a discal cell in the middle of the wing veins. The antenna has a final segment which seems to have several parts fused together to make up the third antennal segment.

The majority of larvae are found in decaying vegetation or scavenge on animal remains (Oldroyd, 1964). Hermetia species have been found in some forensic contexts (Figure 4.26). The *Hermetia illucens* Linnaeus adult, for example, is black and ranges from 15–20 mm in length. The species is identified by the two small abdominal translucent spots that give it its name.

Some stratiomyid species have aquatic larvae. Such larvae are spindle shaped, as with all stratiomyids they have a covering of calcium carbonate over the cuticle. They have thoracic spiracles and their posterior spiracles are enclosed in a slit in the posterior segment, which is sometimes called a siphon (Figure 4.27). This is surrounded by a halo of fine hairs which repel the water. The larvae pupate in a puparium as do the Calliphoridae.

Figure 4.26 A pre-imaginal stage of *Hermetia illucens. Source:* Reproduced with kind permission of Dr M. I. Saloňa

4.3.10 Trichoceridae

These flies are members of the Nematocera and therefore have physical features that are similar to the 'Daddy long legs' (Tipulids). Characteristically adults of this family have long antennae with at least eight segments. On the head there are two or three ocelli or simple eyes. The wings are held up over the back of the body at rest and the legs are long and slender. This is a moderately delicate fly, an average of 8 mm long, blackish brown in colour and its wing venation is distinctive with nine veins reaching the wing margin including two anal veins. The second anal vein is strongly curved

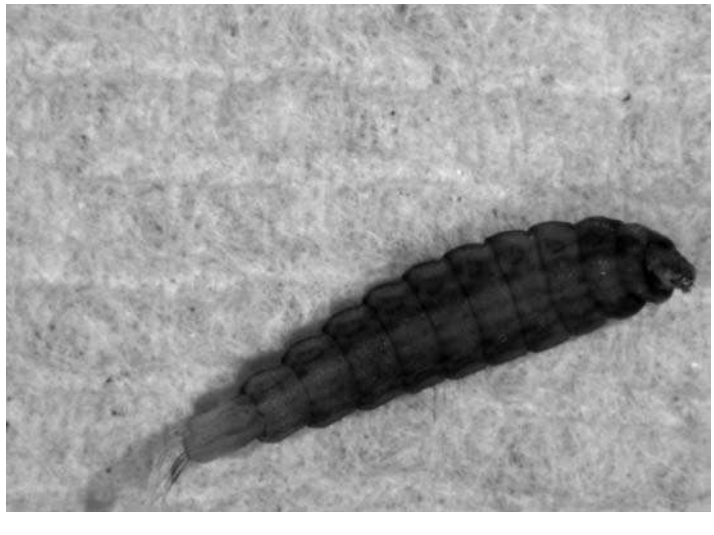


Figure 4.27 An aquatic larva: a member of the Stratiomyidae

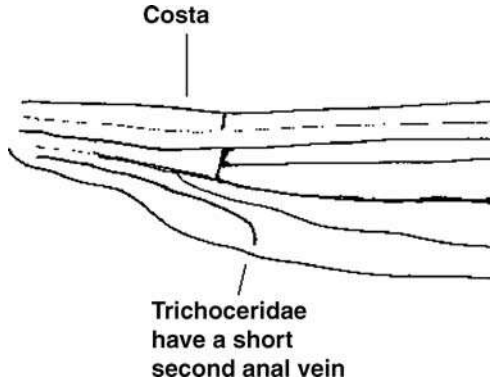


Figure 4.28 The curved second anal vein characteristic of the Trichoceridae

(Figure 4.28). The costa runs round the margin of the wing, although it is less distinctive on the distal margin. Some members of the family have a slight opacity of the membranes of the wing around the cross vein. A key feature in distinguishing the family is the length of sections of the first part of the second radial wing vein (R_2) relative to the second plus third sections (R_{2+3}) (Laurence, 1956).

The larvae have four larval instars and the instars can be distinguished by the colour of the head capsule. The larvae have posterior spiracles, which are held upon projections covered with a fringe of hairs. These projections are termed the anal lobes of the final segment. The final instar has a paler yellow-brown head capsule, which is flattened, whereas the earlier instars have a black head capsule. All have strong mandibles. Pupae are not held inside a protective covering and the sex of the pupa is distinguishable. The family have been found of value, as is any insect species, but is not of routine significance as a forensic indicator.

A number of fly species are associated with submerged bodies in their larval stages, but have no relationship with those which are terrestrial. The non-biting midges (Chironomidae), Tipulidae and the black-flies (Simulidae) are examples of species which can be found in aquatic habitats and in association with a corpse.

4.3.11 Tipulidae

Tipulidae are members of the Nematocera and have similar features to those described earlier. In the adults the antenna are long and have at least six uniform segments. They have a head that supports feeding palps, which are drooping (Figure 4.29). The forensically significant species of tipulid are those with aquatic larval stages. These larvae tend to be fat, often greyish in colour and have a well developed head. They can reach around 50 mm in length. The larval head can be retracted into the prothorax. The posterior segment carries the two posterior spiracles on a transverse lobed segmental plate. The plate is fringed with hairs to facilitate gas

Figure 4.29 An example of the features of a Tipulid including the drooping mouthparts

exchange. Some species have paired prolegs on the ventral surface of the abdomen. Three genera are particularly common in Britain but cannot be used as habitat indicators because they are cosmopolitan and exist both in pools and/or in fast flowing rivers. These are *Tipula* spp., *Dicranota* spp., and *Pedicia* spp.

4.3.12 Simulidae

Britain has approximately 35 species of Black-flies. Adult members of this family are compact flies 2 to 6 mm long. As the name suggests, these flies are black. They have very broad wings, which have distinctly thickened section of their costal veins at the proximal end of the wing.

The larvae are club-shaped and can be up to 10 mm long. The head of the larva has two collapsible stalks each with fans of bristles that serve to trap small organic fragments of food. On the prothorax of the larva there is a single **proleg**, which assists in movement. The larvae anchor themselves onto the substrate using a caudal disc, which is covered with extremely tiny hooks to give the larvae purchase.

The pupae have obvious white respiratory filaments that stick out. The pupa is protected in a silk cocoon pocket anchored to the substratum of the water body. This family inhabits flowing water in its aquatic stage.

4.3.13 Chironomidae

These are small flies, which have a humped thorax that frequently overshadows the head. They can be up to 14 mm long. The front pair of legs is the longest of the three

pairs. The wings do not extend beyond the end of the abdomen and are very narrow. The veins have a distinctive characteristic fourth or medial vein (1 + 2) which does not fork. At rest the chironomid holds its wings alongside its body. Male chironomids are distinguished by their feathery antennae.

The larval stage is aquatic and the most frequently cited in forensic contexts – particularly in stagnant water – are the blood worms (*Chironomus* spp) (see colour section). Identification of this family to species level is difficult. However, behaviourally, blood worms (*Chironomus* spp.) can be distinguished by their characteristic 'figure of eight' wiggling movement. Chironomid pupae are often indistinguishable from those of other midges and gnats. Although some species can be distinguished by the tufts of fine white hair present on the pupal head, whilst others are undistinguishable from the pupae of mosquitos as they too have a respiratory horn.

4.4 Members of other orders that have forensic relevance in aquatic cases

There are several other orders of insects which are relevant forensically, particularly as their juvenile stages are aquatic. The orders are the Ephemeroptera, Trichoptera and Plecoptera. One of the indications that these are members of different orders despite the fact that they have the word 'fly' in their common name is that the whole word is run together where as in the true flies the common name is made up of two words, the latter being the word fly e.g. dragonflies and flesh flies.

4.4.1 Mayflies – Ephemeroptera

The members of this order have an incomplete lifecycle incorporating an immature adult stage, which is distinct and termed a subimago. The life stages are egg, nymph, pupa, subimago, and imago. The nymph is distinguished from members of the Plecoptera by having three appendages ('tails') on the abdomen (Figure 12.2 in Chapter 12). The nymphal body is segmented; the thorax has three pairs of legs attached to it, and also wing buds. Nymphs consume algae, plant debris, and small food particles. Their developmental period can be several months to several years. The duration of the nymphal stage is between three to six months. The subimago stage lasts between a few hours to one to two days.

Ephemeroptera nymphs can be found in a range of habitats and their feeding strategy is influenced by the habitat. Some species burrow into sediment, some crawl around on silt or moss; others cling to stones. The swimming ability of nymphs ranges from very effective to incompetent. Although adult mayflies have no forensic value, they may be obvious in a crime scene and can be distinguished by the fact that they hold their wings straight up in relation to the body.

4.4.2 Caddisflies – Trichoptera

The insects in this order have a complete metamorphosis. Their lifecycle can take between six months and two years to complete. Some newly hatched larvae consume algae and plant debris whilst others are predatory.

Caddis larvae are distinguished by having two fleshy protuberances which project anally from their abdomen (Figure 12.5). Each protuberance ends in a hook, sometimes with additional hairs and anchorage points. Larvae can be found either in a case or be case less, although even the cased caddis may escape from their refugium on occasion. Caddis larvae have obvious long, jointed legs on their thorax. This feature distinguishes them from fly larvae, which never have jointed legs, and chironomid larvae, which do not have either obvious jointed legs on their thorax (although they do have what appear to be two feet on the first segment behind the head). Chironomid larvae have extensions on their last abdominal segments, which also have gills.

The lifecycle takes between six months and two years to complete and the larval stage comprises five instars, which are completed within six to 10 months after which the larvae pupate in a cocoon (case-building caddis will seal off the door of the case and build the cocoon within). The pupal stage can take from a few weeks to around six months. Adult caddis flies cut their way out of the cocoon and emerge as a pre-adult, which is mobile but still immature – a **pharate** adult, which has fringed legs that it uses to swim to the surface of the water. Once at the surface it rapidly matures into the adult form. The length of time which the adults survive is around a couple of weeks but this is dependent upon the weather.

4.4.3 Stoneflies – Plecoptera

Members of this order have an incomplete metamorphosis and the lifecycle can take up to three years to complete. The main emergence is between late spring and midsummer. Stonefly egg stage can last between a single day and three months. Stonefly nymphs can be carnivorous, omnivorous, or herbivorous; the Perlodidae and the Perlidae are the families that are predators.

Stonefly nymphs have three pairs of well-developed legs with two claws on the end. The end of the abdomen has two caudal tails (Figure 12.1). The number of instars is considered to be between 12 and 25; however 33 instars have been recorded in one species of Perlidae (Harris, 1970).

A stony substratum appears to be a key requirement for the presence of stoneflies along with good water quality. Member of this order can be found in slow-flowing rivers with a muddy bottom and rooted vegetation. Stony rivers and streams will also be a suitable habitat for stoneflies as well as the shores of lakes which have rocks along their edges.

4.5 Review technique: larval spiracles or mouthparts – preparation of whole slide mounts

Introduction

On some occasions it may be appropriate to make a microscope slide of a specimen to confirm aspects of its identity. To do this you will need to 'clear'a specimen to remove the soft tissue, leaving the chitinised tissue so that you can examine the spiracles on either a larva or puparium more easily in order to confirm its species identity. Sukontason *et al*. (2006) describe a method of preparing puparial cases in order to identify the species. (The same procedure can be used to make a slide of the cephalopharyngeal skeleton.)

The procedure described below, is for the preparation of a slide of the spiracles of a larva to confirm its instar.

Safety Instructions (COSHH)

- Use normal laboratory etiquette.
- Wear safety glasses when preparing the slide.
- Ten per cent KOH is caustic – wear latex gloves.
- Glacial acetic acid can cause irritation – keep the watch glass covered.

Materials

two dropper pipettes
mounted needles
three watch glasses
a 10 ml measuring cylinder
slides
a 250 ml beaker
cover slips
mountant – e.g. euparol
labels for slides
fine forceps
wash bottles with distilled or ionised water
specimen of larva(or puparium)
glacial acetic acid
glass rod
compound microscopes with phase contrast facility
ten per cent potassium hydroxide
beaker for waste
clove oil

Method

1. Immerse your specimen (which you have carefully punctured with a fine pin) in 10% potassium hydroxide (KOH) overnight to soften the tissue and destroy the internal tissues. (The alternative is to boil the specimen in 10% KOH, but this is a potentially dangerous procedure as the potash solution is caustic.)
2. Place the whole specimen in a small watch glass and flood it with distilled water to wash off the KOH.
3. Pipette off the liquid and dispose of it in the waste beaker.
4. Repeat the washing in distilled water as in instructions 2 and 3.
   Your specimen should now be either a very pale yellow colour, or transparent. (If not, replace it in the potassium hydroxide for a longer period until you are satisfied with the level of transparency of the cuticle.)
5. Remove all water and blot the specimen dry. Take care – the specimen must be dry for the next stage.
6. Using fine forceps place the specimen into a second, *dry* watch glass and add 5 ml glacial acetic acid with a fresh dropper pipette. Cover the dish to reduce the rising odour level (ideally this stage should be carried out in a fume cupboard).
7. Leave the contents for 5 minutes to dehydrate your specimen.
8. Using forceps place the specimen into a third watch glass and add (by eye) 5 ml of clove oil. Cover the watch glass with two glass slides to reduce the odour (again this should be carried out in the fume cupboard).
9. Leave the specimen for 10 minutes. Meanwhile set up the compound microscope and collect the slides and cover slips.
10. Add a small amount of mountant (for example Euparol) to the centre of a glass slide using a glass rod.
11. Take the specimen out of the clove oil. Position it carefully on the glass slide using mounted needles.
12. Carefully overlay the specimen by sliding a cover slip down a mounted needle, onto the specimen. Label the slide with your name, the date and the contents of the slide.
13. Allow the slide to dry and harden. This can take two weeks (or use a slide dryer to reduce the time).
14. Examine the specimen under phase contrast (total magnification $\times$ 400). Make a labelled diagram of the spiracles and indicate the instar of the specimen in your laboratory notebook.
15. Justify your conclusion and use Kamal's data to determine the duration of the lifecycle of your specimen to this point if grown at 26.7 °C.

5

Key for the identification of European and Mediterranean blowflies (Diptera, Calliphoridae) of medical and veterinary importance – adult flies

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5.1 Introduction

Blowflies are a large Dipteran family with 114 species known from Europe (Rognes, 2010). The family's position as a paraphyletic taxon (Rognes, 1997) was recently confirmed by molecular studies (Kutty et al., 2010). Species of forensic importance belong to one of four subfamilies: Calliphorinae, Chrysomyinae, Luciliinae and Toxotarsinae; the last subfamily is a New World endemic. This group of four subfamilies, with addition of the parasitic Melanomyinae, create a monophyletic clade, which is a good candidate for adoption within the 'Calliphoridae' in any future revision of the whole family (Rognes, 1997).

Adult necrophagous blowflies are the earliest visitors to human and animal dead bodies in terrestrial habitats. They are rarely replaced by other insects unless the conditions are atypical, such as those occurring in dry environments or with buried carrion. After arrival, blowflies feed on exposed body fluids (saliva, blood, mucus) and females with mature eggs immediately begin oviposition. This ability to exploit newly dead bodies makes blowflies the most important group for the estimation of PMI using the developmental method.

Identification of the blowflies of Europe and the Mediterranean is not especially difficult. However a single key encompassing all species of forensic and medical importance has not so far existed. The broadest representation of species was

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

prepared by Zumpt (1956) in a series of papers published in the volumes of *Die Fliegen der Palaearktischen Region.* The weak point of this publication is the very limited illustration of characters used for taxonomic purposes, which makes the keys difficult for inexperienced researchers to use. More recent monographs improved this situation but they covered only the local fauna of particular countries or regions such as Romania (Lehrer, 1972), Scandinavia including Denmark (Rognes, 1991), Great Britain (Erzinçlioğlu, 1996), Israel (Rognes, 2002) and Poland (Draber-Mońko, 2004).

In fact only a few species of blowflies regularly colonise the dead bodies of large animals. The list of European blowflies of forensic importance proposed recently by Szpila (2010) includes 12 species. According to recent findings in Europe and the USA, this list should be extended by the addition of a few species with confirmed breeding records from human dead bodies. In the present key, *Lucilia cuprina,* recorded from Spain (Rognes, 1994) has been included along with *Chrysomya putoria,* which has been reported from the Canary Islands (Baez, Ortega and Kurahashi*.,* 1981) as '*Chrysomya chloropyga*' (see discussion in Rognes and Paterson, 2005).

Chrysomya marginalis, a carrion breeder well established in Israel (Rognes, 2002) and Egypt (Schumann, 1986) is also included in the key presented below as part of the cohort of flies with relevance to the south-east Mediterranean. The key also incorporates two obligatory agents of myiasis (see Chapter 10): *Cochliomyia hominivorax* and *Chrysomya bezziana*. Both these species resemble typical necrophagous blowflies and may easily be misidentified by inexperienced researchers. The New World screwworm, *Cochliomyia hominivorax,* was accidentally introduced into Libya in the 1980s and only rapid and decisive control action has eradicated this species from the Mediterranean region (Beesley, 1991; Lindquist, Abusowa and Hall, 1992). The Old World screwworm *Chrysomya bezziana*, known as an oriental species, has extended its distribution to Persian Gulf countries such as Iran (Navidpour *et al.,* 1996) and Iraq (Al-Izzi, Al-Taweel and Jassim, 1999). A single record of this species from Algeria (Abed-Benamara *et al.,* 1997) was probably a misidentification (Hall, 2008) but the occurrence of *Chrysomya bezziana* in North Africa is possible. The same concerns relate to the description of a case of human myiasis from Turkey where a larva with a deep spiracular cavity and spinulation characteristic of *Wohlfahrtia magnifica* Schiner (Sarcophagidae) was apparently wrongly identified as *Chrysomya bezziana* (Satar, Çaça. and Şakalar, 2005). The presence of both screwworm species in material collected in real cases from dead human bodies (as predeath infestations) has a rather low probability of occurring but if it did happen could seriously affect the estimation of PMI.

For medico-legal purposes, thanks to the addition of *Cochliomyia hominivorax* and *Chrysomya bezziana*, the key presented below may be successfully used for the identification of European and Mediterranean blowflies causing wound myiasis of human and domestic animals (as both primary and secondary agents) as well as for the identification of flies infesting dead bodies.

The full list of keyed species is as follows:

Calliphorinae
- *Calliphora loewi* Enderlein, 1903
- *Calliphora subalpina* Ringdahl, 1931
- *Calliphora vicina* Robineau-Desvoidy, 1830
- *Calliphora vomitoria* Linnaeus, 1758
- *Cynomya mortuorum* Linnaeus, 1761

Chrysomyinae
- *Cochliomyia hominivorax* Coquerel, 1858
- *Chrysomya albiceps* Wiedemann, 1819
- *Chrysomya bezziana* Villeneuve, 1914
- *Chrysomya marginalis* Wiedemann, 1830
- *Chrysomya megacephala* Fabricius, 1794
- *Chrysomya putoria* Wiedemann, 1830
- *Phormia regina* Meigen, 1826
- *Protophormia terraenovae* Robineau-Desvoidy, 1830

Luciliinae
- *Lucilia ampullacea* Villeneuve, 1922
- *Lucilia caesar* Linnaeus, 1758
- *Lucilia cuprina* Wiedemann, 1830
- *Lucilia illustris* Meigen, 1826
- *Lucilia sericata* Meigen, 1826
- *Lucilia silvarum* Meigen, 1826

The majority of material used for the purposes of key construction was collected personally by the author. Excellent stack-images of several specimens were taken by Tomasz Klejdysz (Poland, IPP-SRI). Specimens of *Cochliomyia hominivorax* and *Chrysomya bezziana* were provided for study thanks to the courtesy of Dr Soccoro Gomez Dorante (Mexico, COMEXA) and Dr April H. Wardhana (Indonesia, IRCVC). For each species, pictures of particular elements of male genitalia (cerci, surstyli, penis, pregonite and postgonite) are included as these characters are of great taxonomic importance and very useful for confirmation of identification based on general morphology. The method of treatment of freshly collected specimens for further examination of the details of the genital apparatus is well presented by Pape *et al.* (2010). Description of the proper preparation of dried, pinned specimens from museum collections is provided by Rognes (1991).

Terminology follows Merz and Haenni (2000), except that the author uses the word setae rather than macrotrichia and that the word bristle is used to denote a particularly strong seta.

The present key has been tested before publication and seems to work well; in doubtful cases, however, the author recommends that identifications should be checked against the illustrations and descriptions published in the listed references.

5.2 Key

1. Stem-vein (Plate 5.1D) bare above: 2
   Stem-vein haired above (Plate 5.1D): (Chrysomyinae) 3

2. Thorax nonmetallic, dark (Plate 5.9A–E); lower calypter with hairs above (Plates 5.2A, 5.3D): (Calliphorinae) 10
   Thorax bright green metallic (Plate 5.9K–O); lower calypter bare above (Plate 5.2B): (Luciliinae) 14

3. Greater ampulla with stiff erect hairs (Plate 5.2C); lower calypter with dense hairs above (Plates 5.2M, N): (*Chrysomya* spp.) 4
   Greater ampulla bare or with short fine hairs (Plate 5.2D); lower calypter bare or with a few pale hairs (like on Plate 5.2B): 8

4. Anterior wing margin darkened (Plate 5.2E), male genitalia in Plates 5.6I–L: *Chrysomya marginalis*
   Anterior wing margin transparent (Plate 5.1A): 5

5. Anterior thoracic spiracle yellow (Plate 5.2F): 6
   Anterior thoracic spiracle brown (Plate 5.2G): 7

6. Dorsal part of thorax shiny, with little dusting (Plate 5.2I); black transverse marginal abdominal bands on abdominal segments III and IV very narrow, up to about a quarter on AIII and usually not more than about sixth in AIV (Plate 5.2J); male genitalia in Plate 5.6A–D: *Chrysomya albiceps*
   Dorsal part of thorax with conspicuous dusting (Plate 5.2K); black transverse marginal abdominal bands on abdominal segment III broader, even up to one-half of tergite length (Plate 5.2L); male genitalia in Plates 5.7A–D: *Chrysomya putoria*

7. Lower calypter white, with yellowish fringe (Plate 5.2M); male genitalia in Plate 5.6E–H . . . *Chrysomya bezziana* (obligatory parasite)
   Lower calypter brownish infuscated (Plate 5.2N); male genitalia in Plate 5.6M–P: *Chrysomya megacephala*

8. Genal dilation (Plate 5.1B) yellow or orange, with mostly yellow hairs (Plate 5.3A), male genitalia in Plates 5.7E–H: *Cochliomyia hominivorax* (obligatory parasite)
   Genal dilation (Plate 5.1B) dark (Plate 5.2H): 9

9. Upper and lower calypters bright (Plate 5.3B), basicosta yellow (as in Plates 5.3E, J): *Phormia regina*
   Upper and lower calypters dark brown (Plate 5.3C), basicosta black (as in Plate 5.3K): *Protophormia terraenovae*

10. One pair of acrostichal bristles on postsutural area, abdomen shining blue without dusting (Plate 5.9E), male genitalia in Plate 5.5Q–T: *Cynomya mortuorum*
    Three pairs of acrostichal bristles on postsutural area (as in Plate 5.1C), abdomen shining blue with weak dusting (Plate 5.9A–D): (*Calliphora* spp.) 11

11. Upper and lower calypters predominantly white-yellowish (as in Plate 5.2A), male genitalia in Plate 5.5E–H: *Calliphora subalpina*
    Upper and lower calypters predominantly black (Plate 5.3D): 12

12. Facial ridges, mouth edge, and anterior part of genal dilation orange (Plate 5.3G); basicosta yellow (like in Plates 5.3E, J), male genitalia in Plates 5.5I–L: *Calliphora vicina*
    Facial ridges, mouth edge, and anterior part of genal dilation dark (Plates 5.3HI); basicosta black (as in Plate 5.3K): 13

13. Postgena and lower part of genal dilation with black hairs (Plate 5.3I), male genitalia in Plate 5.5A–D: *Calliphora loewi*
    Postgena and lower part of genal dilation with orange hairs (Plate 5.3H), male genitalia in Plate 5.5M–P: *Calliphora vomitoria*

14. Basicosta bright yellow (Plate 5.3E, J): 15
    Basicosta brown or black (Plate 5.3K): 16

15. Central occipital area with two to five bristles below inner vertical bristle (Plate 5.4A), abdomen usually bright green, occasionally shining coppery, male genitalia in Plate 5.8O–R: *Lucilia sericata*
    Central occipital area with one bristle just below inner vertical bristle (Plate 5.4B), abdomen usually shining coppery, male genitalia in Plate 5.7Q–T: *Lucilia cuprina*

16. Three pairs of acrostichal bristles on postsutural area (as in Plate 5.1C), palpus brown to black (Plate 5.4D), male genitalia in Plates 5.8S–V: *Lucilia silvarum*
    Two pairs of acrostichal bristles on postsutural area, palpus yellow (Plates 5.4EF): 17

17. Coxopleural streak absent, male genitalia in Plate 5.8A–D: *Lucilia ampullacea*
    Coxopleural streak present (Plate 5.4G): 18

18. Male: surstyli slender, gradually tapered to sharp tip (Plates 5.8JK); female: tergit VI straight in lateral view (Plates 5.1F, 5.8M): *Lucilia illustris*
    Male: surstyli stout, abruptly narrowed at tip (Plate 5.8EF); female: tergit VI convex in lateral view (Plate 5.8H): *Lucilia caesar*

6

Identifying beetles that are important in forensic entomology

6.1 What do beetles look like?

Beetles belong to the order Coleoptera and all share features in common. Adult beetles are composed of a head, thorax in three parts all fused together (although the second and third parts are less visible dorsally) and an abdomen. They have two pairs of wings; the two forewings are hardened and form a protective covering over the second, membranous pair of wings. These chitinous, and on occasion 'leathery', protective cases are called the **elytra** (singular elytron) (Figure 6.1).

Beetle heads can be structured in one of several ways. They can project forwards horizontally (a **prognathous** head), or orientate downwards (an **hypognathous** head). Adult beetles have biting mouthparts or **mandibles**. Located on the head are the antennae that carry tactile, heat-sensitive, olfactory and humidity receptors. Their antennae have 11 segments in general. Coleopteran antennae vary in form. Some are threadlike (filiform), or platelike (lamellate) whereas others are elbowed (geniculate), or have clublike ends (clavate).

The beetle exoskeleton is formed from hardened plates. The plates on the top surface are called **tergites**; the plates on the under (ventral) surface are called **sternites**. The segment plates at the side (lateral) of the body are called pleurites. (The pleuron is the name for this region of the exoskeleton.)

The dorsal surface of the thorax is divided into the **pro**-, **meso-**, and **metanotum**. (Each plate, or tergite, is called a **notum** (plural nota).) The pronotum, (the surface of the first thoracic segment in front of the elytra) is the biggest of the thoracic segments. It is made up of only one plate. (The ventral surface is correspondingly divided into three; the pro -, meso -, and **metasternum**) (Figure 6.2).

The prothorax is well developed and, together with the head, can be interpreted as a distinct anterior portion of the body. It is usually distinctive in shape and size and can be used as a means of identifying the beetle. The middle region of the thorax (the mesothorax) supports the pair of hardened wing cases that meet along the centre of

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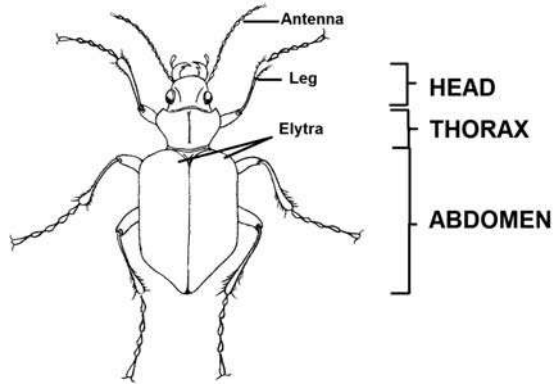

Figure 6.1 Generalised morphology of a beetle – dorsal view

the back of the beetle (dorsal surface). Part of the mesonotum is located between the base of the elytra behind the pronotum. This small plate is called the **scutellum**.

The membranous wings are attached to the body on the last section of the thorax (the metathorax, with which the mesothorax is fused). This pair of wings is folded beneath the elytra when the beetle is not in flight.

Beetles legs are positioned on the sternum. They are generally designed for running or walking, but in some beetles, as in the Scarabaeidae, the front legs are also modified for digging (Figure 6.3).

Figure 6.2 The structure of the coleopteran thorax

Box 6.1 Structure of the insect cuticle

Insect **cuticle,** which is made of **chitin** and proteins, can be rigid or flexible. Cuticle provides protection from physical damage and water loss, and a rigid structure for muscle attachment. It limits growth to those times when the cuticle is newly developing. The mechanical properties of cuticle depend on the quantity of protein present, the sequence of proteins and the degree of tanning (sclerotization).

Cuticle has three parts: epicuticle, procuticle and epidermis. The epidermis and cuticle together are called the insect **integument**. The **epicuticle** is the outermost layer. It is between 0.1 μm and 3 μm thick and is also made of three layers. The outermost layer is a cement layer, which prevents distortion of the next layer, a lipid-protein layer. Below this second layer is a glycoprotein superficial layer. The epicuticle does not contain chitin. It is not capable of providing support or extending, but does provide waterproofing and protection against mechanical damage.

Below this is the **procuticle**. Procuticle is between 10 μm and 0.5 μm in depth and comprises a thicker endocuticle, which is light in colour, overlaid by a thinner, darker exocuticle. Procuticle is made up of a protein matrix in which layers of parallel microfibrils of chitin, an amino-sugar polysaccharide, are embedded to make a sheet. In the exocuticle the sheets of microfibrils are in the same plane, but each sheet may be orientated at a slight angle to the previous sheet. An alternate stacked, or helicoids arrangement of microfibril sheets in the endocuticle, results in it being a thicker layer than the exocuticle. The darkening of the thinner exocuticle is due to tanning (sclerotization).

The basal layer beneath the cuticle is the **epidermis**. This single layer of cells is supported on a basement membrane which separates the exoskeleton from the main body cavity. Epidermal cells regenerate by cell duplication, or mitosis. This layer secretes the cuticle-forming chemical, which is needed for moulting to take place.

Types of cuticle

There are two types of cuticle: soft cuticle and hard cuticle.

Soft cuticle is flexible and the cuticle is thin and has little or no exocuticle. Larvae predominantly have soft cuticle and a hydrostatic skeleton. Soft cuticle is also important where movement is required and, for example, allows gravid females to extend their abdominal plates to lay eggs.

Hard cuticle is hardened and armour-like because of the level of tanning, the positions of the microfibril sheets and hydrogen bonding between adjacent chitin molecular chains. Hardened chitin is found surrounding the spiracles of fly larvae and is present on the head and as the mandibles of beetle larvae. It provides the strength and rigidity of the body and elytra in adult beetles.

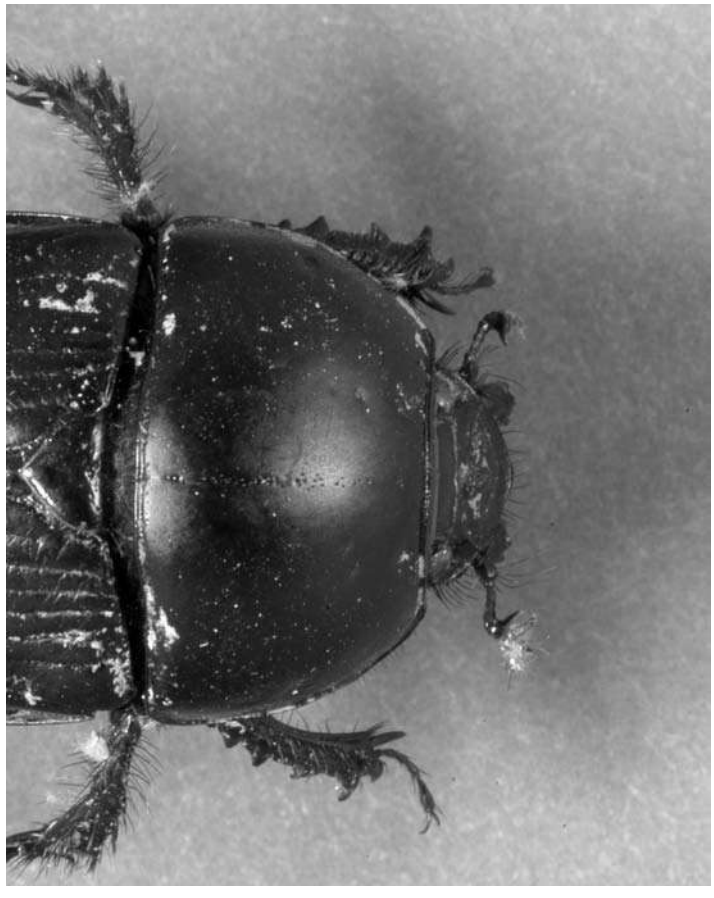

Figure 6.3 The front legs of a beetle that have been modified for digging (*Geotrupes* sp.)

The upper plates of the abdominal segments are **sclerotised** (made of cuticle hardened with a protein called sclerotin). The lower abdominal plates (the sterna) are soft.

Beetles exhibit complete metamorphosis during their lifecycles and pass through an egg stage, larval stages and a pupal stage and then emerge as an adult, or imago. Each stage is morphologically different. Beetle eggs are frequently difficult to locate on or around the body as, unlike fly eggs, they do not often appear in batches. They are often laid singly in the vicinity of suitable food sources.

Beetle larvae have more distinctive morphological features than do fly larvae. For example, they have a sclerotised head capsule, and mouthparts that include mandibles (are mandibulate). Larvae may or may not have legs on the thoracic region of their body. **Prolegs** (limbs on the abdominal region) are rarely present in beetle larvae and this distinguishes them from the larvae of other orders.

For example, ground beetle larvae (carabids) have an elongated flattened shape with well defined legs that end in two claws. These are called **campodeiform** larvae. Scarab

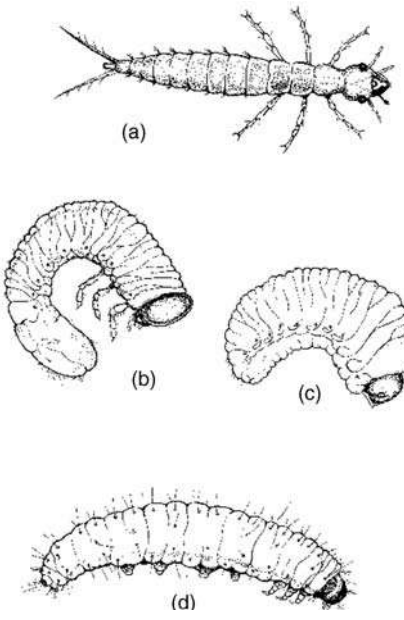

Figure 6.4 Examples of the shapes of beetle larvae. *Source:* Reproduced from Munro (1966) with kind permission from Rentokil Initial plc.

beetle larvae resemble a 'c shape' and these beetles tend to have a brown sclerotised head and a whitish body. On the other hand, larvae of the dermestid family are particularly hairy on the sides and the upper body surfaces and are recognised because of this coat of hairs. Examples of the shapes of forensically significant larvae are shown in Figure 6.4.

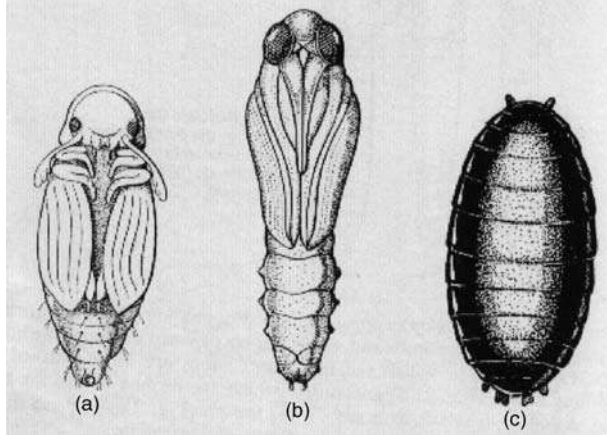

Figure 6.5 The types of pupa and puparia to illustrate the relationship of appendages to the body. *Source:* Dr Andrew Brigham and Rentokil Initial plc, for permission to reproduce diagrams of beetle larvae and pupa (presented in this book as Figures 6.4 and 6.5) from Munro J.W., Pests of Stored Products. The Rentokil Library Benham and Co. Colchester (1965)

Small, hardened structures projecting from the end of the larval abdomen are called **urogomphi**. They are recognisable, for example, in the larvae of Dermestidae, Nitidulidae and Histeridae.

The third stage of metamorphosis is called the pupal stage 6.16. The pupa has mouthparts that do not articulate (i.e. are **adecticous**) and the rest of the pupal appendages are free and visible through the pupal coat (the pupa is **exarate**) (Figure 6.5). This is not so in the staphylinids, where the pupa is covered by a hardened coat and the pupal appendages are held in place by secreted material (an **obtect** pupae).

Some pupae pupate in a chamber within the soil. Others, like the scarabaeids, form a cocoon. In this instance the cocoon is made from material in the posterior section of the **caecum** (Richards and Davies, 1988).

6.2 The life stages of the beetles

Beetles also have a complete metamorphosis and pass through an egg stage, three to five larval stages depending on species, and a pupal stage before becoming an adult, although the availability and quality of food may dictate the number of instars that are completed before adulthood is reached (Figure 6.12).

Coleopteran eggs tend to be oval, spherical or spheroid in shape and are usually considered to be morphologically similar, irrespective of family. Larval shape does differ between families and this is a valuable means of distinguishing the species that are present on a corpse. Beetle larvae usually bury themselves in the ground, or in a specially constructed chamber, when they pupate. Less detailed information is available about beetle lifecycles than is known about the diptera.

The length of the beetle lifecycle varies, depending upon the family and species of beetle. Development through the complete lifecycle, from egg to adult (imago) can take from seven to ten days in rove beetles (Staphylindae). In ground beetles (Carabidae) completion of the lifecycle to the adult stage may take a year and the adults may live for two to three years. In some species the number of instars in the larval stage is not fixed but depends on environmental conditions. In Dermestidae, for example, there may be as many as nine instars (Hinton, 1945) prior to the beetles becoming adults and this can extend the lifecycle quite considerably. Usually though, there is only one generation of beetles per year. Smith (1986) indicates that the length of the pupal stage of *Dermestes* sp. can last between two weeks and two months and that these beetles can overwinter (enter **diapause**) in a pupal chamber, if the weather is not suitable or if it is late in the season.

The problem of lack of a ready morphological distinction between larval instars in beetles means that other methods to distinguish the instar are needed. Watson and Carlton (2005) investigated the lifecycles of three species of American silphid that feed on the insects visiting the carcass and on the carcass itself; *Oiceoptoma inaequale* (Fabricius), *Necrophilia americana* (Linnaeus) and *Necrodes surinamensis* (Fabricius). Using multivariate analysis, they identified three larval stages. For *Necrodes surinamensis* they showed a first instar average duration of 12 days, a

duration of 10 days for the second instar and 11 days for the third instar, indicating that the larvae were present from day 9–22 of decomposition (Watson and Carlton, 2005).

Chapter 11 provides further information about some of the beetle families that play a significant role in succession on a corpse and so have a role in forensic entomology.

6.3 Selected forensically relevant families of beetles

The order Coleoptera is divided on the basis of molecular studies, into what is treated as four suborders: Archostemata, Myxophaga, Adephaga and Polyphaga. The Archostemata is made up of three families, which mostly inhabit decaying wood. The Myxophaga are made up of four families that are aquatic, or are found in moist habitats and are algal feeders. Although all insects may be of importance in forensic entomology the remaining two suborders, Adephaga and Polyphaga, contain families of beetles that are most commonly found at crime scenes. The suborder Adephaga contains ten families and comprises predatory beetles, which inhabit terrestrial and aquatic habitats, and includes the ground beetles – Carabidae, Dytiscidae and Gyrinidae. The Polyphaga contains 149 families including the families Dermestidae, Scarabaeidae, Staphylinidae, Histeridae and Cleridae.

Suborder Adephaga

The term adephagous is derived from the Greek word for being ravenous (extremely hungry) and describes beetles that are carnivorous and therefore predators. These beetles are distinguished by the positioning of their legs. The coxae of the third pair of legs (the hind legs) are fused to the metasternum. When you look at the underside of the beetle you see that this region of the leg divides the first visible abdominal sternal plate (Figure 6.6b).

There are lines down the sides of the thorax called sutures. (The indents are positions where there is internal strengthening of the exoskeleton.) An example of this is the suture between the notum and the sternum. (Sutures are readily recognised as the large transverse indentations across the thorax of the fly.) The majority of beetles in this suborder have thread-like antennae are described as filiform.

Larvae of insects in this suborder have legs with five segments that end in two claws (only rarely is it one claw). These larvae are mostly elongated and flattened (Luff, in Cooter and Barclay, 2006). Because most beetles in the Adephaga are predaceous they are attracted to a dead body to feed on the insects and their life stages inhabiting a cadaver. The beetle families of forensic interest, which are in the Adephaga, are the Carabidae, and the water beetle family the Dytiscidae.

Suborder Polyphaga

This suborder contains the majority of families of beetles with which the forensic entomologist may be concerned. The following features characterise this suborder. The hind coxa is rarely fused to the metasternum (it moves, or articulates) and so does not divide the first visible abdominal sternite (Figure 6.6a). The thorax in this

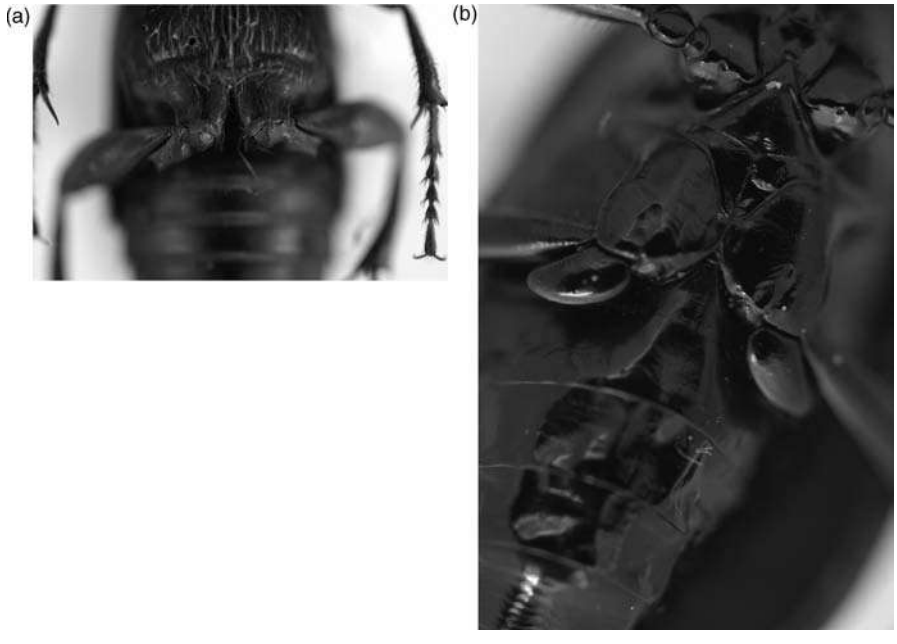

Figure 6.6 Ventral view of the beetle thorax to illustrate the distinction between the Polyphaga (Figure 6.6a) and the Adephaga (Figure 6.6b)

suborder does not have lines (sutures) across its dorsal surface. The types of antennae in the suborder vary, so they cannot be used as an indicative feature.

Polyphaga larvae are of many different shapes. They have legs with four segments that end in a claw. Some larvae in the suborder Polyphaga have legs that are reduced; others have vestigial legs, or they may even be absent altogether.

Polyphaga adults eat a variety of food. Some beetles are predaceous, but in the suborder as a whole many are phytophagous. Only beetles that are predators or necrophages are of immediate importance to the forensic entomologist. A number of beetles, for example the Dermestidae, visit a dead body, either because the body itself forms food and a habitat or to feed on the insects already present – for example, the Staphylinidae. The families of insects from this suborder, which are important in forensic entomology, include the Silphidae, Scarabaeidae, Staphylinidae, Histeridae, Trogidae, and Dermestidae. Cleridae and Nitidulidae.

6.4 Features used in identifying forensically important beetle families

6.4.1 Carrion beetles (Silphidae)

Silphidae have a flat body with sharp margins and their heads are small relative to the size of their thorax. The beetles of this family have antennae in which the sequence of

Figure 6.7 *Nicrophorus humator* Gleditsch

antennal segments tends to thicken as the segments progress to the end, or the antennae are distinctly clubbed. The distance between the points of insertion of the antennae is wide. These are large, robust beetles and some, such as *Nicrophorus vespilloides* Herbst, have orange or red markings on their elytra. Others such as *Nicrophorus humator* Gleditsch (Figure 6.7 – see also the colour section) are black in colour. One of the main identification features of this family is that no abdominal segments protrude from the hardened upper wings (the elytra). If the beetle is turned over, six abdominal sternites are visible.

6.4.2 Rove beetles (Staphylinidae)

Staphylinidae are active beetles that are easily recognised because their short elytra expose at least half of the abdominal segments so that seven to eight protrude, when the insect is viewed from above (Figure 6.8). They range in size from tiny to large. For example, the largest British staphylinid species *Ocypus olens* Müller, (whose English common name is the devil's coach horse), has been recorded at 28 mm long (Richards and Davies, 1988). Beetles of this family, however, are accomplished fliers and have strong membraneous wings packed away under their shortened elytra. Some species have the habit of curling up their last few abdominal segments over their 'back'. This makes them look very aggressive and the action is reminiscent of a scorpion. If you see specimens reacting like this as you approach them then you have most likely found a staphylinid beetle.

Figure 6.8 An example of a member of the Staphylinidae

Staphylinid beetles are predators and are attracted to the corpse to feed on the larvae of diptera. A number of species of rove beetles (Staphylinidae) have been found on a body. For example Goff and Flynn (1991) recorded the presence of adult *Philonthus longicornis* Stephens from a 23-year-old Caucasian male in Hawaii and *Creophilus maxillosus* Linnaeus, which Centeno, Maldonado and Oliva (2002) recognise, as forensically relevant in Argentina and which Chapman and Sankey (1955) also recorded from rabbit corpses in exposed conditions in Surrey, England.

6.4.3 Clown beetles (Histeridae)

These are small, shiny black beetles (Figure 6.9) with an exoskeleton that has a hard, often leathery or sculptured texture and a more-or-less oval shape. Their antennae are elbowed (geniculate) and the final segments of the antennae are formed into an obvious club. Histerid legs have flat tibiae. The significant identification feature of this family, when looked at from above, is the square cut to the ends of the elytra, which reveals the last two abdominal segments.

Both larvae and adults are found on the corpse as they feed on those insects attracted to decaying organic matter. The larvae also eat fly larvae and prey on other insects. The adult beetles respond to being handled by withdrawing their heads and pulling their legs, and any other projections, into the body, which is sculptured to allow this, and by 'playing dead' (exhibiting **thanatosis**).

6.4.4 Trogid beetles (Trogidae)

These are medium-sized beetles, which are dull brownish in colour (Figure 6.10). The dorsal surface of their body appears roughened and the elytra can sometimes be

Figure 6.9 A hister beetle

hairy. The segments at the tip of the antennae are plate like. The legs of trogid adults are not broad, or modified for digging.

Trogidae larvae have characteristically long, sharp claws. Chinnery (1973) indicates that species of the genus *Trox* are not common in the UK. They are found

Figure 6.10 A trogid beetle

at the dry stage on small carcasses and, in particular, feed on hide, fur, leather, feathers, and dry matter. These beetles will also exhibit thanatosis if disturbed.

6.4.5 Hide and skin beetles (Dermestidae)

Dermestidae range in size from very small to medium (1.5–10 mm) and have an oval to elongated shape (Plate 6.1 and 6.2 in the colour section). Their antennae are made up of between five and 11 segments, ending in a club made of two to three segments (Peacock, 1993).

Adult members of the genus *Dermestes* lack a simple eye (an **ocellus**) on their head. The coxa on their front leg is conical and sticks out prominently from the coxal cavity (Figure 6.11). The femur of the hind leg is covered by the hind coxa, which is flattened into a plate. These beetles have the capacity to pull all their appendages into the underside of their body, so nothing protrudes.

Larvae of forensically relevant Dermestidae are brown to black in colour and have hairs of varying lengths (**setae**) over their dorsal surface. There are frequently tufts of hair on the sides or posterior edge of the body. Indeed the larvae of *Dermestes maculatus* DeGeer are commonly known as 'woolly bears' as a result of this profusion of hairs. The larvae are 6–13 mm (1/4 to 3/8 inch) long and have two horns (urogomphi) on their terminal segment.

Dermestes lardarius Linnaeus is known to pupate in a puparium for 40–50 days at 18–20 °C. They have one generation per year. Male *Dermestes lardarius* pass through four larval instars whilst the female have five instars.

Figure 6.11 The front coxa of the dermestid projects from the coxal cavity

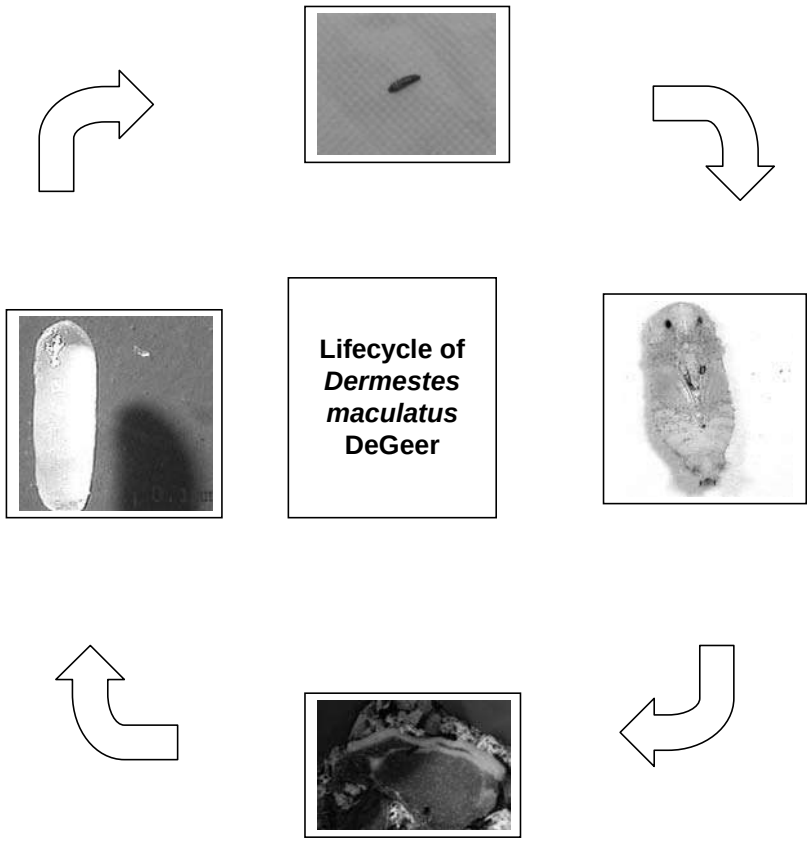

Figure 6.12 The lifecycle of *Dermestes maculatus* DeGeer

6.4.6 Chequered (or bone) beetles (Cleridae)

These beetles are usually brightly coloured on at least some part of their body (Figure 6.13). They are elongated and cylindrical in shape and appear to have a 'neck' because the first part of the thorax (the pronotum) is less broad than their elytra. The adults can be hairy. An example of a forensically significant member of the Cleridae is *Necrobia rufipes* DeGeer, the red-legged ham beetle, which can be found in association with bodies later in the decomposition sequence (see the colour section). In Hawaii it has been found in the soil under a corpse at a PMI of 34–36 days (Goff and Flynn, 1991). This species is a predator of fly larvae.

6.4.7 Sap–feeding beetles (Nitidulidae)

These are very small beetles, and are not often longer than 7 or 8 mm (Figure 6.14). The Nitidulidae have undergone taxonomic revision recently. Therefore the choice

Figure 6.13 A clerid beetle

Figure 6.14 An example of a nitulid beetle

of keys and terminology should be considered carefully. Their antennae are usually composed of 11 segments, ending in a three-segmented club. Their elytra are often truncated, but the family rarely has more than three abdominal segments visible dorsally. The fore and mid coxae are transversely orientated, whereas the hind coxa is flattened. The tarsal formula for this family is most frequently 5–5–5 (This means that the tarsus of each of the legs is made up of five tarsomeres). The first segment (tarsomere) of the tarsus is not shortened and all of the tarsal segments are more or less dilated.

This family is a coloniser of corpses that are in the later stages of decomposition. According to Cooter and Barclay (2006) in the British Nitidulidae, the subfamily Nitidulinae includes two genera, *Nitidula* and *Omosita,* which are particularly associated with bones and dried carrion. Wolff *et al*. (2001) undertook a preliminary study in Medellín, Colombia, and found that 0.2% of the total number of families visiting a dead pig, which they had set up in an experimental 'crime scene', were members of the Nitidulidae. All members of this family were recorded from the advanced stage of decay that occurred between13 and 51 days after the pig died.

6.4.8 Ground beetles (Carabidae)

Ground beetles have a characteristic beetle shape and are readily recognisable as such. They can be found in a number of habitats, including grassland and forests. Carabids are members of the Adephaga because their first abdominal sternite segment is divided by the hind coxa. Their antenna are beadlike (filiform) and located on their head, between the eyes and jaws. The beetle head is prognathous. In carabids the elytra are usually sculptured, for example with striations, so that one sees nine regular ridges and furrows along the elytra (Figure 6.15). They are frequently

Figure 6.15 The striations on the elytra of a carabid

fixed in position and, where this is the case, the beetle has only the vestiges of membranous wings and so cannot fly.

Carabid larvae are long or elongated in shape. The larva has a pair of sharp pincer-like mandibles and six simple eyes (ocelli) down each side of its head. The larval abdomen has ten segments and on segment nine there is a pair of **cerci**. The larvae have legs that end in two claws. Carabid larvae are very quick in their movements and tend to be nocturnal, so they may not be obvious members of the corpse assemblage.

6.5 Identification of beetle families using DNA

Techniques for the identification of insects through the analysis of mtDNA – RFLP and RAPD – were described in Chapter 2. As with flies, those molecular techniques have their original application in phylogenetic investigations of beetle species. For example they were used to separate members of morphologically similar ground (carabid) beetles of the *Nebria-Gregaria* group, on Queen Charlotte Islands in British Columbia, Canada. Clarke *et al*. (2001) concluded from RAPD and mtDNA analysis that only one species of the group could be separated out on molecular grounds, from the particular group of carabids.

The RAPD analysis of beetle DNA has been a successful tool in crime analysis (Benecke, 1998). The families investigated included the carrion beetles (Silphidae) – for example *Oiceoptoma thoracicum* Linnaeus, (Figure 6.16) for which a DNA profile was determined from a badly decayed body in October 1997 (Benecke, 1998).

Mitochondrial DNA has also been used to identify the larvae of beetle species present on a body and also for additional purposes, such as identification of the human host from the gut content of the larvae upon whom it had been feeding. DiZinno *et al*. (2002) successfully analysed specimens from the nitidulid genus *Omosita,* in order to match mtDNA to a human host.

Dobler and Müller (2000) explored the phylogenetic relationship of the Silphidae using 2094 base pairs (bp) of COI and COII, as well as tRNA. With the longer lengths of mtDNA they were able to obtain a greater resolution of the genetic make up of the family, providing an increased identification profile for use by the forensic entomologist. Zehner, Haberle and Armendt (2004) explored intraspecies variation within the clerid beetles (based upon different mitochondrial genomes for the same species of organism–Heteroplasmy). They showed that within the cytochrome oxidase I gene, in both *Necrobia rufipes* and *Necrobia ruficollis* Fabricius, there was a high degree of heteroplasmy that did not express itself as much in *Necrobia violacea* (Linnaeus), another species of clerid. This variation has to be considered when interpreting a profile from a specimen from the crime scene.

Less research has been undertaken on the molecular profiles of forensically important coleoptera than for the diptera. However, since the techniques are in place, further profiling of beetle species should expand this base as more crime-scene investigations occur and further species of beetles are found to have forensic value.

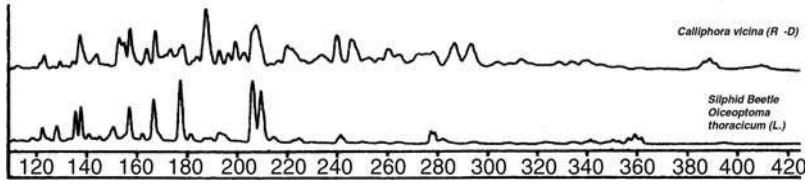

Figure 6.16 Electropherogram profile to allow comparison of the RAPD of a Silphid beetle to that of a calliphorid fly. *Source:* Elsevier for permission to reproduce a section of Figure 4 from Benecke M., Random amplified polymorphic DNA (RAPD) typing of necrophagous insects p164 (1998) with permission from Elsevier (presented in this book as Figure 6.16)

Therefore the more usual approach to identification is the use of a dichotomous key based on morphological features.

6.6 Key to selected forensically relevant families in the order Coleoptera

The following key is intended for use in identifying beetles which may be found in the water body in which a corpse has been submerged or that are known to be associated with carrion and have been found in close association with the corpse. In aquatic sites, other insects with apparently hardened wing cases may be present. However if they have sucking mouthparts for piercing prey they are distinct from beetles. Corixidae and Notonectidae are examples of such families (see Figures 12.6a and Figure12.6b). Use the key on the basis of the location of the body. Choose the description that best fits the insect being examined and move to the next couplet. This is intended to be an initial key that may help distinguish some common beetle families. More comprehensive keys should be consulted to confirm the identity of your specimen.

Species found in water: go to **Part A**.

Terrestrial species – found on land: go to **Part B**.

Part A

1. The eyes of the beetle are split into two. Small beetles (3–8 mm). Oval in shape when viewed from the top. First antennal segment is large; the rest merge into a club shape. Long forelegs but mid and hind legs are shorter and flattened for swimming. Surface swimmers.

Gyrinidae

2. Does not exhibit all of these features: go to 3.
3. Front legs reduced in size; back legs flattened. Legs have long bristles. Thorax is wider than it is long. Antennae are long with 11–12 segments. Mouthparts are short.

   Dytiscidae (Figure 12.8)

   Not so: go to 4.
4. Oval body. Clubbed antennae, not longer than size of head. Mouthparts (mandibular palps) tend to be long, hind legs flattened.

   Hydrophilidae (see colour section). This is an aquatic family but some adults are attracted to terrestrial rotting material and dung.

   Not so – seek a more comprehensive key such as an Aidgap key.

Part B

1. Each leg has five tarsal segments: go to 3.
2. Legs may vary in number of tarsal segments: go to 11.
3. Insects black or with a metallic sheen and long legs. Antennae long, slender and made up of uniform segments. Jaws are curved. This family often feeds on necrophages.

   Carabidae (Figure 6.15)

   Not so: go to 4.
4. Antenna are club shaped, with or without lamellae plates: go to 5.

   Antenna not elbowed: go to 8.
5. Insects with legs modified for digging. Antenna projects forwards and has a clubbed end made up of a number of plates.

   Scarabaeidae (dung beetles such as *Geotrupes* sp. may be found) (Figure 6.3).

   Legs not modified for digging: go to 6.
6. Antennae clubbed but with an elbow. Terminal segments are expanded in size to make the club. Rounded body. No distinct 'neck' region and the wing case does not extend to cover the whole abdomen – last two segments exposed. Shiny black beetles.

Histeridae (Figure 6.9)

Not so: go to 7.

7. Body flattened. Beetle appears to have a 'neck'. Antennae are clubbed.

   Cleridae (Figure 6.13)

   Not so: check another key

8. Small beetles, more or less oval in shape and the head is visible from above. The legs fit into grooves.

   Dermestidae (Figure 6.11)

   Legs do not fit into grooves on the ventral side: go to 9.

9. The beetles are either black in colour or have black and red/orange elytra or have a thorax coloured differently from the elytra. Body is rounded. (The wing case does not extend to cover the abdomen in some species). If examined from the side view the body has upright hairs.

   Silphidae (burying beetles have heads which project forwards whereas carrion beetles' heads project downwards) (Figure 6.7)

10. Wing case covers only a small part of the abdomen. At least seven abdominal segments are exposed. When frightened, the beetle curves its abdomen forwards like a scorpion.

    Staphylinidae (Figure 6.8)

    Not so – tarsal segment number is variable: go to 11.

11. Black beetles; first and second pars of legs have five tarsal segments. The third (hind) legs have four tarsal segments.

    Tenebrionidae

    Not so, all legs have four tarsal segments: go to 12.

12. Beetles very small and found on dried flesh, remains of sinews and bone. All legs have four tarsal segments

    Nitidulidae (Figure 6.14)

If beetles have features that differ from those listed, or the beetle does not fit the descriptions, use a more comprehensive key. You may have opportunist species in the crime scene that are using the body as a place of shelter. These will not key out using this key.

7
Sampling at the crime scene

Forensic entomologists may be called to the crime scene at any time, day, or night. It therefore helps to have the resources assembled so the response time can be rapid. Keeping a carrying case complete with equipment that will not deteriorate, such as vials, bags, pens and entomological forceps along with your butterfly net may be the most efficient way to respond to such an invitation (Figure 7.1). If you are working for the prosecution you need to collect sufficient samples from the crime scene for fellow forensic entomologists to make their own assessment for the defence, should this be requested. This is not only good science but imperative if the body is to be buried or cremated and the relatives wish to view it prior to disposal without any remnants of maggots or other insects present. Relatives should be able to view the deceased in a condition that is the least disturbing. (It may also be that because of the need for hygiene control activities the environment (crime scene) concerned will change when it is tidied up. So speed and good sampling techniques are required.)

7.1 Entomological equipment to sample from a corpse

The equipment required to collect insects from a corpse includes plastic or polycarbonate screw-top sampling jars for preserved specimens and live cultures, 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 (Adams and Hall, 2003).

Kahle's solution contains both a fungal control agent and a preservative. It has been used at the University of Lincoln for samples for eight years and has preserved the samples used in a teaching collection in the same flexible condition they were in when the larvae were first killed. Alcohol has also been used. However this required that, because of evaporation, the samples were more frequently curated than when using Kahle's solution.

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

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

Figure 7.1 Entomological crime scene equipment in a carrying case

Box 7.1 Kahle's solution composition

Chemical	Amount
Ethyl alcohol (95%)*	30.0 cc
Formaldehyde*	12.0 cc
Glacial acetic acid*	4.0 cc
Water	60.0 cc

means of combining uses, and limiting the quantity of equipment and chemicals required at the scene.

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 and not frozen, or chilled, when the maggots are placed on it. For the return journey the cultures should be kept in as low a temperature as possible, at the base temperature of the specimens. A mobile refrigerator for the car or van, or a cool box with artificial ice blocks, would be suitable. A thermometer or temperature logger 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 in the resources brought to the crime scene. The sample jars of preserved and live

specimens, from each site on the body, should be packaged together as a pair. Where samples are being taken by a crime scene investigator (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 holes punched in it and the lids to the culture jars also need 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 7.2). 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 colonisation site on the body, they are immersed for at least 30 s in water at a temperature of at least 80 °C, to fix the larvae at their maximum length (Adams and Hall, 2003). 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!)

A general description of the crime scene should be recorded. This includes whether the body has been wrapped, or covered in some way (see Figure 1.3) or, 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 site description, along with associated photographs, should be recorded.

Figure 7.2 Sealed, labelled bag containing entomological specimens collected at the crime scene. *Source:* © Colonel DAOST and Warrant officer 1st Thierry Pasquerault

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 that 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 that 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, wind direction and wind speed can all be recorded over a period of time. At a minimum, a temperature and humidity recorder should be used on site.

Once permission from the senior investigating officer has been obtained, the crime scene should be examined and a general assessment made prior to capturing specimens and sampling the body. It is important to start with the organisms most likely to be disturbed by human presence as these may therefore be lost as you progress with your investigation. These are usually the adult insects present at the scene.

7.2 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 7.3). 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 can be transferred to individual specimen jars later. Insects are mobile, so 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 that have been trapped on the radiator grill, bonnet or on the windscreen (windshield) of the vehicle. This may provide details of movement of the body. The temperatures in the car may be important as the interior of the vehicle is likely to get quite hot and this may affect the speed of the insect development, where flying insects have been able to gain entry and lay eggs.

Insects such as beetles, which are visible on the surface of the body or on the ground, can be collected by hand picking and placed in individual, labelled

Figure 7.3 Retrieving a fly from a net

containers. This is a sensible precaution because beetles may be carnivores and eat 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 handpicked again. Pitfall traps can be used to catch crawling insects near the body if it is an outdoor crime scene. Tulgren funnels 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 is

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. Saloňa *et al*., (2010) have shown the value of this approach. Mites, in particular, may be recovered. The researchers showed a direct correlation between the profile of insect specimens from the soil samples beneath where the body had been laid and those collected from the body at autopsy. In the absence of a body the insects recovered from the soil may give an indication of post mortem interval of the absent body.

7.3 The sampling strategy for the body

Once the flying and crawling insects have been collected, the body should be searched in an orderly sequence – searching clothing, examining orifices and wounds and also beneath the body.

7.3.1 Eggs

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 underside 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 normally laid in batches, 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 that has absorbed body fluid exudates. Fly eggs 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. It is therefore 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 ballpoint ink as this will not survive damp conditions). The label should include the name of the crime scene investigator who collected them (Figure 7.4), the officer in charge of the 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.

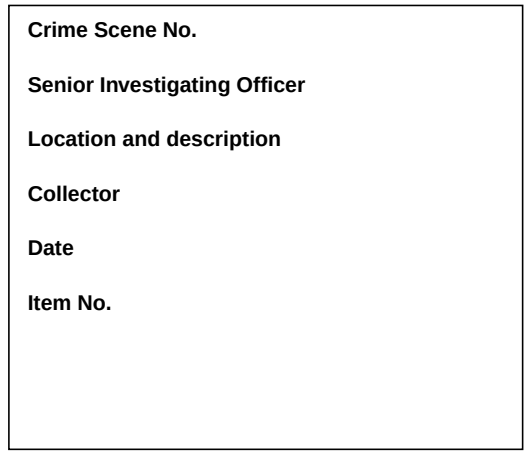

Figure 7.4 Label for the inside and outside of the collecting tube

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. This data must also be recorded in your scene log.

7.3.2 Larvae

Larvae will be located as the body is searched for eggs. They, too, tend to be in the body orifices such as the eyes, ears, nose and so on, including any wounds. 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 larva, if sampled at this stage, can easily die. So it is necessary, therefore, to protect it 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 that are to be preserved from the specific site are then added. They are left immersed in the water for at least 30 s before the contents of the jar are poured through a small sieve and collected in a large, labelled 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, 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 recorded 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.

7.3.3 Pupae and puparia

Fly puparia are usually found some distance from the body. The third instar post-feeding larval stage migrate from the body 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 that are available in buildings. If the puparia are still on the body then either there may have been some restriction to larval migration, such as blankets or wrappings, 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 organised search strategy should be used to determine if the lifecycle reached the puparial stage. The ideal is to search within each grid of 1 m side over a 36 m^2 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 puparia that do not hatch provide the examples of preserved specimens from the scene.

7.4 Sampling at aquatic crime scenes

Water bodies may be difficult to sample and require trained divers to sample them if they are submerged and held in position. Alternatively the body may have been noticed because it has risen to the surface and may be colonised by both aquatic organisms and terrestrial insects. Macroinvertebrates, including insects, are the most frequent colonisers of submerged bodies and they are often associated with the body rather than being specifically attached to it. This makes their collection difficult and may require some form of fine netting to be used to scoop up the body and its inhabitants and associated species.

Once back on the land the body is searched in much the same fashion as the terrestrial corpse and any terrestrial eggs, larvae or puparia should be collected alongside any adult flies and beetles. As the specimens are aquatic, they need to be preserved in a more concentrated solution of preservative. A solution of 95% alcohol is a good starting point and this should be replaced several times before the preserved

Box 7.2 Artificial pond water

Artificial pond water is often used for toxicological investigations. Water with the same composition is of value for maintaining cultures of macroinvertebrates, including aquatic insects, from crime scenes.

Into 10 l of deionised water add 50 cm^3 of solutions of each of the following:

calcium chloride ($CaCl_2.H_20$)	58.80 g l^{-1}
magnesium sulphate ($MgSO_4.7H_2O$)	24.65 l^{-1}
sodium hydrogen carbonate ($NaHCo_3$)	12.95 l^{-1}
potassium chloride (KCl)	1.15 l^{-1}

In order to prepare artificial sea water a 0.05 M saline solution is prepared by adding 0.25 g sodium chloride to each 100 cm^3 of artificial pond water.

specimens are placed in their final storage tube, suitably labelled. This allows the dehydration of the specimens to the point where the preservative can function appropriately. Specimens intended for further culture to confirm identity should be kept in lidded containers. If the labelled samples are transported as previously described, surrounded by ice packs or in cool boxes the specimens should survive satisfactorily. Once back in the laboratory they should be placed in aquaria. Dechlorinated tap water (water that has been left to stand for 24 hours at room temperature) may be used, although bringing back some water from the crime scene may also be an option in order to ensure the specimens survive. The organisms should be kept so that they do not eat each other, and the locations from which they were collected on or near the body should be identified on the tanks and in your notes.

The background fauna at the submergence site should be sampled so that any distinction in proportions of species resulting from the presence of the body can be determined. It may be necessary to seek the assistance from the local Environment Agency or wildlife trust in order to obtain some measure of the baseline of the populations of macroinvertebrates, if the crime scene has been excessively disturbed. Such may be the case if a car is driven into the water or dredgers, or inflatable rescue boats have had to be used to lift the body.

7.5 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 m (4 feet). This provides a measure of ambient air temperature at a comparable height to measurements 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 3.

7.5.1 Meteorological conditions in water bodies

If the crime-scene environment is aquatic, you may need to place a temperature logger in the water to confirm the water temperature relative to the ambient temperature. This should allow you to make a judgement regarding the water temperature in which the submerged body was retained over time.

8

Rearing insects and other laboratory investigations

Insects collected at crime scenes are often reared to their adult stage in order to identify them accurately, or to confirm an initial identification made using the larval stages. Alternatively it may be appropriate to rear the specimens to adulthood and then breed up the species from the egg stage up to the stage at which they were found on the body. Thus, by using the average temperature at the crime scene, it would be possible to confirm the time since the eggs were first laid on the body (and, by implication, time since death).

In all instances the specimens should be sent to the forensic entomologist, or taken back to the laboratory under controlled conditions, ideally at a low temperature to ensure that further development does not occur and to ensure that the insects do not escape.

8.1 Transporting entomological evidence to the laboratory

The conditions under which the insects are kept *in transit* are extremely important. They must always be transported, and subsequently maintained, in conditions that ensure that they develop through their life stages, or are stored without damage. Larvae from each of the separate locations on the body should be transported in individual containers.

The potential influence of temperatures on specimens during transit should always be taken into consideration. If the samples were collected by a crime scene investigator, they should be taken to the laboratory as rapidly as possible. A temperature that is the base temperature for those insects found at the scene should be used to transport them, if the species are obvious. This temperature may need to be determined for local conditions. Alternatively, a cold box with ice packs or refrigeration may be necessary. Myskowiak and Doums (2002), point out that temperatures as low as the normal refrigeration temperature of 4 °C may alter the larval life stages duration and the time taken to reach adulthood. They showed that

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ten days of refrigeration prior to introduction to culturing at 24 °C led to an alteration of between nine and 56 hours from the normal 15.5 days of *Protophormia terraenovae* larval development. In all cases controlled environmental conditions are necessary, along with a suitable source of food for any larvae and adults being transported even though they may not consume it.

Whilst all of the insects may feed on carcasses, it is inappropriate, both from the health-and-safety standpoint, and in terms of human tissue retention laws, to use flesh from the corpse to feed specimens in the laboratory. A supply of food, for example pig's liver, should be brought to the crime scene to provide food for the samples of living larvae. Food should be used for the rest of the rearing period. (Dead specimens are stored in preservative.) Larvae recovered from the crime scene should be placed in foil packages containing food and around 50 larvae. Such packages are then placed in containers, for example lidded polystyrene cups with holes in the lids for gas exchange, for transport and for subsequent rearing.

8.2 Laboratory conditions for fly rearing

The polystyrene cups with foil packages of meat, each containing around 50 larvae recovered from a specific site on the body, can be stored in the dark in a controlled environment cabinet or room (Figure 8.1), until the larvae reach the post-feeding stage.

In controlled environment cabinets a pierced lid should be placed on the top of each container to reduce drying out; one per sample from each location, for each

Figure 8.1 Larvae from a crime scene are ideally reared in a controlled environment cabinet

larval species collected. Pierced lids allow some gas exchange and prevent the buildup of ammonia as the larvae grow. The pots should be maintained at a relative humidity of at least 65%, or in baths of water at the appropriate temperature, so that the microclimate around the containers prevents the eggs, or initial larval instars, from drying out. Work by Introna *et al.* (1989) confirmed that being reared in growth cabinets under conditions reminiscent of the wild does not statistically alter the duration of the lifecycles of flies. They used *Lucilia sericata* for the trials.

As a precaution against loss and intermixing of cultures, each pot can be placed in a second container such as an aquarium. The aquarium sides should either be treated with a layer of **Fluon** (50:50 with water) so that the larvae cannot gain a purchase, or the top should be covered with fine mesh. A valuable alternative to a mesh cover is a pair of tights with the feet cut off. These cover the container top and prevent any of the maggots escaping whilst allowing a good air flow. The tights however should be taped in position to prevent larval escape. (If the species include those of the Piophilids, using a second container (aquarium tank) may be valuable since Piophilid larvae leave the body by springing off and can force their way through the tights.) Each container should be clearly labelled with date, case number, collector, and item number. These should also be recorded in the notebook.

Good practice means it is necessary to confirm the developmental stages through which the insects pass during the rearing process in the laboratory. As each life stage develops in the laboratory, samples recovered from each location on the body (if possible at least 20 per sampling) should be despatched in boiling water and preserved in Kahle's solution. Data relating to temperature and time to reach this stage should also be recorded, both in a laboratory note book and also on the sample pots, so that the pots collected at the crime scene can be related to this information. This record, along with the specimens, may be either requested by the court, or be used in court to illustrate your methodology.

8.3 Methods of maintaining and rearing insects – terrestrial species

Adult flies should be kept in large cages in order to facilitate mating, to gain eggs for development through to the stage recovered at the crime scene. These cages should be around 46 cm $\times$ 36 cm $\times$ 46 cm and be covered in mesh to allow light to enter and air to circulate, whilst retaining the flies. If the cage is too small the insect wings will become battered and flight will be affected, so mating will not be able to take place successfully. Access to the cage, so that food can be replaced, is through a 'sleeve' in the front of the cage.

The adults should be provided with a constant supply of water. This can either be in a screw-top jar with a wick emanating through the top, or in a Petri dish with water and stones, or a damp sponge, so the flies can drink without drowning. A 50:50 mix of sugar and dried milk powder should be provided for the adults, along with a water

Table 8.1 Average minimum lifecycle durations of a selection of dipteran species at fixed temperatures

Species	Temperature (°C)	Egg stage (hours)	L1 (hours)	L2 (hours)	L3 (hours)	Pupariation (hours)	Source
Calliphora vomitoria L.	12.5	64.8	55.2	60.0	434.4	717.6	Greenberg and Kunich (2002)
	23.0	21.6	25.2	19.2	210.4	247.2	Greenberg and Kunich (2002)
	26.7	26.0	24.0	48.0	420.0	260.0	Kamal (1958)
Calliphora vicina R-D	16.1	41.4	83.0	128.0	522	719.7	Anderson (2000)
	20.6	22.5	57.0	84.0	368.5	514.8	
	26.7	24.0	24.0	20.0	176.0	288.0	Kamal (1958)
Lucilia sericata Meigen	17.0	28.0	39.0	54.0	279.0	442.0	Grassberger and Reiter (2001)
	20.0	22.0	24.0	35.0	161.0	209.0	
Lucilia illustris Meigen	15.0	70.3	75.0	135.0	573.0	458.0	Byrd and Allen (2001)
	25.0	14.0	16.0	19.0	123.0	125.0	Anderson (2000)
Phormia regina Meigen	20.0	21.2	30.0	55.0	274.0	244.0	Byrd and Allen (2001)
	25.0	18.9	25.0	44.0	251.0	209.0	Byrd and Allen (2001)
Protophormia terraenovae R-D	12.5	91.2	290.4	240.0	832.8	722.4	Greenber and Kunich (2002)
	23.0	16.8	26.4	27.6	118.8	144.0	Greenberg and Kunich (2002)
Sarcophaga haemorrhoidalis Fallén	25.0	N/A	12.0	32.0	112.0	300.0	Byrd and Butler (1998)
Sarcophaga bullata Park	26.7	N/A	26.0	18.0	166.0	288.0	Kamal (1958)
Piophila casei Linnaeus	15	177.6	211.6	156	408.0	417.6	Russo et al. (2006)
	25	33.6	91.2	98.4	156.0	165.6	

supply. (If sugar alone is used, female insects may not necessarily develop their egg-laying capacity.) Meat or liver is also placed in the cage, both as a food source and to provide nutrients for female ovary development. This is also a place where eggs can be laid. The meat can be minced (ground) or can be palm-sized pieces of liver.

The food should also be partially covered. This restriction encourages both flies to lay eggs and the food to retain its moisture for a longer period. Care should be taken to keep the relative humidity above 50% and ideally 65% in order to ensure that the eggs and larvae survive.

Porcine liver has been used most successfully as a food and oviposition source. Ox or sheep liver can also be used but care should be taken to be consistent in the type and source of diet used. The majority of researchers successfully use *ad libitum* liver as a food source for carrion feeders from forensic sites, without any effect on the duration of lifecycle stages.

Flies should be bred at the most appropriate temperature, either in relation to the crime scene, or to achieve rapid development. Often this requires a controlled environment cabinet, although a room with a temperature with a limited and recorded fluctuation can also be used. Experimental research provides an indication of a suitable temperature. Information about the expected duration of the life stages comes from a number of sources (Table 8.1) including work by Kamal in the 1950s, who investigated the lifecycles of 13 fly species at 26.7 °C and 50% relative humidity (Kamal, 1958) along with papers by Anderson (2000); Higley and Haskell (2001); Greenberg and Kunich (2002); Donovan *et al*. (2006); Villet *et al*. (2006); Byrd and Castner (2009); and Gallagher, Sandhu and Kimsey (2010).

The day length that has been successfully used to avoid influencing the lifecycle is 16 hours daylight and eight hours dark (16L: 8D) (Vaz Nunes and Saunders, 1989). However the most appropriate day length to use is the average day length for the season in which the specimens were recovered from the crime scene, so that the conditions in the environment prior to recovery of the body are mirrored. Shorter day lengths, though, may encourage the larvae to enter diapause and so restrict the speed at which the post mortem maybe determined.

8.3.1 Conditions for the Postfeeding Stage

Once the larvae have reached the third instar they need to be transferred into conditions that ensure that the post-feeding larvae can migrate successfully, whilst preventing loss of the evidence. The ideal is an aquarium with vermiculite, sand, or sawdust in the bottom, kept in a controlled environment cabinet at the same temperature as that found at the crime scene. This provides a medium in which larvae can bury themselves to pupariate. So it is worth containing third instar larvae in an aquarium tank at this stage of their lifecycle, by moving the cup, if the aquarium has not previously been used as a means of secondary containment. Extra space is particularly important because lack of a pupariation site can influence the degree of success in completing the lifecycle in the normal period of time.

8.4 Dietary requirements of insects reared in the laboratory

Both flies and beetles have specific nutrient requirements in order to satisfactorily complete their lifecycles. Flies require carbohydrate as an energy source, together with water and protein. Protein is particularly important to females, for the development of the ovarioles and for egg production. They also require a number of vitamins and minerals.

For example Estrada *et al.* (2009) found that the diet on which *Chrysomya albiceps* Wiedemann larvae were fed on affected development. They explored the effects of artificial diets on larval growth. These diets contained bovine liver, uncooked stomach muscle, chicken heart and an artificial diet that included animal flesh. The control was muscle that had not been made into an artificial diet. Estrada *et al*. found that larval development time and weight gain was satisfactory on artificial diet for all of the samples, although fewer adults emerged on bovine liver and raw muscle treatments. They also showed similarity in development rate and emergence between the artificial diet and the control. For this species, liver can therefore be used as a growth medium for specimens reared from the corpse. Kaneshrajah and Turner (2004), however, showed a reduced *Calliphora vicina* larval growth on liver, in contrast to growth when heart, lungs, kidney, or brain tissues were used.

In contrast, when comparing growth on horse flesh and porcine flesh Boatright and Tomberlin (2010) showed that the number of degree days required for *Cochliomyia macellaria* to reach adulthood did not differ significantly. Care must therefore be exercised in checking the data used with respect to the materials upon which the insects are reared, if the object is to replicate the time period at average temperatures in order to reach the developmental stage recovered from the corpse.

A number of other sources of food, beyond cuts of muscle and viscera, have been exploited for maintaining carrion-feeding flies. They include the use of commercial cat food, dog food, minced beef, and artificial diets comprising agar, baker's yeast, and sodium chloride. Such food sources have varying benefits and are also often a source of malodours. Hermes (1928), however, showed an effect on the sex ratio of *Lucilia sericata* caused by the amount of food available to the larvae, so *ad libitum* feeding in the laboratory is necessary. Where the insects under consideration are also dung feeders, alternative sources of nutrition are required.

Stratiomyidae, for example, are species which inhabit animal waste products. Of particular note in forensic entomology are members of the genus Hermetia, for example *Hermetia illucens* (Linnaeus). Once eggs are separated out, the larvae will survive on a diet of wheat bran maize meal and alfalfa plant meal as an alternative to the use of animal manure (Sheppard *et al.*, 2002). This is of considerable advantage when rearing and maintaining this species as it reduces the smell and the issue of food storage is less problematic.

On occasion an artificial diet is required, either because this is the most suitable means of adding a known quantity of drug, or for oviposition, or because complaints

about malodours mean that the regime has to be modified. Zhang *et al*. (2009) designed an artificial diet that could be heat sterilised. The successful diet comprised whole milk powder, dried yeast, wheat germ, powered agar, and water and provided comparable growth success to that on beef liver. The proportions varied depending upon whether larvae or adults were being kept and whether or not the diet was being used as an oviposition resource. Cohen (2001) discusses the future requirements for successful insect rearing technology and lists some references of general interest.

Carrion beetles require a diet that incorporates the relevant nutrients, or to feed on flesh regularly, in order for them to thrive. They should therefore be fed dead meal worms every three or four days (Eggert, Reinking and Muller, 1998). This is also a suitable food for ground beetles (carabids), which can also be fed on ant eggs, such as those used for feeding fish, or maggots and pupae. The inclusion of insects in the diet is particularly appropriate for Silphidae. Cleridae, Histeridae and Nitidulidae can be fed in the same way.

In contrast, larvae and adults of skin beetles (Dermestidae) – *Dermestes maculatus* or *Dermestes lardarius*, can be fed on dried dog food or fish food. They prefer pelleted food rather than flake, but will consume both. Such food should be checked every two or three days to ensure sufficient is available and it is of appropriate quality. The food should be available in excess so that the dermestid lifecycle is not impaired.

In order to ensure that, where necessary, beetles such as dermestids can breed efficiently, it may be necessary to provide meat on an intermittent basis, in order to provide all of the required nutrients for reproduction and to trigger egg laying. To simulate the conditions at the crime scene using meat such as pork that is not too moist may be better than using an artificial diet.

8.5 Beetle rearing in the laboratory

Silphid, clerid, histerid or staphylinid beetles can be kept in transparent plastic containers, glass jars, vials, pots or buckets. They need to be kept individually to prevent one beetle eating another. If you are attempting to breed a new generation, an individual male and female beetle should be placed in a container. These containers should have a layer of moist peat, peat substitute, soil, or sawdust in the bottom, depending upon the family of beetles, and places in which insects can hide (refugiums), such as half plant pots or pieces of ridged cardboard or crumpled paper.

Silphid beetles, such as *Microspores* sp. adults, need a temperature of 20 °C and a daylight regime of 16:8 (L: D) (Eggert, Reinking and Muller, 1998). They can be kept in a group with a maximum size of six beetles of the same sex. If silphid beetles are required to breed, pairs can be placed in a container with a small carcass such as a defrosted mouse or a large piece of beef, pork, or chicken.

If you are keeping Nicrophorus beetles, any meat left in a container placed on the top of peat, or peat substitute, will be buried and eggs will be laid near the meat ball (Kramer Wilson, 1999). Such eggs should be recovered and retained on moist filter

paper at 20 °C until they hatch. Eggs of silphid species such as *Nicrophorus vespilloides* take on average 56 hours to hatch at 20 °C (Muller and Eggert, 1990, in Eggert, Reinking and Muller, 1998).

A water source in a wicked vial should be provided in the tank for both adult beetles and larvae. The vial is sunk into the peat so that the beetles can readily gain access. Each larva, in its individual container, can be supplied with a carcass with a hole in it through which to gain entry. Cultures can be maintained under a regime of total darkness in an incubator, or controlled environment cabinet. Not all of the beetles associated with corpses require carrion to complete their lifecycles.

8.5.1 Dermestidae

Dermestids can be bred satisfactorily on dried meat or on an artificial diet. They breed optimally at a slightly higher temperature than 20 °C and are ideally kept at 25 °C and 80% relative humidity (Coombs, 1978). Dermestids, too, can be kept as cultures in aquaria or glass jars.

Dermestids require wood sawdust or sand, and some solid medium such as polystyrene or cork, in which to burrow to pupate. This should be covered with several layers of paper to simulate conditions in the body. A supply of water must be provided, either as a piece of folded, dampened paper, or as a vial of water with a wick. Keep the water away from the food to discourage fungal growth. Black paper, in the form of a 'concertina', provides an egg laying site from which it is relatively simple to see and recover eggs.

8.5.2 Rearing beetle larvae

Beetle larvae can be reared in a variety of containers from beakers and plastic pots to aquaria and jam jars. In all instances it is helpful to maintain the humidity either by supplying moisture on damp kitchen towels or paper towels, or by using moistened plaster of Paris as a base to maintain the humidity.

Carabid and staphylinid larvae (and adults) should be kept individually as they are predatory and cannibalistic. In addition to a container with a moist base, moss should be included as a refugium where the larvae can hide. Food should be placed in containers and checked for mould every few days. Meal worms (*Tenebrio molitor* Linnaeus) and dipterous larvae (maggots), obtained from a fishing tackle shop, can provide food as can earthworms.

Histerid beetle larvae are infrequently found as they inhabit the soil or the region under the body. They are photophobic so rapidly disappear as the corpse is turned over. Histerids are not only corpse feeders – they are also carnivorous. These larvae therefore also need to be kept in individual containers. Such containers can be anything from small plant pots to the cut ends of soft drinks bottles with some holes poked in the base. The containers are filled to 2 cm from their top with garden

soil (or loam) to which maggots are added as food. The Histerid larva is then placed in the container and a piece of meat placed on the soil surface. These containers are then placed in dishes of water to maintain the soil moisture. Ideally the pots are then covered in netting to contain any fly larvae that pupariate and emerge because they have escaped being eaten by the histerids. The meat will also provide moisture and adds nutrients to the soil through decomposition. The meat should be checked every few days and replaced before it becomes mouldy. The beetle specimens can be kept in the dark in incubators and controlled environment rooms.

8.6 Methods of maintaining aquatic species

Those aquatic insects that inhabitant ponds can be kept in glass tanks, large plastic containers (Figure 8.2) or aquaria that have an additional aeration source, although it may be better to use a bubble stone than to use aeration pumps unless this is covered to prevent the smaller specimens entering the pump. Macroinvertebrates that inhabit pools and stagnant regions of flowing water may also be kept in tanks. Those that inhabit flowing water, or riffles, may require the water to be pumped through the tanks in order to replicate the conditions of high oxygenation within the habitat.

Figure 8.2 Aquarium with pump

Species of macroinvertebrates from running water may require additional means to provide well aerated moving water. An example of such a family is the blackfly (Simulidae). Their eggs are often laid on grass in the midst of strong water currents. This has to be reproduced in the laboratory (Muirhead-Thompson, 1964). By using an aquarium as a water reservoir and piping the water down into a small container in which vegetation containing Simulian sp. eggs is suspended, the required high levels of oxygenation can be achieved. Water passes through the smaller container and back to the aquarium to ensure a stream is maintained and the excess water can escape. Aerators (such as bubble stones or pumps with small tubes at the end) have also been used to achieve this. They are placed with the stream of bubbles directed towards a removable glass plate positioned in the tank. The effect is to provide a localised area with increased oxygenation, in the region of the vegetation on which the larvae are located. This ensures that the larvae are located on vegetation that is less likely to decompose and also allows maintenance of the culture in good quality water.

On occasion it is valuable to reproduce the conditions of a submerged body in the same location from which the corpse was recovered – to explore the rate of colonisation and succession by macroinvertebrates. To do this a sampling device is required. Vance, VanDyke and Rowley (1995) designed a suspension frame and cradle that would hold a carcass in position under the water and allow its removal at intervals to harvest the colonising species.

8.6.1 Aquatic larvae

These can be reared in an aquarium that combines a source of water in a small bowl, or tank for the insects to swim in (declorinated and preferably the water from which the location from which corpse was recovered) surrounded by damp loam soil around 3 cm deep. Luff (in Cooter and Barclay, 2006) considers that the soil should be banked up at one end of the tank to make a vertical wall made of soil as a pupation site. A means of escape from the water should be included, which can be pebbles, a stick, or green plants. Daphnia is valuable as food for young larvae; worms, including meal worms, can also be used. The lifecycles of aquatic species vary but can be of long duration – approaching several years. This should be borne in mind when considering this approach in investigating evidence form the crime scene in relation to the timing of the court case.

9

Calculating the post mortem interval

After identifying the specimens from the body, the next stage is to link this information to the temperature at the crime scene in order to determine how long it has taken for the larvae to grow to this stage.

Temperature data, covering the period since the person was last seen alive, are obtained from the local meteorological station. These data are 'corrected', using a **correction factor** calculated using the meteorological office data and corresponding half hourly temperature readings, which have been recorded at the crime scene for three to five days after the body was discovered. These corrected data provide an estimate of the temperatures at the crime scene before the corpse was found. From this information you can determine the length of time the flies took to grow from an

Box 9.1 Additional comments on PMI

Insects are cold blooded (poikilothermic) and cannot control their body temperatures, so they use the environment as a source of warmth. Insects use a proportion of the environmental energy (thermal units) to grow and develop. The overall energy budget to achieve life stages can be calculated. Its calculation is a common feature of integrated pest management predictions, as well as for crop production. The thermal units are called degree days (°D) and can be added together to reflect periods of development. In this case they are called accumulated degree days. If the period is shorter and the length of time being discussed is in hours then the thermal values will be as accumulated degree hours (°H).

The minimum temperature for growth (basal temp) will vary with each species. The maximum temperature before growth ceases and death results is in the region of 52.7 °C (126.9 °F), although growth in many species ceases at a lower temperature. The temperature during each 24 hour period may vary but the area under a curve between the upper and lower thresholds of growth represents a predictable block of time – accumulated degree days (or accumulated degree hours).

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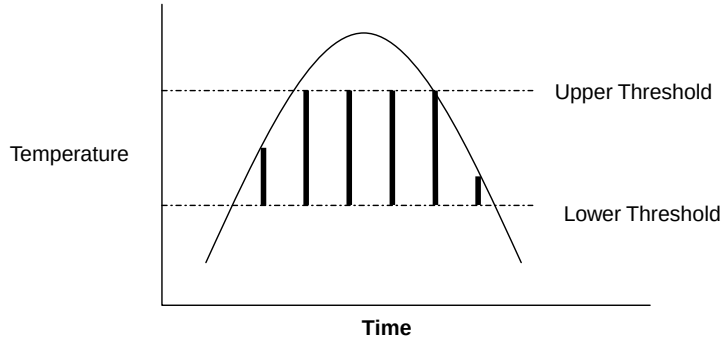

Figure 9.1 Insect growth in relation to upper and lower threshold temperatures

egg to the developmental stage recovered from the body. By implication, this is the best estimate of the post mortem interval (PMI) that is available.

Such estimations of time since death are based on the speed of insect growth. Insects are 'cold blooded', so their growth is influenced by temperature. Below a temperature threshold, development stops; above a specific temperature threshold, the rate of growth also slows down. Between these two points, however, the rate of growth of the juvenile insect is considered to have a linear relationship with temperature (Figure 9.1).

The maximum temperature threshold for different species of insect varies. Wigglesworth (1967), for example, suggested the maximum temperature for growth and development for *Calliphora* sp. larvae was 39 °C, whereas for *Phormia* species it was 45 °C. Upper threshold temperatures are rarely experienced when investigating most crime scenes, so this factor is only infrequently important. However, if temperatures do remain at, or near, the maximum for a long period of time, this will affect the accuracy of the PMI estimate, as the growth of the insect will be slower than expected. Equally, at particularly low temperatures, development may not be possible at all.

We call the temperature threshold below which growth and development will not take place the **base temperature**. This will vary from species to species and can vary with geographic location. For example, Davies and Ratcliffe (1994) demonstrate a threshold of 3.5 °C for *Calliphora vicina* in the north of England, whereas Marchenko (2001), working in Russia, records a base temperature of 2 °C for the same species. Donovan *et al*. (2006) explored *Calliphora vicina* growth in London, at temperatures between 4 °C and 30 °C and found the base temperature there to be 1 °C for *Calliphora vicina*. This reinforces the need to ensure that the most appropriate base temperature is chosen with respect to the crime scene, or that this limitation must be acknowledged in the expert's report when it is submitted.

Oliveira-Costa and de Mello-Patiu (2004) point out that calculations that use an inappropriate base (lower threshold) temperature will overestimate the accumulated physiological energy budget (termed **accumulated degree hours**, or **days**) and so the forensic entomologist may give a false post mortem interval. Such species

adaptation has to be taken into consideration at crime scenes and the base temperature for common dipteran species in that locality may have to be predetermined.

9.1 Working out the base temperature

The specific base temperature for a particular species is worked out in the laboratory from the insect's growth rate at set experimental temperatures. The calculation is based on the premise that the cooler the temperature, the more slowly the insect develops. The base temperature is calculated by plotting temperature against 1/total days to develop, i.e. the time between the larvae initially emerging from the egg and the emergence of the adult, using a range of temperatures. If the line of the graph is extended down to the *x*-axis, the point where it meets the *x*-axis (abscissa) can be read off (Figure 9.2).

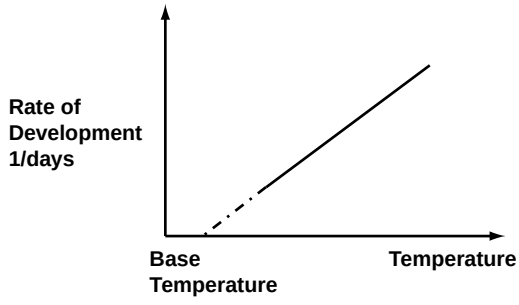

Figure 9.2 Base temperature determination using the linear approximation method

This is the base temperature for that particular species. This graphical method of determining the base temperature is called the **linear approximation estimation** method.

Table 9.1 Lower developmental thresholds (base temperatures)

Species	Base temperature °C
Calliphora vicina	2.0
Calliphora vomitoria	3.0
Protophormia terraenovae	7.8
Lucilia sericata	9.0
Chrysomya albiceps	10.2
Phormia regina	11.4
Muscina stabulans	7.2

Source: Elsevier, for permission to quote details of lower temperature limits for a number of flies, published in Marchenko M.L.K., Medico-legal relevance of cadaver entomofauna for the determination of the time of death. Forensic Science International 120(1-2): 89-109 2001 and a section of Forensic Science International (presented in this book as Table 9.1)

Box 9.2 How to get the temperature correction factor

In the EXCEL work book enter the data for the crime scene temperatures and for the meteorological station temperature data in the first two columns. (This should be up to ten days of data for each site.)

- Highlight the two columns of numbers.
- Click on the scatter graph symbol on the tool bar ribbon.
- Choose the top left scatter diagram and click on it to obtain the graph.
- Place the pointer on one of the data points on the graph and right click.
- From the drop down menu choose 'Linear Trend Line, Display Equation on Chart' and 'Display R-Squared Value on Chart'. This will give you the trend line and the equation that you need.
- Relocate the equation so that it is easy to read in relation to the graph. This equation is the correction factor for the crime scene temperatures before the body is found and should be applied to the data which you have for the meteorological station prior to the discovery of the body.

9.2 Accumulated degree data

As Figure 9.1 shows, there is a relationship between rate of insect growth from the egg stage to adulthood and temperature. This is because growth and development through the various life stages has a cost in terms of a 'physiological development energy budget'. This budget can be expressed in thermal units called **degree days** (°D) or **degree hours** (°H).

The methods for working out degree days, or degree hours, range from using averages through to transformations of the temperature using sine waves, cosine waves and integration calculations. Work on accumulated degree days at the University of California (Wilson and Barnett, 1983) suggests that these methods are interchangeable and, for most calculations, there is little variation in level of accuracy between those that use transformations and those that are based on average figures. Therefore, for the sake of simplicity, the averaging method for a linear estimation of ADD or ADH will be described here, as this can be applied to both indoor and outdoor crime scenes.

The hypothesis on which insect growth in degree days is based is that, between the upper and lower thresholds, the rate of growth of the insect is linear in relation to temperature increase. This 'physiological energy budget' can be represented as the area under a curve, for temperatures above the base temperature, in each 24-hour period. As can be seen from the Figure 9.3, for each hour or day, the budget is

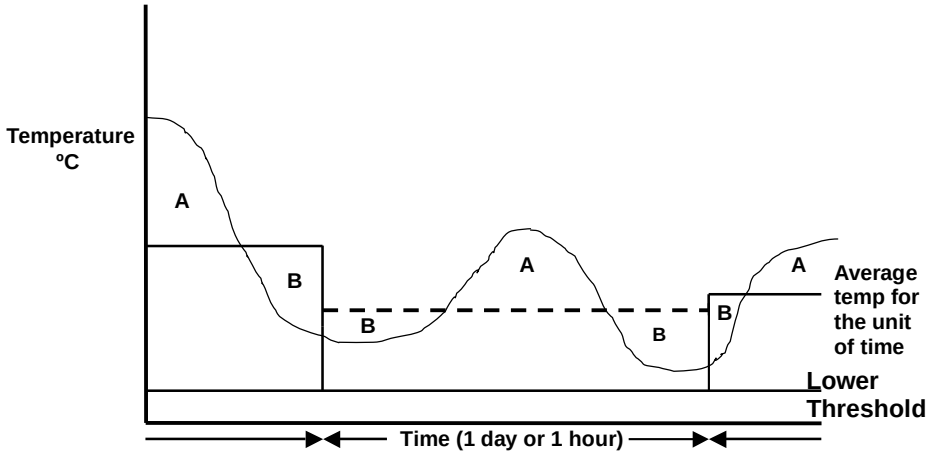

Figure 9.3 Graph to show the justification for using the accumulation averaged temperatures over time. *Source:* The Regents of the University of California for permission to reproduce a modification of Figure 4A from Wilson L.T., and Barnett W.W. Degree days: an aid in crop and pest management California Agriculture (January-February) 4-7 1983 (Presented in this book as Figure 9.3)

represented as a rectangle of time in relation to temperature; any underestimate at one point in the accumulation is compensated for by an over estimate at another point on the graph. Therefore, total accumulated degree hours (or days), reflect the time taken for the insect to develop to the stage recovered from the crime scene.

Based on this relationship, accumulated degree hours (or days) can be determined from a formula. The formulae are:

$$\text{Time}_{(\text{hours})} \times (\text{Temperature} - \text{base temperature}) = \text{ADH}$$

$$\text{Time}_{(\text{days})} \times (\text{Temperature} - \text{base temperature}) = \text{ADD}$$

Information about time to complete individual stages, at set experimental temperatures, comes from the literature. The sources range from Kamal (1958); Vinogradova and Marchenko (1984); Byrd and Butler (1998); Anderson (2000); Greenberg and Kunich (2002) to Lefebvre and Pasquerault (2004). Each of these sources has its value, although Kamal's calculations have been queried. If the data for the duration of the individual life stages is used cumulatively, these problems are overcome. Alternatively, different sources of duration at fixed temperatures for each life stage may be used, which reflect temperatures nearer to the norm for the crime scene i.e. 12.5 °C and 26.7 °C. These experimental temperatures are multiplied by the time, usually in hours, taken to reach the individual life stages. For example, the duration of the egg stage cited in the literature, plus first instar duration, plus second instar and so on, are all added up to provide a total experimental time period to reach a particular stage in the lifecycle. (The number of life stages which have to be taken into consideration is predetermined by which

stage was found on the body.) The base temperature (Table 9.1 provides examples) must be subtracted from the temperature at which the specimens were grown, before multiplying this figure by the time taken to pass from the egg stage to the chosen lifecycle stage.

Once we have identified the species and worked out the experimental energy budget to reach the lifecycle stage recovered from the body, we need to turn to the conditions at the crime scene. The physiological energy budget (accumulated degree hours or days – i.e. ADH and ADD), which was built up over time at the crime scene, has to be worked out for the period between death and discovery of the body. It is based on the individual temperature fluctuations at the crime scene within each 24-hour period, either as an average per hour, or a daily average. Had the temperatures at the crime scene prior to the discovery of the body been known, these thermal units could merely be added up until the point where the summation approximated to the experimental 'physiological energy budget' (ADH or ADD) for that species. However, we rarely have this data for the period before the body is discovered. So crime-scene temperatures have to be estimated from the information that is available. Usually these are the data from the nearest meteorological station. Each daily or hourly energy budget is calculated by multiplying by one the temperature from which the base temperature has been subtracted.

It is also most important, when calculating the ADH or ADD from the scene, that the experimental temperatures used are in the same units as those used for recording the crime scene temperatures. If the Fahrenheit scale was used, then this should also be used for recording the temperature at the crime scene. If the centigrade scale was used, then this should be the measure used at the crime scene. The units in which the temperature is recorded at the local meteorological station usually dictate what is used, but the figures may, on rare occasions, need to be converted.

Ideally the temperature measurements for the time since the victim was last seen should be based on hourly averages and you should calculate an ADH (°H) measure of post mortem interval. However, in practice this may not be possible because the available meteorological data is given only as daily maximum and minimum

Box 9.3 Converting temperatures

In order to convert a temperature which is in Fahrenheit into Celsius (T_c), subtract 32 from the temperature in Fahrenheit, multiply the result by 5 and divide by 9:

$$T_c = (5 \div 9) \times (T_f - 32)$$

In order to convert a temperature in Celsius (Centigrade) to Fahrenheit (T_f), multiply the temperatures in Celsius by 9, divide the answer by 5 and then add 32 to the total:

$$T_f = [(9 \div 5) \times T_c] + 32$$

temperatures. Under such circumstances you should calculate accumulated degree day measurements (ADD or °D) because they reflect the level of accuracy of the PMI estimate that can be ascribed. (Remember, you can convert ADH to ADD by dividing ADH by 24. You cannot accurately convert from ADD to ADH.)

The choice of whether to use ADD or ADH is also dictated by the level of accuracy that is most appropriate. As a rule of thumb, calculations should be in accumulated degree hours (ADH) if victims have been missing for less than a month. If they have been missing for more than a month the data is more appropriately presented as accumulated degree days (ADD). This is because the accumulated level of variation in shorter time periods gives a less accurate measure of the 'physiological energy budget'.

9.3 Calculation of accumulated degree hours (or days) from crime-scene data

9.3.1 How to obtain corrected crime scene temperatures

Corrected data for the crime scene before the body was found by comparing temperatures from the local meteorological station with those from the crime scene, once the body has been discovered. A scatter diagram is plotted of the meteorological temperatures (x-axis) against the crime scene temperatures (y-axis) recorded for three to five days after the body was discovered. A regression equation is calculated. This equation is then used to correct each of the meteorological station readings to generate predicted crime scene temperatures (Figure 9.4). These corrected average

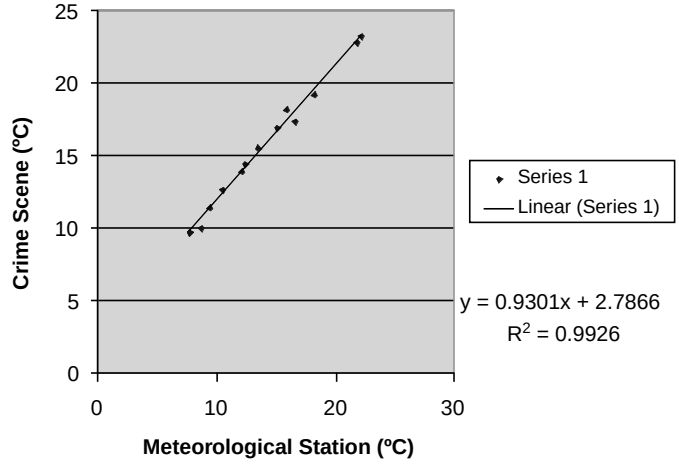

Figure 9.4 Regression of crime scene temperature data against the meteorological station temperature to determine a correction factor for the period after the body was found

Table 9.2 An example of the headings and completed spread sheet to calculate the ADD for *Calliphora vicina* Robineau Desvoidy

Species	Met Data	Corrected Met data	Base Temp*	ADD	∑ADD
Calliphora vicina	13.20	14.68	1.00	13.68	
	14.30	15.67	1.00	14.67	28.35
	14.50	15.85	1.00	14.85	43.20
	13.50	14.95	1.00	13.95	57.15
	13.50	14.95	1.00	13.95	71.10
	14.00	15.40	1.00	14.40	85.50
	15.00	16.30	1.00	15.30	100.80
	15.50	16.75	1.00	15.75	116.55
	15.50	16.75	1.00	15.75	132.30
	16.20	17.38	1.00	16.38	148.68
	16.50	17.65	1.00	16.65	165.33
	16.70	17.83	1.00	16.83	182.16
	16.50	17.65	1.00	16.65	198.81
	17.30	18.37	1.00	17.37	216.18
	17.50	18.55	1.00	17.55	233.73

hourly, or daily, temperature readings are used in the calculation of accumulated degree hours (ADH) or accumulated degree days (ADD).

The temperatures are based upon hourly or daily averages, so the time used is either one hour or one day. Each of the above figures (minus the base temperature) is multiplied by 1 – i.e. one hour or one day. Then each result is added to the former accumulated figure, working backwards from the time of discovery of the body, until the figure for the experimental accumulated degree hours or days is reached. The number of days or hours to reach this figure is then counted up.

An Excel spread sheet can be used to input the initial Meteorological Office data, work out the best estimate of crime scene temperature for the species of insect, subtract the base temperature and obtain a figure for the post mortem interval. This approach means that you calculate the figures quickly and accurately. Table 9.2, together with Box 9.4, provides an example of such a table, complete with figures and instructions on how to make it. The ADD and or ADH must be calculated for each species of fly present on the body. When considered together, these data provide confirmation of the predicted post mortem interval that you have calculated.

9.4 Sources of error

A number of factors need to be taken into account when calculating the post mortem interval. It is important to consider using the temperature of the maggot mass as the temperature for larval development in particular instars. If maggot mass temperature

Box 9.4 The post mortem interval (PMI) calculation

Table 9.3 The nature of the Excel spreadsheet

Column	Information	Source
A	Species being used for the calculation	Fieldwork
B	Meteorological data	Fieldwork or meteorological station
C	Corrected crime scene data using the correction factor and data from B	
D	Base temperature	From the literature, based on geography
E	Degree hours (°H) or Degree days (°D)	C1- base temperature calculation
F	Sum of °H or °D, i.e. accumulated degree hours (ADH) or accumulated degree days (ADD)	In cell F2 the sum of E1 and E2 [=SUM(E1,E2)] and the individual accumulated degree data subsequently i.e. in cell F3 [=SUM(F2,E3)]

Put the following formulae into the initial row of boxes in the Excel workbook (Table 9.3). In the example you would start in row 3. For this reason the formula indicates the number 3 in the initial cells. If you start at a different point amend the formulae accordingly.

- **Column B** – put the meteorological data for the period before the body was found; start with the day of the discovery and work backwards.
- **Column C** – put the formula = (B3* the correction factor accordingly modified so that it is suitably modified for EXCEL. In the example the formula is = (B3*0.9) + 2.8 as the correction factor is $0.9x + 2.8$ (see Figure 9.4).
- **Column D** – put the base temperature in the cells of this column.
- **Column E** – use the formula =C3-D3 to provide the °D or °H for each period of time used. Copy the formula down the columns by dragging the cross on the right-hand side of the cells.
- **Column F** – add (accumulate) the individual degree hours, or days by writing = Sum(E3,E4) in the cell. In the next cell down you write the formula = SUM(F4,E5) so that you add (accumulate) the sum of the next day's (or hour's) degree day (or hour) to the sum of the previous total so you get a value for the accumulated degree days (ADD) or accumulated degree hours (ADH).

was recorded as greater than the ambient temperature, the temperature of the mass should be used in the calculations. This is true where third or potentially late second instar larvae are recovered from the body, as the maggot mass temperature may be the highest temperature experienced by the larvae (Higley and Haskell, 2001). If puparia are recorded, the crime-scene soil temperature at 5, 10, and 20 cm depth should be used to adjust the estimated crime-scene air temperatures, for the period likely to reflect the time the insect was in pupariation.

Where there is no experimental growth data available for the particular species, the larva should be reared until the adults are mature and oviposit. The eggs can then be maintained at a temperature which represents that estimated for the crime scene. The duration from egg stage to the stage of the lifecycle which was recorded at the crime scene, will provide a means of estimating the post mortem interval and also of providing confirmation of any post mortem interval that has been calculated.

Base temperatures must be considered for individual species and the correct base temperature must be used. It may be necessary to use several base temperatures to calculate the post mortem interval, in order to reflect the other information relating to the case, particularly when the person was last seen some distance away, or the body may have been moved. (If the temperature for the period being considered is below that of the base temperature, then a value of zero is included in the calculation for the particular hour or day.)

Concern about the accuracy of temperature predictions has been expressed where the meteorological station temperature recordings for the period before the body was discovered are at variance with the records for the few days or weeks after the body was discovered (Archer, 2004). If the weather conditions differ markedly, the temperatures during the 3–5 days when the temperature is recorded at the crime scene, may not give an accurate reflection of the crime-scene conditions. 'Correcting' these data will not produce a sufficient level of accuracy between the two sites. The best way forward in this instance is to grow the insects through to the life stage recorded from the body, using the crime scene temperature.

Wall (2004) expressed concern about using average daily temperatures when calculating accumulated degree data, rather than taking account of the temperature fluctuations. He pointed out that in his experiments in 2003, using average (mean) temperatures gave considerably longer estimates for insect development (ADD) than when temperature estimates were based upon maxima and minima to determine ADD.

9.5 Use of larval growth in length to determine post mortem interval (isomegalen diagrams and isomorphen diagrams)

Where the corpse has been discovered indoors, or in a controlled environment where the temperature is not fluctuating, the relationship between temperature and growth can be used in another way. Under such conditions the length of the larva, when killed

Box 9.5 Calculation of the ADD for a corpse found in an urban location

This data is based on a crime scene where a dead body was found and from which early third instar larvae were collected and determined from lifecycle experiments to be *Calliphora vicina*. Table 9.2 illustrates the approach using an Excel spread sheet to calculate accumulated degree days (ADD), when only the daily average temperatures are available.

$$ADD = time_{(hours/24)} \times (temperature - base\ temperature)$$

A regression equation must be used to provide a correction factor for the meteorological data prior to the discovery of the body. This is determined from the scatter plot of the temperatures at the crime scene and the meteorological station. In this example the temperatures had been collected by the police and a regression analysis undertaken to relate crime-scene temperatures to those at the meteorological station. The regression equation (and hence correction factor) was calculated to be $0.93\,X + 2.8$. Therefore 2.8 can be added to each meteorological station daily average temperature ($0.93\,X + 2.8$) when it is modified. The equation from the regression analysis will indicate what the correction factor is. (For this example I have chosen one similar to the correction factor figure which was calculated for the case study. It will be different for each location.)

The Base temperature for *Calliphora vicina*, chosen as most applicable is 1 °C (using Donovan *et al.*, 2006). The forensic entomologist should either determine their own for the particular geographic location or choose the most appropriate estimate for the species from the literature.

Using the data from Kamal (1958) because at the time the body was found the temperatures at the Crime scene were in the mid-20s (°C), the following times apply for each of the stages (in hours):

Egg stage	24.0
LI	24.0
L2	20.0

The total experimental time period to reach the start of the third instar is 68 hours.
The ADH is $68.0 \times 25.7 = 1747.6$
The ADD is $1747.6 \div 24 = 72.82$

From Table 9.2, and working down to the nearest value for the ΣADD, within the sixth day, including the day of the discovery of the body, is the most likely minimum estimate for the time of death of the victim.

in the standard way by immersing in boiling water, can be related to the time since the larva hatched. Graphs are produced under controlled conditions in the laboratory for the time since hatch of the species against the average minimum length. The time since hatch can then be read directly off the graph on the basis of the length of the individual larvae collected from the crime scene. These graphs are called **isomegalen diagrams** and have been calculated for *Lucilia sericata, Protophormia (=Phormia) terraenovae* and *Calliphora vicina* (Reiter, 1984; Grassberger and Reiter, 2001 and 2002).

A second type of graph can be used, which is derived where lifecycle stages from hatching of the egg to the time of emergence of the adult (eclosion) have been plotted against time, at specific temperatures. Each line indicates a change in the lifecycle to the next stage. The areas between the lines relate to the identical morphological stages. These are called **isomorphen diagrams** and they have been calculated for the same three species as have the isomegalen graphs. Isomorphen diagrams are useful when postfeeding larvae and/or puparia are collected from the crime scene. From these stages the post mortem interval can be read directly off the graph, provided the temperature has been constant.

9.6 Calculating the post mortem interval using succession

Investigating post-mortem interval for a period of at least three months may mean that there is a large assemblage of flies, beetles and other insects present on the body. These can be used for the calculation of PMI using another method. This method requires that first of all every specimen is identified to family. After that, an attempt is made to relate this 'snap shot' of decomposition fauna to the succession of insects which routinely colonise a corpse at that site. Knowing which insects are present and which are absent locally in what season, helps the entomologist to estimate the post mortem interval.

As a result the dominance of particular populations of species alters and a succession of insects can be found on the body as it decomposes. This sequence is termed **insect succession**.

Rodriguez and Bass (1983) showed that information about succession in relation to decomposition could be used to determine the post mortem interval of human corpses. This method of applying succession to determining the post mortem interval is based upon knowledge of the local fauna. It may also require experimentation to confirm the sequence of colonisation in a particular location.

For example, if the species present included X, Y and Z and these particular species had been shown to be present in the locality between 14 and 16 weeks after invading a fresh corpse, then time since death of that person would be estimated as 14–16 weeks. Such an assemblage of insects would define the 'probable' time since death and would be a guide to post mortem interval. In situations where the body is badly decomposed and the forensic pathologist cannot provide an estimation of time of death,

Table 9.4 Daily temperature readings from a meteorological station for the period between 7 and 20 April

Species	Average daily temperature °C for days from body discovery
Calliphora vomitoria	15.0
	15.0
	14.0
	14.0
	14.0
	15.5
	15.0
	14.0
	13.5
	12.0
	12.5
	13.0
	13.0
	12.5

information from the succession of insects may provide the best estimate that is available, despite the large margin of error that is ascribed when interpreting the data.

Work relating to insect succession on rabbit corpses in Egypt revealed rapid decomposition to the dry stage in 4.5 days, at an average dry season temperature of 28 °C. This changed for temperatures ranging between 13.5 °C and 16.6 °C, when decomposition to the same point took an average of 51.5 days (Tantawi *et al*., 1996). In 'autumn', Tantawi recorded a slower rate of decomposition, resulting in a longer period of decay than in the cooler temperatures of 'winter'. This reduction in the speed of decomposition was thought to be the effect of rainfall delaying larval development. So it is necessary to take weather conditions into account when using succession as a measure of post mortem interval, just as it is when using larval growth rates (accumulated degree hours). In North American studies, the first families of beetles recorded on the body are carrion beetles (Silphidae), rove beetles (Staphylinidae) and clown beetles (Histeridae) (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1996). Amongst the later colonisers of the body are the dermestids.

Insect succession on buried remains is more restricted than on bodies left on the soil surface. The investigation of buried corpses requires a greater investment of resources and time. According to insect succession on buried remains was first studied in Canada in 1995 (VanLaerhoven and Anderson, 1996, 1999). They considered that before this date no scientifically valid work, simulating disposal of a murder victim, had been undertaken. Their work on insect succession on buried, clothed pigs showed that the species range, measured as colonisers and, or trapped in pitfall traps, was less on previously exposed pigs than on those that had been buried immediately. They noted that buried pigs showed a distinct pattern of succession, which contrasted with that from pigs retained on the ground surface. However they

also noted variation in terms of the species colonising the body and times of colonisation between the two sites they chose.

9.6.1 Movement of the corpse

The particular assemblage of insects present on a corpse is also an important indicator of whether the body has been moved. If an unexpected species is present, which is more characteristic of a different habitat, or geographic region, then the body may have been moved. This again depends on knowledge of the local fauna. Organisations such as local wildlife trusts, nature reserves or amateur naturalist societies can be a source of important information about the species that are expected in a particular area. Back copies of their house journals may provide published accounts that have received peer review and can provide a basis for your conclusions that will be acceptable to a court.

9.6.2 Predators feeding on insects infesting a corpse

The longer a body remains undiscovered, the greater the chance that insects such as wasps and ants, will consume those insects that are feeding directly on the body. This destruction of evidence can cause an interpretation problem relating to time since death. Ants, for example, may carry away eggs and the population of the next generation of colonisers may be reduced as a result. They live in colonies as does the other hymenopteran predator the wasps and so the corpse may be visited by more than one individual equally they may not be specific to a particular stage of decomposition. Gomes *et al*. (2007) noted that some wasps such as *Vespula vulgaris* Linnaeus may also cut sections of flesh from the body. This post mortem damage to the copse may be interpreted as significant damage to the body that predated death. They also concluded that, in Brazil, *Agelaia pallipes* (Oliver) was a coloniser at the fresh stage, chewing the skin in the region of the body orifices, and speeding up the arrival of other species of insects, which assisted in body decomposition. The specific nutritional role occupied by both ants and wasps may varies depending on circumstances. They may be predators and feed on the colonising insects consuming the body in some instances, but they may also be necrophages because they feed on decomposition fluids, or remove pieces of flesh on others. Okiwelu, Ikpamii and Umeozor (2008), in their studies in Nigeria, were unable to show that the presence of either ants or wasps on the bodies varied over the stages of decomposition that he investigated. This reinforces the forensic role of these insects in medico-legal cases, solely as agents that may be responsible for the removal of some of the insects present and as potential agents of post morten damage to the corpse.

Equally beetles such as staphylinids and carabids may feed on the adults and larvae that are present on the body. Sometimes feeding takes place at night so you will be less aware of their presence. Some will feed on the younger life stages or attack adults during daylight. In either case there will be an alteration in the sequence of the

succession of insects and some species that would be expected to be present may not appear. This information about predation can be important when interpreting the data if the individual has been dead for a period greater than a couple of days.

Mites are considered a significant group that are of value at crime scene and in cases of urban or stored product forensic entomology in the same way as are ants and wasps. Mites are a subclass of the Arachnida. As is characteristic of the Arachnida, their body is divided into two – a cephalothorax and an abdomen. Mites are wingless and have four pairs of legs.

Mites have a role to play both in interpreting the environmental conditions in which the body is found and also the time since death. Turner (2009) points out that mites are seasonal creatures and he therefore terms their presence a 'date stamp' for time since death. Many mites are carried on humans, clothing, and insects and can be relocated to other sites, which also adds to their value as a seasonality indicator. Indeed some mites are carried specifically by particular species of insects. This transport of one organism by another is called **phoresy** (Figure 9.5).

Figure 9.5 An insect transporting mites. *Source:* Reproduced by permission of Mr. Ian Ward

The European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, is well known as a cause of ill health and allergy and hence can be of relevance to urban forensic entomology. The humidity required by house mites appears to be above 60% RH. Below this level the mites die out. They are therefore an indicator of the long-term conditions in which the body was found. They may also be an indicator of altitude. If dust mites are found on a body it is unlikely that the body will have been transported down from high altitudes where the relative humidity is low (Spieksma, Zuidema and Leupen, 1971).

The lifecycle of the house mite includes the following stages: egg, active larva, resting larva (which can also be known as a pharate tritonymph), active tritonymph, a pharate adult stage and an active adult. The lifecycle lasts between 19 and 30 days depending upon temperature and humidity levels. Mites are also indicators of particular habitats and locations as recorded by Prichard et *al*. (1986) in linking a suspect to a crime scene in Ventura County, USA. The mites were a pre-adult stage of the Trombiculidae; a stage known as Chiggers. These creatures locate a suitable host by responding to carbon dioxide in exhaled air and once on the body can inflict bites (these skin bites were the link between the suspect and the crime scene because the police officers were also bitten). They are unique amongst mites in that the larval stage is an ectoparasite on vertebrates. Braig and Perotti (2009) consider that the term 'micropredator' suits the relationship of such mites better.

Humans can also carry mites in their hair follicles. The species concerned are Demodex *folliculorum hominis* Henle and *Demodex brevis* Akbulutova, which are located in the sebaceous glands associated with hair follicles. Desch (2009) notes that live specimens have been found on corpses dead for as long as 14 days. Özdemir *et al*. (2003), in their assessment of over 100 post mortems, found Demodex only in 10% of the cases and noted that the longest period of time since death was 55 hours. They considered that populations of hair-follicle mites increased with age and were more prevalent on those with fair complexions. However they could not find a relationship between length of postmortem interval and presence on the body.

Mites can be of value in PMI determination as demonstrated by Perotti's (2009) reassessment of the views of Mégnin on the death of a newborn baby in Paris in 1878. She considered that the species of mite that had colonised the body soon after death was *Tyrophagus longior* Gervais (as had Mégnin but by its earlier name), a soil mite that required eight months to reach the population size found on the mummified remains of the newborn baby.

Braig and Perotti (2009) point out that 75 species from 20 families of mites are associated with human cadavers at all stages of decomposition on, within and underneath the body. They realigned the mite species Mégnin identified with his insect succession relative to stages of decomposition. Significantly they note that the odours related to butyric fermentation and advanced decomposition are strongly attractive to mites. This is a corpse decomposition stage in which they can become more visible to the naked eye.

9.7 The effects of hymenopteran parasitoids on post mortem interval determination

Hymenoptera can influence the post mortem interval indirectly as some are parasitoids of carrion-infesting flies. Parasitoids pass through a large period of their lifecycle using only a single host – in this instance an available fly species. Voss, Spafford and Dadour (2009) explored the value of such wasp species as a means of indirect post mortem interval determination and concluded that *Tachinaephagus zealandicus* Ashmead and *Nasonia vitripennis* Walker were the two most valuable species of general relevance to forensic entomology. *Nasonia vitripennis* parasitises pupa within the fly puparia (King and Rafai, 1970). The fly species it attacks include *Calliphora* sp., and *Hydrotaea rostrata* Robineau Desvoidy. Optimally Nasonia develops in a warm Mediterranean-type climate with temperatures around 31 °C (Voss *et al.,* 2010). The upper threshold temperature was determined as 37.5 ± 0.9 °C and the base temperature to be in the region of 10.4 ± 0.8 °C (Voss *et al.,* 2010). Grassberger and Frank (2004b) suggest that overall *Nasonia vitripennis*, in research in Austria, required 224.3 1.7 degree-days to complete its lifecycle. The use of parasitoids to compensate for the loss of material from the crime scene is helpful but the results should be treated with caution when presenting the data to a court.

9.8 Review technique: interpretation of data from a crime scene case study

9.8.1 Introductory background for the assignment on a fictitious murder

The body of a young girl was found on the 20 April at 11 a.m. in the Pleasure Gardens, in a small seaside resort called Corton-on-Sea. There were signs of a struggle, but no cuts or knife wounds were noted on the body by the pathologist who attended the scene. Neighbours had last seen the girl during the previous week. Larvae were recovered from beneath the eyelids and in the nasal cavities of the corpse. At each location that had a maggot infestation (e.g. eyes, nasal cavities, etc.), at least 60 larvae were collected, of which 30 were preserved and 30 were reared in the laboratory to their adult life stage.

Daily meteorological station temperature readings for 20–30 April were obtained from an inland weather station. Temperatures for the same time period were recorded at the crime scene by the police, using a portable weather station. A regression equation for the relationship (Figure 9.4) between crime scene temperature and meteorological station temperature, was calculated to be $0.9x + 2.8$. This will be

used as the correction factor for meteorological station data, working backwards from date the body was discovered.

The species from beneath the eyelids and in the nose were later identified as second-instar larvae of *Calliphora vomitoria* Linnaeus. The entomologist grew the second instar larvae to the adult stage in the laboratory in order to confirm this identification. A base temperature for *Calliphora vomitoria* of 3 °C was chosen.

9.8.2 Instructions

Interpret the data in Table 9.4 to provide an estimate of time since death of the victim. Use data from Greenberg and Kunich (2002) for the length of the *Calliphora vomitoria* lifecycle.

9.9 Further reading

For further reading on the calculation of the post mortem interval please see Richards and Villet (2008), Tarone and Foran (2008), Michaud and Moreau (2009) and Gosselin *et al.* (2010).

10

Ecology of forensically important flies

Flies that are attracted to a corpse to lay eggs are influenced by their environment. This information is of vital importance when interpreting a crime scene and estimating the length of time that a body has been dead. In fact it is one of the deciding factors determining whether forensic entomologists should consider the calculation as one to determine time since death (PMI) or whether they are calculating the period of insect activity (PIA). Where there is some concern that the body has been stored just after death and insects have been excluded from gaining contact then the best estimate that can be provided is an estimate of the period of insect activity when the body was accessible. Other means would be necessary to determine the length of time between death and the insects gaining access to the body.

To find a body, flies must initially be able to interpret volatiles in the air and then respond to them by following the odour plume to the decomposing corpse. The time taken for a body to release such volatiles and for the insect to register the volatiles and travel to the corpse, combined with the decision about whether or not to oviposit, determines the point at which the time-since-death calculation (PMI) starts. This period is currently indeterminate and depends upon both the habitat in which the body is located and environmental conditions.

Some environmental factors are easier to investigate and quantify, particularly if they relate to meteorological features such as rain or wind, which can limit the opportunities for flies to locate a body and to oviposit on it. Therefore elements of uncertainty exist in each individual case in which forensic entomology is used. Hence a minimum time since death determination is the most appropriate calculation to make.

On a body, female insects choose to lay eggs in places that provide sufficient food for the next generation, and also protection, moisture and a consistent microclimate in which the larva can develop. As it decomposes, the human corpse can support colonies of a number of different fly species but their distribution on the corpse is dictated by its condition and the presence of larvae of any other species (Ives, 1991). Work by Archer and Elgar (2003) showed that, after the corpse had been exposed outdoors for 24 hours, the preferred colonisation sites on a carcass changed from the orifices to beneath skin folds. Such locations included between legs or under the ear

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pinnae. They also found that, over time, larvae of both the Calliphoridae and Sarcophagidae altered their distribution on the body and concluded that migration to more favoured sites was in response to food depletion, although competition may also be involved.

The nature of the carrion insect assemblages will vary depending upon whether the body is in sun or shade and with the seasons. The number of databases of species in relation to decomposition state is low. Michaud *et al*. (2010), in comparing the carrion fauna of bodies laid on the soil surface in **Arcadian forest** and agricultural land, provided only the second database of potential corpse colonisers for North America. Their work confirms that time since death based upon succession or carrion insect assemblages is geographically and seasonally sensitive.

10.1 Ecological relationships of some forensically relevant families

Blowflies are among the first to colonise a body. In the UK, possibly the most common species initially present on the body, are *Calliphora vicina* and *Calliphora vomitoria*. One of the first clues to their colonisation is the presence of eggs on the corpse. Campan *et al*. (1994) used a lexical software package to analyse verbal descriptions of the sexual behaviour of *Calliphora vomitoria* in order for mating to be successful and oviposition to be possible. Their work revealed the need for correct orientation of the body of the fly and the mode of presentation of its wings in order to cue mating – hence the need for fly cages of suitable dimensions as discussed in Chapter 8.

Mating behaviour most frequently leads to flies laying eggs on a body during daylight, although some researchers have also found that oviposition in some species can occur at night. Wyss, Chaubert and Cherix (2003) recorded flies laying eggs up until 10 p.m., unless it was raining. Greenberg (1990) also recorded egg laying at night on bodies at ground level. Singh and Bharti (2001) used bait placed 1.85 m (6 feet) above the ground, on top of a pole, to investigate night oviposition and to counter the argument that the flies crawl to the corpse to lay eggs during darkness. They used suitably sticky tape wound round the pole, to prevent flies crawling rather than flying to the bait. In both March and September, with temperatures between 16 °C and 27 °C, very low light levels (0.6–0.7 lux) and relative humidities of 75–85% RH, the calliphorids, *Calliphora vicina*, *Chrysomya megacephala* and *Chrysoma rufifacies* laid eggs at night. These results supported the observations that *Calliphora vicina*, *Phormia regina* and *Lucilia (Phaenicia) sericata* oviposited at night at locations lit by street lights, which had been previously published by Greenberg. However, he commented that few eggs are laid at night. When investigating a death, therefore, the possibility of oviposition on a body during darkness should not be excluded.

Similarly, the effect of weather conditions should also be taken into consideration. Greenberg (1990) stated that calliphorids do not fly in the rain. Digby (1958) examined the effects of wind speed on the ability of *Calliphora vicina*

(then known as *Calliphora erythrocephala*) to fly. He recorded an optimal wind speed for flight of 0.7 ms^{-1} and suggested that speeds greater than this inhibited the ability of Calliphora *vicina* to fly. Temperatures above 30 °C and below 12 °C are also known to inhibit blowfly activities. Niederegger, Pastuschek and Mall (2010) point out that, in Germany, development times of their cultures under varying developmental conditions, differed from those in other geographical locations. They achieved satisfactory pupation and development at 13 °C in contrast to the work of others, which consider 16 °C as a limiting temperature (Reiter, 1984). This potential for variation should be taken into consideration when interpreting the effects of conditions at a crime scene both in terms of time of oviposition and development times.

Blowfly daily activity patterns are influenced by seasonality and changes with geographic location. Hedström and Nuorteva (1971) considered most calliphorids to have a maximum daily flight activity after midday, or to show bimodal flight activity, although in the subarctic regions of Finland, peak flight activity was around noon. In central Asia *Calliphora vicina* shows a single (unimodal) peak of daily activity in the cooler months, when the flies are most active around midday. In contrast, in the warmer months in central Asia, this species is active at two periods in the day, with least activity during the hottest part of the day (Erzinçlioğlu, 1996). Changes in peaks of seasonal activity will also be related to seasonal peaks in fly populations. Johnson and Esser (2000) suggest that, in the tropics, blowfly population peaks are synchronised with the early and late stages of the rainy season, when relative humidities and temperatures are high but rainfall is not at its maximum. Thus the influence of the seasons may affect the interpretation of when the eggs were laid on a corpse.

Work by Tiwari, Mohan and Joshi (1995) examined the role of **heat shock proteins** (hsps) in protecting flies from dramatic variations in temperature and other causes of metabolic stress. They showed that these proteins were present in a number of calliphorid and sarcophagid species but confined their experimentation to *Lucilia cuprina* (Wiedemann). They showed that there was a protective effect of a 'pre-conditioning temperature' which they considered to be 37 °C, enhanced both adult and larval survival at higher temperatures. A temperature increase of 0.1 °C min^{-1} allowed an increased thermotolerance, which could be achieved by the sort of behaviour changes observed by Johnson and Esser.

Blowflies most frequently overwinter in soil, as third instar larvae. *Lucilia* sp. has a maternally induced diapause in the third instar. This differs in *Calliphora vicina,* in which populations only undergo diapause if they are northern. This is potentially a response to differences in day length and environmental conditions such as lower average winter temperatures compared with southern populations. Both *Calliphora vicina* and *Calliphora vomitoria* tolerate a certain degree of supercooling; *Calliphora vicina* has a lower freezing threshold; the egg stage is particularly cold resistant (Block, Erzinçlioğlu and Worland, 1990). Pohjoismäki *et al.* (2010) has shown that *Calliphora vicina* can be active until November in Finland and it has been seen to overwinter as an adult. So, survival strategies of particular species and the

location of the corpse should be considered when interpreting the post mortem interval from species that are present on a body early in the year.

The presence of calliphorid flies and their egg-laying activities in the UK, in winter, has been the topic of a study undertaken in London during mid-December (Brandt, 2004). Egg masses were found on pig carcasses in both indoor and outdoor locations in central London, at ambient indoor temperatures of 10 °C to 16 °C and an ambient outdoor temperature range of −1 °C to 14 °C. The eggs on the indoor pig hatched into larvae, whilst those on the outdoor pig remained in the egg stage.

Climatic change is altering the distribution of Calliphoridae and is responsible for changes in the range of some species. This is particularly true of *Phormia regina,* a common species at crime scenes in the USA. It is attracted to human faeces and animal dung (Coffey, 1966) but is also found on human remains. Byrd and Allen (2001) showed that it is the dominant species in forensic contexts in the summer months in the northern USA, whilst being the dominant species in winter months (October to March) in southern USA. *Phormia regina* activity is inhibited by temperatures as low as 12.5 °C, according to research undertaken by Haskell (cited in Byrd and Allen, 2001).

Huijbregts (2004) confirmed the presence of *Phormia regina* in the Netherlands, Fennoscandia and the UK, noting that in the twentieth century it has only been recorded on four occasions. Indeed Erzinçlioĝlu (1996) considered *Phormia regina* to be introduced into the UK from the USA. However, in 2001, Huijbregts (2004) recorded *Phormia regina* in the Netherlands on four separate occasions in the same year. He suggests that this species is extending its range. Catts and Haskell (1990) and Haskell and Williams (2008) comment that this fly prefers shade rather than brightly lit, open habitats. This preference for shade may account for the prevalence of records of *Phormia regina* in urban environments in the Netherlands, where shade is readily available in and around buildings.

Development times for *Phormia regina* increase under a cyclical temperature regime, compared to development times at a constant temperature (Byrd and Allen, 2001). However, using constant temperatures, they were able to replicate the results for time to peak egg hatching recorded by Kamal (1958). At 25 °C, peak emergence was found at 19 h whilst at 30 °C it was reduced to 15.5 h (Byrd and Allen, 2001). Variation in the length of the lifecycle occurred at higher temperatures (35 – 45 °C) when adults failed to emerge. They also showed variation in length of lifecycle when cultures were kept at a constant temperature of 40 °C or at 10 °C. (Interestingly *Phormia regina* activity is thought to be inhibited when monthly temperatures are below an average of $10\,^{0}$C – Deonier, 1942). Byrd and Allen felt that developmental durations at experimental temperatures of 25 °C and below were in accord with the rest of the scientific literature for this species. But they urged caution in using published data to determine the 'experimental value for Accumulated Degree Hours or Days' for the species, where environmental temperatures at the crime scene were above 25 °C. However, Nabity, Higley and Heng-Moss (2007) point out that light intensity and day length influence *Phormia regina* growth, so these may have an effect in addition to temperature.

A second species that is increasing its numbers in Europe, as indicated by Dutch crime scene records, is the holarctic blowfly, *Protophormia terraenovae*. Whilst this species is not often recorded from rural environments, it has become more common in large cities along the Netherlands coast. In Britain it is recorded from the Pennines and Erzinçlioğlu (2000) states that it favours upland and northern regions.

Nuorteva (1963) suggests that this species does not compete well with other species and so only flourishes where competition is low, for example as an Arctic species. She also suggests that it is usually the first blowfly to emerge and that if a larva of such a species is found on a body it indicates that the death occurred in the spring.

Current research indicates that refrigeration of *Protophormia terraenovae* larvae affects their development. When reared at 24 °C and then refrigerated at 4 °C, development is dependent upon the period of refrigeration. Refrigeration of the first instar larvae decreases time to adult emergence. With increasing periods of refrigeration, the second instar and pupa show an increase in time to adult emergence. The overall effect of refrigeration gives a potential error of more than six hours in the PMI calculation (Myskowiak and Doums, 2002).

Changes in geographic distribution are also occurring for Chrysomya species, which are usually considered to be tropical and subtropical species. They are found in Africa, Asia and southern Europe and were introduced onto the American continent. However, its range seems to be moving northwards. In 2000, *Chrysomya* species were recorded from northern France (Erzinçlioğlu, 2000) and in 2002, *Chrysomya albiceps* was recorded from Austria in central Europe (Grassberger, Freidrich and Reiter, 2003). The level of interaction of this species with other species, rather than the influence of higher temperatures alone, is considered by Grassberger *et al*. to be the important factor in determining its change in distribution.

Competition with *Chrysomya albiceps* is now thought to be causing a reduction in the prevalence of the secondary screwworm fly, *Cochliomyia macellaria* – a species that is native to the American continent (Faria Del Bianco *et al*., 1999). Second and third instar larvae of *Chrysomya albiceps* are predators on other larvae when the occasion presents itself (i.e. **facultative** predation). The first instar of *Chrysomya albiceps* larvae feed on tissue fluid or decomposition liquor and so are necrophages.

According to De Andrade *et al*. (2002), postfeeding *Chrysomya albiceps* larvae will attack *Chrysomya macellaria* postfeeding *larvae* if they both leave the same corpse at the same time. *Chrysomya albiceps* cannot complete its development below 15 °C (unless it is present in sufficient numbers that it can form a maggot mass, which in itself raises the local temperature of the microclimate for the larvae). This is thought to be a means of surviving competition in this less dominant species. The time between oviposition and the adult stage of *Chrysomya albiceps* is recorded as19.2 $\pm$ 0.92 days at 20 °C and 8.3 days $\pm$ 0.5 at 35 °C (Grassberger, Freidrich and Reiter, 2003).

In afro-tropical regions, oriental regions from India to China, central South America, and southern Europe, *Chrysomya albiceps* is commonly the initial coloniser of a corpse (Baumgartner and Greenberg, 1984; Hall and Smith, 1993).

It is also recorded as one of the two most frequently encountered species in forensic cases in South Africa (Mostovski and Mansell, 2004), where it is recognised as a spring and summer species. Smith (1986) considered that this species fulfils the initial colonising role undertaken by *Calliphora* and *Lucilia* in the temperate zone.

A species recorded in Britain, as colonising a body in the second wave of insects, is *Cynomya mortuorum* Linnaeus, a blue-green blowfly (see colour section). In Finland the lifecycle from egg to adult of wild specimens, takes an average of 26.2 days, at a mean temperature of 15 °C (Nuorteva, 1977). Staerkeby (2001) recovered *Cynomya mortuorum* from a suicide victim in south-eastern Norway and concluded that its egg-laying seasonality in Norway was similar to that in Finland. In the UK this species is more common in the north of England and Scotland than in the south of England, where it is considered to be scarce (Colyer and Hammond, 1951; Smith, 1986). So the species may be considered to be present in small numbers on a corpse and also to be a geographic indicator for the north of England and Scotland.

10.2 Specific family features

10.2.1 Greenbottles – Lucilia spp.

In Britain there are seven species of greenbottle. These are *Lucilia (Phaenicia) sericata, Lucilia caesar, Lucilia illustris* Meigen, *Lucilia richardsi*, *Lucilia silvarum* Meigen, *Lucilia ampullacea* Villeneuve, and *Lucilia bufonivora* Moniez. Smith (1986) records only four of them – *Lucilia ampullacea, Lucilia illustris, Lucilia caesar*, and *Lucilia sericata* – from dead bodies in the UK. They vary in their choice of habitat. *Lucilia* species are amongst the first wave of blowflies to colonise a body. *Lucilia sericata* is, however, reported in experimental trials by Fisher, Wall and Ashworth (1998), on the basis of poor responses to fresh liver compared to aged liver, to be a species that does not act exclusively as a pioneer species on a fresh corpse.

In southern Britain, *Lucilia sericata* has been estimated to produce three or four generations a year (Wall *et al*., 1993). It is very infrequently found indoors, or in woodlands and hedgerows (Smith and Wall, 1997) and in Britain, is thought to be a species indicative of bright sunlight (Colyer and Hammond, 1951). In the USA, this preference for sunlight enables *Lucilia (*syn. *Phaenicia) sericata* to colonise bodies during the hottest part of the summer. Its minimum threshold is considered to be 9 °C and at temperatures of 13 °C *Lucilia sericata* lifecycle did not progress beyond the larval stage (Niederegger, Pastuschek and Mall, 2010).

Lucilia illustris is also a fly, recognised in the USA as being associated with bodies found in open, brightly lit habitats (Catts and Haskell, 1990). In contrast, in the UK, *Lucilia illustris* is considered to be an open woodland and meadow species. In Canada the species is most obvious in summer and autumn (Michaud and Morreau, 2009). The work of Niederegger, Pastuschek and Mall (2010) goes some way to explaining the reason this species is successful during the seasons in that in

fluctuating temperatures between 5 °C to 29 °C the larvae can take advantage of the high temperatures and tolerate the lower, enabling good growth, which was not seen at a fixed temperature of 13 °C.

In contrast, *Lucilia caesar* is considered to be a species associated with forests and tolerant of shade. The latter species occurs further to the north of Britain than *Lucilia illustris* (Smith, 1986). The effect of environmental factors on fly activity was considered by von Aesch *et al*. (2003). They showed that spring radiation and temperature were significant at the 5% level or more for determining the presence on the wing of *Lucilia caesar, Lucilia sericata*, *Lucilia silvarum* and *Cynomia mortuorum*. However, solar radiation rather than temperature, determined the flight activity of *Calliphora vicina*.

The Finnish community of corpse-inhabiting species in open habitats comprises five species of *Lucilia*. The species are *Lucilia sericata*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia silvarum,* and *Lucilia richardsi*. Of these five, Prinkkilä and Hanski (1995) report *Lucilia illustris* to be the most abundant, followed by *Lucilia silvarum*. Hanski (1987) also noted that *Lucilia illustris* was the earlier of the two species to emerge in early summer. Prinkkilä and Hanski considered that *Lucilia caesar*; *Lucilia sericata* and *Lucilia richardsi* are rare, citing their larval competitive abilities as the reason. This poor competition results in varying levels of fly densities in the field.

In general, field populations of greenbottles appear to be small in numbers. Gilmour, Waterhouse and McIntyre (1946) suggest that *Lucilia cuprina* Weidemann, a species found on carrion in Australia and as an agent of myiasis, has a population density of between 0.17 and 14 flies per hectare. In a northern British population MacLeod and Donnelly (1960) estimated that the population of *Lucilia sericata* was 2.5 flies per hectare, which was considerably smaller than the Australian populations. However, Smith and Wall (1998) showed that the density of *Lucilia sericata* varied according to the season, rising from 0 per hectare in England in early June of 1994, when the study took place, to 6.3 per hectare in the middle of August. Clearly populations will differ with season and geographic location, which may account for their absence from a corpse on occasions where they might be expected.

The distance over which greenbottles disperse also varies with species. In experimental trials in Australia *Lucilia cuprina* has been recorded between 0.7 and 3.5 km from the point of release (Gilmour, Waterhouse and McIntryre, 1946). Work on the dispersal of individual *Lucilia sericata* suggests that it might be expected to disperse a much smaller average distance of up to 800 m per lifetime (Smith and Wall, 1998). However, the fecundity of the flies and their physiological state can also influence their dispersal. Smith and Wall point out that movement in response to carrion odour is a migration trigger in both gravid females and those females that lack a supply of protein in their diet, whereas females that have just laid eggs, or who are newly merged and are undergoing egg development (**vitellogenesis**), show less tendency to respond to the odours emitted from a corpse. The researchers suggest that availability of both sugars and protein sources may account for the differences in migration rates and hypothesise, therefore, that a population of *Lucilia sericata* could spread at a rate of 31–42 km a year.

Once the eggs were laid and the larvae emerged, Rankin and Bates (2004) demonstrated that, in cohorts of *Lucilia sericata* above a population size of 400 larvae, heat production was dependent on which instar was present and not on the size of maggot mass or cohort. They concluded that at ambient temperatures above 15 °C, the duration of the lifecycle (and hence post mortem interval) could be based on the ambient temperature of the environment for this species. They also showed that at temperatures between 22 °C and 35 °C, larval growth rates in the wild did not significantly differ from those in laboratory experiments. This point is important when attempting to estimate time since death on the basis of larval development in controlled environments for this species.

10.2.2 Muscid flies – Muscidae

The muscid flies, are attracted to nitrogenous material that leaks from the corpse. Predominantly they are attracted by urine and faecal waste. Whilst few have been found to be of forensic value, several species can be of significance in specific circumstances. Sharanowski, Walker and Anderson (2008), working in Canada, found that species of Musca and Hydrotaea were present during bloat if the corpse was in sunlight but absent from corpses in shade. In summer, *Musca domestica* was present on corpses in bloat and also in early decay whilst Hydrotaea species were absent.

Adult Hydrotaea species were recorded at the dry stage of decomposition. There would therefore, appear to be a variation in corpse attraction that is both seasonal and geographic. The consistent feature seems to be that members of this genus will develop in circumstances where there is high bacterial load causing fermentation. Lobanov (1970, cited in Skidmore, 1985) considers that this genus has two generations a year in the northern temperate regions of the world. Skidmore (1985), citing personal observations in the north of England, considered the species *Hydrotaea dentipes* Fabricius to be present from late March to November or December (depending upon the weather), which made him suggest that at least four generations a year might be expected.

In south-western Australia *Hydrotaea rostrata* Robineau-Desvoidy has been found on a third of the human cadavers, making it a significant species where the body has not been found for several weeks or months (Dadour, Cook and Wirth, 2001). Their lifecycle, in constant light under experimental conditions, has been found to be 21.6 days in summer. Its duration is tripled in the winter months (Dadour, Cook and Wirth, 2001).

Other species of Hydrotaea, formerly known as Ophyra species, have been found in association with corpses. Couri *et al*. (2009) identified Hydrotaea (*Ophyra) carpensis* Weidemann and *Hydrotaea* (*Ophyra) ignava* Harris from the oesophagus of a mummified corpse in a nineteenth-century museum collection in Lisbon, Portugal. They considered that this species invades corpses in the later stages of decomposition after it has been colonised by *Callipora vicina* and Muscina species. The adults were thought to emerge from the soil when the temperature below the soil surface exceeded

10 °C. Hydrotaea (Ophyra) has also been recovered from buried corpses. Skidmore (1985) considers that *Hydrotaea (Ophyra) ignava* has several tranches of adult emergence; in June, August, and October. Although in the north of England and in Scotland there may be only two, with adult populations peaking in autumn.

10.2.3 Soldier Flies – Stratiomyidae

Stratiomyid species have been recovered from corpses. However, they are more likely to be considered nuisance flies and encountered in the context of urban forensic entomology. The black soldier fly, *Hermetia illucens* Linnaeus, is a detritivore and, although found in dung heaps, is also of significance as a forensic indicator. This species has also been found to be an agent of myiasis (Calderón-Arguedas, Barrantes and Solano, 2005) where it is accidentally swallowed. Tomberlin and Sheppard (2002) concluded that the species was capable of mating two days after emergence and that oviposition took place four days after that. At 27 °C, *Hermetia illucens* eggs will hatch after four days. The duration of the larval feeding stage is approximately 14 days before postfeeding migration occurs. The species requires normal daylight in order to lay fertile eggs (Sheppard *et al*., 2002). Most significantly, members of this family will consume the larvae of Muscidae and Calliphoridae and therefore can affect the calculation of time since death.

10.2.4 Lesser Dung Flies – Sphaeroceridae

This family is found on corpses less frequently than the Calliphoridae, with whom they cannot compete well. The family contains species that feed on bacteria and that breed in dung and decomposing material. They are habitat specific. For example, in Germany, Buck (1997) found that *Minilimosina parvul*a Stenhammar was a common vertebrate-infesting species in forests whereas *Telomerina eburnea* Rohácek, was most common in open habitats. Their development time is considered to be at least five weeks. According to Fredeen and Glen (1970), the sphaerocerid *Leptocera caenosa* Rondani will survive exposure over a three month period to temperatures of 7 °C but not those as low as 1.5 °C. This is a species that has been recorded from buried bodies in northern France (Bourel *et al*., 2004). It is found, if present, in the advanced decay stage – although only in small numbers.

10.2.5 Fleshflies – Sarcophagidae

In Britain and elsewhere, this family is found to be present on the body after a few days of decomposition. One of the most significant things about it is that the flies are capable of being **viviparous**. That is, they generally deposit larvae onto the corpse and not eggs, although egg laying in Sarcophagidae has been noted under laboratory conditions, for example in *Sarcophaga (Liosarcophaga) tibialis* and *Sarcophaga*

(Lyopygia) argyrostoma Robineau-Desvoidy (Villet, MacKenzie and Muller, 2006 and Niederegger, Pastuschek and Mall, 2010, respectively).

Sarcophagidae are considered to be unimpeded by rain and to fly despite the weather (Erzinçlioğlu, 2000). Archer and Elgar (2003) consider that females are selected to deposit the larvae (or eggs) in safe nutritious locations. As a result, flesh flies may be the initial colonisers of the body outdoors, if there is a long period of rainy weather. Despite this, many flesh flies prefer sunlight rather than shaded conditions (Smith, 1986) although *Sarcophaga (Subgenus Robineauella) caerulescens* Zetterstedt is considered a shade lover. Singh and Bahrti (2008) point out that, in Asia, sarcophagid species may oviposit in low light conditions, which does not preclude them being able to oviposit during the night if there is sufficient artificial light.

The time taken for *Sarcophaga (Subgenus Robineauella) caerulescens* larvae to emerge as an adult is considered to be between 8–12 days (Pohjoismäki et al., 2010). Kamal (1958) provides an indication of the lifecycle durations of the various stages of the American sarcophagid species *Sarcophaga cooleyi* Parker, *Sarcophaga shermani* Parker and Neobellieria (=*Sarcophaga) bullata* Parker. Sarcophagids are also known to colonise corpses located indoors. One of the most common Sarcophagids, recovered as larvae from indoor crime scenes in the USA, is *Sarcophaga africa (=haemorrhoidalis)* (Fallen) (Byrd and Butler, 1998). At a constant temperature (25 °C) this species showed variation in its emergence time – development time to adult emergence varied by as much as 252 hours (Byrd and Butler, 1998). Therefore this provides a source of variation and limitation in the use of this species for calculating time since death.

A crucial aspect of some species' lifecycle is whether or not the species has a second generation within a season. In the case of *Sarcophaga scoparia* the duration of the lifecycle of the second generation, which tends to occur in August in Finland is longer – 36 days and not around 26 (Hanski, 1987). The potential should be considered when estimating the minimum post mortem interval that the colonising fly is second generation.

10.2.6 Cheese skippers – Piophilidae

Some species of Piophilidae, which Mégnin considered in his succession, are very host specific and confined to the latter stages of decomposition. Such is the case of the species in the genus Thyreophora (Braig and Perotti, 2009). Braig and Perotti point out that the attraction is either bone marrow or the protection provided by the large bones. They note that these species are confined to regions such as the Kashmir region of India and hence to bones of large animals.

Other members of this family are much more familiar as they are food pests found on pork products such as ham and bacon, cheese, biltong, fish, bones and carrion. One such species, *Piophila casei*, may also be found on a corpse during the active decay stage and will colonise a dead body where calliphorid and sarcophagid species are prevented (Mullen and Durden, 2002) (Figure 10.1).

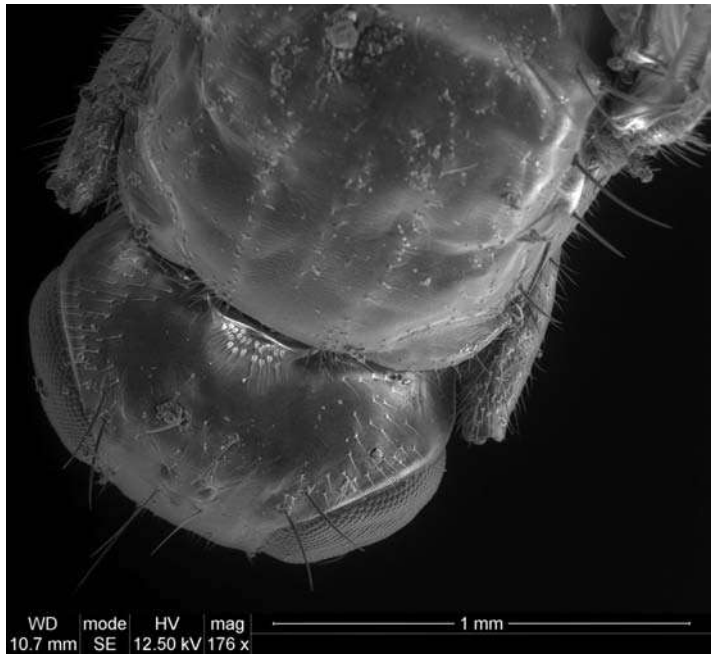

Figure 10.1 Electronmicrograph of the head of an adult Piophilid

Female Piophilids are considered to be monogamous, copulating only once and laying their eggs in batches of three to 100 (Russo *et al*., 2006). The average number of eggs laid in a batch (over 3–4 days) by a female is 140; the maximum batch they recorded totalled 480. At 13 °C the egg stage lasts on average 4–7 days whereas at 18 °C the time is reduced to 2.9 days (cited in Russo *et al*., 2006).

The species can survive at temperatures below freezing. Hegazi *et al.* (1978) considered that the larvae could survive temperatures of −4 °C for more than 10 days. Whilst Sacchi, Gigolo and Cestari (1971) showed that freezing larvae at −21 °C (5 °F) midway through the larval stage and then returning them to suitable conditions did not affect development. The same larval stage was also able to survive temperatures of 50 °C for four hours. Freezing is not, therefore, a means of despatching them from food, nor does refrigeration whilst transporting larvae back to the lab once collected from a body, appear to affect this species.

10.2.7 Scuttle flies – Phoridae

If the corpse is located on the surface of the ground, this family is particularly associated with the later stages of decomposition and advanced decay. A stage in which diptera and coleoptera are both found on the remains of a corpse.

One phorid species is particularly important if buried bodies are being investigated. This is *Conicera tibialis,* the coffin fly. Adult females appear to be able to locate a body using volatiles emanating from the soil. There is evidence that this species can complete several generations below ground, without emerging from the soil, as puparia of *Conicera* sp. have been recorded from exhumed human corpses buried some considerable time earlier (Colyer and Hammond, 1951).

Phorids of the genus Megaselia have been recorded on bodies on the ground surface – they will tend to aggregate their larvae in an attempt to limit competitions with other fly species because they are poor competitors (Kneidel, 1985). Examples of phorid flies that have been found in forensic cases are *Megaselia rufipes* Meigen, *Megaselia scalaris* Loew (a parasitic fly) and *Triphleba hyalinata* Meigen (a cave dweller). Of these, *Megaselia rufipes* is the most commonly encountered species of phorid on corpses left on the soil surface, or from shallow graves in Britain (Disney and Manlove, 2005).

Disney (2005) working on *Megaselia giraudii* Egger and *Megaselia rufipes,* has investigated the lifecycles of these scuttle flies to enable them to be used for post mortem interval determination. He found a large degree of variation in larval growth from the same batch of eggs and also recorded successive waves of larvae migrating from their feeding site.

10.2.8 Scavenger flies – Sepsidae

Sepsid flies are common on bodies at a stage of advanced putrefaction. They are attracted to decaying material and so justify their common name of scavenger flies. The larvae are found in decaying organic matter, particularly when it is liquefying (Oldroyd, 1964), including the decay stages of decomposition of dead bodies. Adult flies have a characteristic wing-waving movement and, according to Oldroyd (1964), will run up and down on any surface or piece of vegetation that is available.

Smith (1986) places them between the caseic decomposition stage and before the ammoniacal fermentation of advanced decay. They are often found on corpses at the same time as Piophilidae and Drosophilidae. Seven sepsid species are specifically associated with carrion or human faeces. Because of this association with dung, its presence on a corpse requires careful consideration. Does it indicate faecal contamination of the body in the process of death or does presence reflect environmental conditions that are unrelated to the presence of the body? This was a point made quite strongly by Smith (1986) and, given the concern to ensure that forensic entomology is a rigorous science, is a major consideration in interpreting the crime scene.

10.2.9 Winter Gnats – Trichoceridae*

Winter gnats (Trichoceridae) have been found to be of value for the determination of time since colonisation of human corpses during the winter months (Broadhead,

*Dr Sharon Erzinçlioğlu for permission to quote the case of Mike Evans and Zoe from Maggots, Murder and Men, harley Books Colchester.

1980; Erzinçlioğlu, 1980, 2000), although, as yet, not many species have been recorded from human corpses. Members of this family are usually found in rotting vegetation, rodent holes (Keilin and Tate, 1940), down mines (Oldroyd, 1964) and in cattle dung – the location in which larvae develop (Laurence, 1956). Such conditions also provide a means of larvae escape from low and freezing temperatures. Although present throughout the year, winter gnats are most obvious in winter and can be seen flying in late afternoon in winter, even when snow is on the ground (Colyer and Hammond, 1951). Laurence (1956) recorded that they can survive freezing for up to two weeks and that they could tolerate conditions where ambient temperature was 7.2 °C.

Members of this family are more apparent and more abundant in spring and autumn than in summer. Male winter gnats swarm – moving in such a way for the swarm to be called 'a dance'. Edwards (1928), recorded by Keilin and Tate (1940) suggests that females remain in sheltered roosting sites, and that any that do enter the swarm of males will immediately undergo copulation.

Nine species of Trichocera are listed in volume 12 of the British 'Checklist of Diptera' (Chandler, 1998), with two more known but not yet identified. The species of winter gnat of value for PMI determination will vary with locality. In the north of England *Trichocera annulata* Meigen may colonise bodies. Erzinçlioğlu found this species on ox heart in an experiment run between 8 December 1979 and 1 March 1980. In *Maggots, Murder and Men* (2000), Erzinçlioğlu describes how he recovered Trichocera larvae from the corpse of a nine-year-old girl at a similar time of year to that of his experiment. The girl, Zoe Evans, was subsequently found to have been murdered by her stepfather. The murder had taken place in January and six weeks later, in February, the decomposing body of the girl was found wedged, head first, into a badger's sett. She had suffocated to death, from a combination of having had her shirt stuffed into her mouth and inhaling blood. The blood resulted from her receiving a violent blow – sufficient to break her nose. The police confirmed their suspicions as to the identity of the murderer when they found some of the stepfather's clothes and underwear, which were covered in blood. The only significant evidence of an entomological nature found on Zoe Evan's body were larvae of Trichoceridae, which confirmed, as everyone knew, that the murder had taken place in winter. In this instance the murder provided more information about the insects than did the insects about the murder, and confirmed that these are a family that could be used to determine time since death in the winter.

10.3 Fly infestation of the living

Some species of fly will not only oviposit on corpses and deposit larvae feed on dead organisms but will also oviposit on the living. Such a circumstance is termed myiasis: causal agents include members of the Oesteridae, Calliphoridae, Sarcophagidae, Phoridae, Fanniidae, Stratiomyidae, Piophilidae and Psychodidae (Derraik, Heath

and Rademaker, 2010). Hall (1995) points out that where calliphorid and sarcophagid species were concerned, the conditions of the host tend to predispose them to infestation as a result of the presence of open cuts, skin necrosis, or bacteria contaminating skin, fur, or wool.

Allowing flies to lay eggs and the larvae to survive on living organisms is not only illegal under animal welfare laws, since undue distress and often death can be caused to animals, including pets, but it also causes economic loss to many livestock farmers. The calliphorid *Lucilia sericata* is a source of sheep fly strike or myiasis in the UK, Australia, and South Island of New Zealand. In North Island *Lucilia cuprina* is the more frequent causal agent of myiasis (Morris, 2005).

No native New Zealand flies are recorded as myiasis causal agents. Introduced species of fly, however, are often the cause. For example two species of introduced Phorid, *Megaselia scalaris* and *Megaselia spiracularis,* have been recorded as a cause of gastrointestinal myiasis (Derraik, Heath and Rademaker, 2010). In Africa and Asia *Chrysomya megacephala* and *Cochliomya macellaria* are frequently encountered in cases of human myiasis. According to Dasgupta and Roy (1969), *Lucilia illustris* will colonise a number of live animal species, including, frogs, toads and fish, initially feeding on the brain. They noted that the rate of development was faster on living tissue than on dead.

In the UK the most common form of human myiasis was, until recently, enteric myiasis. Smith (1986) indicates that larvae of the following fly genera have been

Box 10.1 Myiasis

The invasion of living tissue by insects is also of concern to the forensic entomologist. This invasion is called m**yiasis** and becomes relevant where cases of misuse and abuse are involved.

Myiasis has been defined according to two criteria: the biological requirements of the fly, or where the flies attack humans or animals. James (1947) defined biological myiasis as invasion of tissue or organs of man or animals by dipterous larvae. He acknowledged Patton's (1922) earlier views that presence of eggs, pupae or adults might be included, but considered that the larval stage was the 'active stage' of myiasis.

In medical terms myiasis can be defined according to the location of the fly infestation. For example it can be defined as: wound myiasis; myiasis of the nose, mouth and accessory sinuses; aural myiasis; ocular internal and external myiasis; myiasis of the rectal region and vagina; myiasis of the bladder and urinary passages; furuncular, dermal and subdermal myiasis; creeping dermal or subdermal myiasis or enteric myiasis.

Flies, such as *Lucilia sericata*, *Musca domestica* Linnaeus and *Phormia regina* Meigen, the initial colonisers of the body, have all been implicated in cases of myiasis.

identified as the cause of this disease: *Eristalis*, *Piophila*, *Drosophila, Calliphora* or *Musca*. Where urogenital myiasis has been diagnosed, the species *Fannia canicularis* – the lesser house fly, *Musca domestica* – the house fly and *Sylvicola fenestralis* (Scopoli) have been the culprits. The frequency of colonisation of living flesh by *Lucilia caesar* and *Protophormia terraenovae* is commonly related to latitude. Both of these species become more common as a causal agent of myiasis the further north one travels (Stevens, 2003).

Mechanisms for control of myiasis in animals include use of insecticides as this is a cause of animal welfare cases. However *Lucilia cuprina* and *Lucilia sericata* are becoming resistant to insecticides (Morris, 2005). So there is a drive for research into the volatiles that attract species to a body, in order either to provide bait or a deterrent. This area of research is also of great relevance to forensic entomology.

10.3.1 Piophilidae infestation of the living

Because Piophilids and in particular *Piophila casei*, inhabit food materials such as cheese, they have, in the past, been found to have been consumed accidentally.

Derraik, Heath and Rademaker (2010) record Piophilidae as being responsible for gastrointestinal myiasis, nasal-oral myiasis and cases of urogenital myiasis in humans. These days this is less likely to happen because hygiene laws and packaging techniques mean that the consumer predominantly eats cook-chill or ready meals, which reduce the likelihood of the maggots surviving in the food in order to cause myiasis (Figure 10.2).

Figure 10.2 Piophilid infestation of meat. *Source:* Dr Sharon Erzinçlioğlu for permission to quote the case of Mike Evans and Zoe from Erzinçlioğlu Y.Z., Maggots, Murder and Men. Harley Books: Colchester (2000)

10.3.2 Myiasis and PMI determination

In some developing countries, human myiasis results in the external infestation of skin wounds, eyes, noses, sinuses and ears both accidentally and in the context of wounding. For example, after an assault, a 65-year-old Indian female was diagnosed as having traumatic wound maxillofacial myiasis and 200 maggots of *Musca domestica* were recovered from a wound in the lady's lower lip and from the oral cavity (Babu *et al*., 2010).

If the victim suffered from myiasis prior to death then the oldest maggots on the body may not necessarily reflect the period of time between death and discovery. The time since death would be shorter. Signs of previous insect infestation (myiasis) are indicated where the largest maggots are present in urogenital areas or in wounds. Determination of the existence of larval infestation prior to the death of a victim should be taken into consideration when interpreting the length of time since the body was colonised (period of insect colonisation PIA).

10.4 Flies influencing the crime scene

Because of the nature of their feeding – a process of external digestion – flies will regurgitate and then reabsorb material. In addition they will add faecal material to the crime scene (Figure 10.3). Benecke and Barksdale (2003) describe three cases in which fly artefacts have been added to the crime scene and generated small stains, which could be confused with high velocity impact scatter. In particular, flies are attracted to blood and will regurgitate droplets of this in different locations. The resultant droplets could not be interpreted by reconstructing angles of impact.

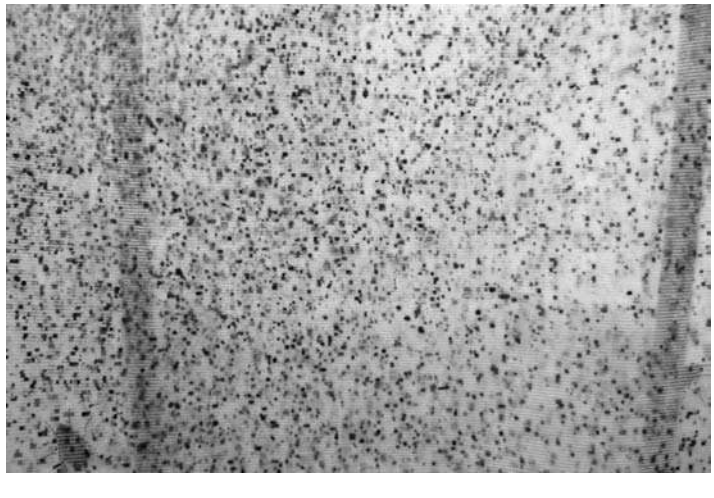

Figure 10.3 Fly faeces and vomit contaminate surfaces

Calliphora vicina and *Lucilia sericata* both feed in a manner that will produce such artefacts. Both the regurgitated blood droplets and faecal material can look like blood spatter (Striman *et al*., 2011). Such feeding can take place both during the day and also in darkness, so the crime scene can be altered if flies are present between discovery of the body and the subsequent visitation of technicians with particular skills in blood-spatter analysis. The droplets generated tend to lack tails. Therefore a room in which there are droplets with tails and small droplets may need to be investigated carefully. Many presumptive tests for blood will fail to distinguish between fly-generated artefacts and blood spatter droplets. Fujikawa *et al*. (2011), however, were able to show that faecal material from *Lucilia sericata* would fluoresce at a wave length of 465 nm. Fluorescent light may therefore be a means of distinguishing between blood sources if this fly species is present.

11

The ecology of some forensically relevant beetles

The relationship between beetles and their environment – beetle ecology – is an important aspect of forensic entomology. For forensically important species this environment is the crime scene, which may include a corpse. The nutrient status of the corpse changes as it decomposes.

Clear relationships between decomposition states, habitat conditions and the presence and sequence of beetle families have been demonstrated. Oliva (2001), for example, showed that in Argentina the nitidulid beetle, *Carpophilus hemipterus* Linnaeus, was found in the later stages of decomposition, in association with the Piophilidae and often also with *Necrobia rufipes,* a clerid beetle. Oliva also linked silphids of the genus *Hyponecrodes*, such as *Hyponecrodes erythrura* Blanchard, with corpses recovered from rural outdoor environments. However caution has to be used in interpreting scientific data from a crime scene in one location, or country, to another. Ideally, data about insect succession on carrion for the particular region where the death took place should be used (Table 11.1).

The speed of corpse decomposition is influenced by environmental conditions. Seasonality, temperature of the environment, its humidity, the level and duration of rainfall and insect abundance in the locality are all major influences on the rate of decomposition of the body and therefore on the speed of succession of the insects colonising it. In Canada, VanLaerhoven and Anderson (1996, 1999) recorded Dermestes species when the body was in early advanced decay. Oliva (2001), working in Argentina also found early colonisation of bodies by dermestids between ten and 30 days after death.

Research highlights how important it is to know as much as possible about the ecology of the species of insects that colonise a body and what influences their development on the corpse. The ecology of selected families and species that colonise the body is presented below.

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

Table 11.1 Beetle succession on decomposing corpses

	Fresh	Bloat	Active decay	Advanced decay	Skeletal
Staphylinidae	*	*	*	*	*
Histeridae			*	*	*
Cleridae		*	*	*	
Scarabaeidae		*	*		
Carabidae			*		
Silphidae		*	*	*	
Dermestidae			*	*	*
Nitidulidae				*	*
Trogidae				*	*
Geotrupidae			*		
Tenebrionidae					*

11.1 Ecology of carrion beetles (Silphidae)

The family Silphidae is made up of two subfamilies (Lawrence and Newton, 1982): carrion beetles (Silphinae) and burying beetles (Nicrophorinae). Both can be found on a body in the decay stages of decomposition. In some countries, silphids are forensic indicators of a short post mortem interval and are found on fresh and bloated corpse. *Oxyletrum discicollis* Brullé for example, is recognised in this role in south-eastern Brazil. Barreto, Burbano and Barreto (2002) found they made up 8% of the insect assemblage infesting human corpses in urban and rural areas near Cali, Columbia. This conclusion was based on investigations of 16 corpses – two women and 14 men – brought to the Cali Institute of Legal Medicine from both urban and rural murders (12 corpses had beetle life stages on them; the other four corpses did not have beetle life stages on them – only eggs and larvae of flies). In contrast Wolff *et al*. (2001) found the same silphid species present on pig carcasses, later in the decomposition sequence. They recorded it in the active decay stage, at 1450 m above sea level and a temperature range of 18–24 °C. So, height above sea level and temperature may influence its presence.

Dekeirsschieter and Haubruge (2011) identified different habitat relationships for carrion beetles (Silphidae) and noted that they were not associated with urban environments in Belgium. They found in experiments undertaken in spring that the Silphinae were exclusively found in agricultural habitats; *Oiceoptoma thoracica* Linnaeus, *Thanatophilus sinuata* Fabricius, *Thanatophilus rugosus* Fabricius and *Necrodes littoralis* Linnaeus. Nicrophorinae were exclusively found in forested habitats. The species that they found were *Nicrophorus vespillo* Linnaeus, *Nicrophorus vespilloides* Herbst and *Nicrophorus humator* Goeze.

Corpse size will also determine the species of carrion beetle (Silphidae) present. Silphinae tend to colonise larger corpses. For example, *Necrophila* (=*Silpha*) *americana* Linnaeus is found at the active decay stage on a large corpse (Payne, 1965), whereas Nicrophorinae (burying beetles) such as *Nicrophorus humator,* were

commonly found on small carcasses of voles and mice. Because more research has been carried out on the Nicrophorinae they serve, despite their preference for small corpses, to illustrate the ecology of Silphidae as a whole.

These beetles use **semiochemicals** to communicate between individuals, demonstrate social behaviour and many look after their young. Bartlett (1987) showed that in the laboratory female *Nicrophorus vespilloides* were significantly more likely to be attracted to containers with males than those containers without, suggesting an odour was released by males. Other males were also more likely to be attracted to the same location where a male beetle was already present. Indeed Nicrophorus sp. males may 'call' when they have found carrion. Bartlett recorded signalling ('*sterzeln*' signalling) achieved by the tip of the male's abdomen quivering and by the beetle's hind legs stroking it. Such communication may lead to several individuals locating the same carcass. Eventually, however, the corpse usually becomes the habitat for a single pair of beetles because the individuals fight each other (Pukowski, 1933, in Bartlett, 1987; Wilson, Knollenberg and Fudge, 1984). Males and females jointly build a chamber below ground and alter the carcass to support the development of their offspring. Interestingly, burying beetles (Nicrophorinae) have been shown to slow down carcass decomposition using secretions that are inhibitors of bacterial growth (Hoback *et al*., 2004). In general such inhibitors have not been shown for members of the other subfamily (Silphinae).

The location of a suitable carcass triggers female ovary maturation in some species. Wilson and Knollenberg (in Wilson and Fudge, 1984) discovered female *Nicrophorus orbicollis* Say had underdeveloped ovaries until the beetles encountered an appropriately decomposed carcass. Its presence triggered the female to produce eggs over a 48-hour period (Wilson and Fudge, 1984). The numbers of eggs produced varies with species. *Nicrophorus defodiens* Mannerheim produces an average brood size of 23.9 eggs, whereas *Nicrophorus orbicollis* produces only an average number of 14.9 eggs (Wilson and Fudge, 1984).

The value of having both parents present is that they can defend the corpse against competition, including preventing flies laying eggs. In some species only the female stays with the larvae until they pupate, whilst in others, such as *Nicrophorus vespilloides,* both males and females may stay (Wilson and Fudge, 1984). The duration of this parenting role is quite short. Field studies during May to August showed that the time between adults burying a carcass and offspring reaching the pre-pupal stage was ten days (Wilson and Fudge, 1984).

The habitats in which burying beetles are found vary with species. Nicrophorus species, other than *Nicrophorus vespillo,* are common in forests. (Ruzicka (1994) showed that *Nicrophorus vespillo* was more commonly found in fields.) Pukowski (1933) considered that the nature of the soil could account for the difference in distribution of some species. Investigations of *Nicrophorus vespilloides* and *Nicrophorus humator* around Frankfurt, Germany, revealed the former favoured drier soil. Therefore habitat and corpse condition as well as environmental conditions may dictate the presence of Silphidae on a corpse.

11.2 Ecology of skin, hide, and larder beetles (Dermestidae)

A number of species of dermestid beetles colonise dead bodies. These include *Dermestes ater* DeGeer, *Dermestes maculatus, Dermestes lardarius*, *Dermestes frischii* (Kugelann), (Centeno, Maldonado and Oliva, 2002). *Dermestes maculatus* will be used as an example of the response of dermestids to a corpse since this species is well researched because of its role as a stored product pest.

Dermestes maculatus growth from egg to adult can take between 20 and 45 days, although the speed of development depends on the temperature of the habitat. The larvae have characteristic hairs on their body segments and are referred to colloquially as 'woolly bears'. These hairs occur in tufts at the end of the body or along the sides of each segment and according to Hinton (1945) can be moved or vibrated when the larva is being threatened.

Food quality and composition is important to the success of dermestid larval growth. McManus (1974) considered that optimum energy consumption for *Dermestes maculatus* was between 0.17 and 0.28 kcal/g per day. Food with high lipid content shortened the length of the larval stage (Obsuji, 1975); *Dermestes* species have been shown to require dietary sterols and in particular require cholesterol. Given a small amount of sterol dermestids can also utilise campesterol or 7 – dehydrocholesterol to complete their lifecycle (Levinson, 1962).

Pre-pupal larvae migrate to pupate and can bore into a variety of substances in order to avoid cannibalism as a pupa (Figure 11.1). This, to some degree, accounts for

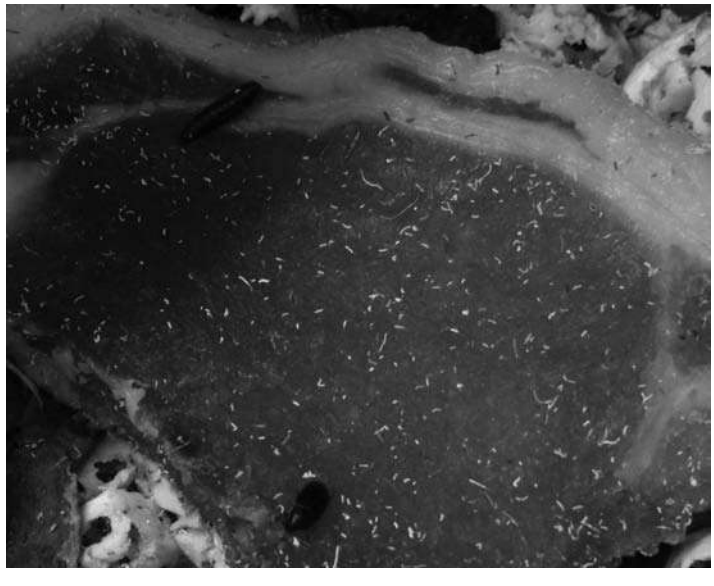

Figure 11.1 Dermestid beetles

their status as a pest as they can bore into wood, bone and even concrete. Dermestid larvae can delay the time of their pupation by up to 20 days if there is no suitable place to pupate (Archer and Elgar, 1998).

Adult dermestids exhibit **phototaxis** (respond negatively to light) and will, when touched, readily 'play dead' (show **thanatosis**). The adults and larvae both exist happily in darkness. However, when food is in short supply, the adult beetles have been known to walk, or fly away from the current food source towards a light source. These habits mean that they need a reliable food source and pupation site, to successfully complete their lifecycles.

Dermestid **frass** provides evidence of forensic significance indicating that this species was formerly present. Frass has a characteristic twisted shape and is white in colour (Figure 11.2). It comprises uneaten food, which is encased in peritrophic membrane. Where frass alone is present, it may reflect dermestid activity for a period of time between 1 month and ten years. Indeed Catts and Haskell (1990) recorded Dermestid frass from a 10-year-old mummified body retained in a house by a solicitous but criminally-culpable relative.

Ecological conditions will determine whether dermestid species will be present. Coleopteran profiles vary in south-eastern Spain in both distribution and abundance throughout the year (Arnaldos *et al*., 2005). In spring and summer they recorded few dermestid species on corpses in the earliest stages of decomposition but numbers of dermestids increased as the remains began to dry out. Dermestid larvae were characteristic of the dry stage of decay and lots were found in the muscle mass and on bones. In south-eastern Brazil *Dermestes maculatus* is also recognised as a forensic indicator (Carvalho *et al*., 2000) during the final decay stages since they were found in both rural and urban locations.

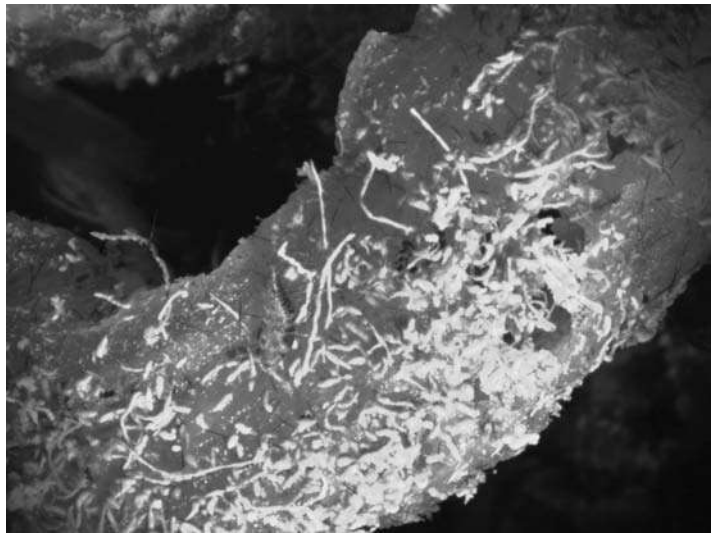

Figure 11.2 Faecal remains (frass) from dermestid beetles

Dermestids appear to tolerate a range of temperatures and relative humidity. Kulshrestha and Satpathy (2001) have shown that they are present where the ambient temperature is 16.5 °C and the average humidity is 71%. Dermestids can also tolerate ambient temperatures of 20 °C and a much-reduced average humidity of 46% (Kulshrestha and Satpathy, 2001). This fits with work by Hinton (1945) who showed temperatures between 28 and 30 °C allowed dermestids to complete their lifecycle in 22 days, whilst at lower temperatures he recorded lifecycles of 40–50 days. Raspi and Antonelli (1995) considered the optimum temperature for growth for cultures of dermestids maintained at a constant in a laboratory was between 25 °C and 30 °C. Under these conditions the average lifecycle duration was between 35.1 and 43.9 days.

Dermestids compensate behaviourally for high temperatures by changing location. Toye (1970) showed that during the morning in Nigeria, when internal temperatures were between 24 °C and 26 °C, *Dermestes maculatus* was seen on the carcass surface. Later in the day, when the ambient temperature reached 29–47 °C, *Dermestes* moved inside the carcass where the internal temperature was lower, reaching 29 °C to 42 °C. The relative humidity within carcasses ranged between 40% and 70 % RH. *Dermestes maculatus* appear to have a preferred relative humidity between 50–60% at 25 ± 1 °C (Toye, 1970).

Dermestids appear to communicate, as do other carrion-seeking species, using odour. Conquest (1999) rinsed the bodies of male and female dermestids in hexane and was able to show the washes attracted males to both male and females. Female dermestids were attracted to the concentrated body washes from other females. Males secrete a **pheromone** from a canal beneath the fourth abdominal sternite. Levinson *et al*. (1978, 1981) demonstrated that the most active of the pheromone components were isopropyl Z-9-dodecanoate, isopropyl Z-9-tetradecanoate and isopropyl Z-7-dodecanoate. These chemicals attracted adults and promoted recognition of sexually mature males (Figure 11.3 ab).

Female *Dermestes maculatus* have multiple mates and copulation is achieved more readily with a new mate (Archer and Elgar, 1999). Males show mounting behaviour after copulation and can be found riding on the backs of the female, particularly where other males are present as a means of preventing further mating.

Jones and Elgar (2004) explored age-related mating success in *Dermestes maculatus.* They tested the effect of male age, sperm age, and male mating history on female fecundity and their ability to achieve fertilisation. When males of intermediate age were used, females were more successful fertilised and laid more eggs than when mated with either young or older males. The age of sperm was not considered an important factor.

The size of a population of *Dermestes maculatus* affects the length of the larval period. Both high and low densities increase the length of time of metamorphosis. Rakowski and Cymborowski (1982) suggest that *Dermestes maculatus* faeces emit two compounds that influence growth and development; the one produced by the larvae, speeds up growth and encourages aggregation; the odour source generated by adults, inhibits larval development. The age of larvae present on

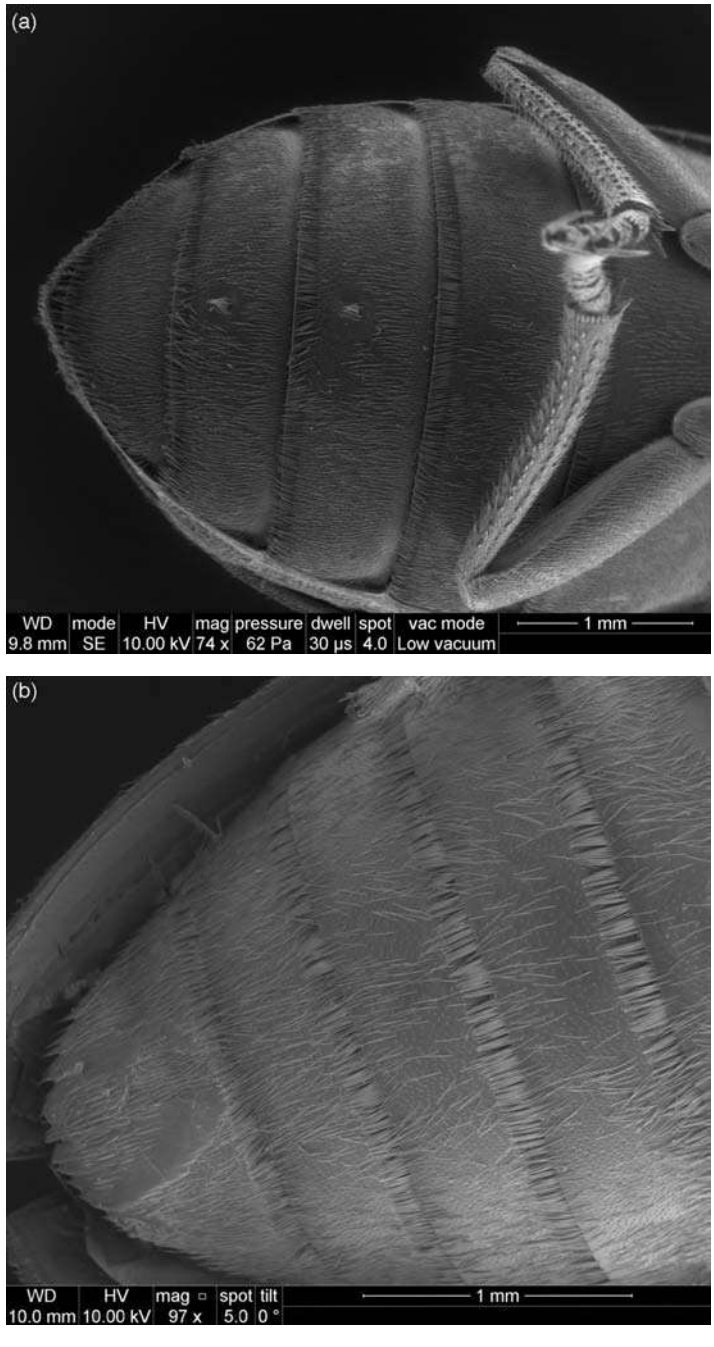

Figure 11.3 Electronmicrograph of the ventral side of *Dermestes* lardarius Linnaeus male (a) and *Dermestes maculatus* DeGeer female (b)

a body should therefore be interpreted on the basis of dermestid population size, as well as temperature.

An example of this phenomenon was provided by Lee Goff (2000). He noted the last larval skins were shed 51 days after the death of an individual and commented on the fragility of these freshly shed dermestid larval cuticles. This fragility, in conjunction with the presence of other species that were also found at similar Hawaiian locations between 48 and 51 days after death, indicated a post mortem interval of greater than this period. However, the freshness of the shed dermestid cuticles on the body, with the absence of larvae, suggested that time since death was not much greater than 51 days. Rapid completion of the larval stage, with the resultant remnants of larval cuticle, may have been a response to dermestid population density. Goff commented that in Hawaii there was forensic significance in the absence of *Dermestes maculatus* larvae where they might be expected to be present at a crime scene.

An association between dermestids and other species is significant. Arnaldos *et al.* (2005), examining insect succession on corpses in south-eastern Spain, recorded Nitidulidae and Dermestidae at the same stage of decomposition, linking their presence on the body at temperatures common to that location. Post mortem interval is most accurate when based on evidence of presence of several beetle species that are normally found in association, rather than solely on a single species of beetle.

11.3 Ecology of clown beetles (Histeridae)

Clown beetles arrive on the body at bloat and remain through the decay stages and into the dry stage. Histerid larvae and adults feed on the larvae of flies colonising the body in these decomposition stages. Stevenson and Cocke (2000) explored the lifecycle of *Arcinops pumilo* (Ericson). In laboratory-bred cultures, adult *Arcinops pumilo* will consume between three and 24 muscid eggs per day whilst larva will consume two to three eggs a day in order to develop satisfactorily. At 20–25 °C, histerid beetles take from 31 to 62 days to pass through their lifecycle from egg to adult (Crowson, 1981). The eggs and larvae produced at this temperature tend to be large, indicating that this is an optimum temperature. However clown beetles are also photophobic and colonise the underside of the body during the day, emerging at night to feed.

Adult histerids secrete small drops of a pungent liquid from the ventral surface of their thorax and abdomen as a defence. In so doing, they frequently turn over, so their ventral surface is uppermost. Members of this family also appear, when touched, to be ‘dead’. This ability to demonstrate thanatosis is a common defence mechanism found in a number of insect species and care should be taken when handling and storing them.

The decomposition stage, in which histerid beetles are present on the corpse, varies depending upon location. Korvarik (1995) found that histerid beetles arrived on the body soon after flies had colonised it. This supports the findings of Payne

(1965) who recorded them during bloat, which occurred from day 1, in active and advanced decay stages, as well as in the initial dry stages of decomposition, which was recorded from day 5 onwards. Wolff *et al*. (2001), in contrast, recorded the arrival of adult histerids on the corpse in active decay between 7–12 days after death. They recorded the presence of larvae between days 77–118, at the later stages of decomposition. Richards and Goff (1997), investigating insect succession on pigs placed in woods at different altitudes in Hawaii, recorded *Hister noma* Erichson and *Saprinus lugens* Erichson in their collections. They, too, stated that histerid beetles invaded a body at the end of the bloat stage.

Shubeck (1968) considered that habitat played a large role in determining whether or not histerids were attracted to a corpse. He found that, in still air, histerids perceived odour from sources a metre away, although capture/recapture experiments demonstrated little evidence of histerid orientation to bait sources. Because little information is available that links the duration of the stages of metamorphosis to histerid colonisation of the body at specific temperatures, they are not valuable as post-mortem indicator species.

11.4 Ecology of chequered or bone beetles (Cleridae)

This family has been classified by some workers as members of the Cornetidae rather than Cleridae; although other researchers have retained the family name Cleridae (Kulshrestha and Satpathy, 2001). The use of the word Cleridae for the family name has been chosen in this account, as it is a familiar term in forensic entomology.

Cleridae feed on carrion and are often called bone beetles. Cleridae have been found from bloat through to the dry stage of decomposition, although, the association with a particular decomposition stage may differ from country to country. For example in the UK, the clerid beetle *Necrobia* can be associated with dry carcasses and bone remains (Cooter, in Cooter and Barclay, 2006). In India, Cleridae and Dermestidae are the most common beetles infesting the dry stage of decomposition of human remains (Kulshrestha and Satpathy, 2001). They recorded *Necrobia rufipes* on remains where the average temperature was 16.5 °C and the relative humidity was 71%; although this species has also been recorded at higher temperatures and a relative humidity of 46%. *Necrobia rufipes* is called the red-legged bacon beetle and is a noted stored product pest. It is 4–5 mm long and dark blue in colour. Its legs, and the segments at the base of the antennae, are red.

The biology of *Necrobia rufipes* has been studied experimentally in a light/dark regime of 8:16 hours, at a temperature of 30 ± 0.5 °C and a relative humidity of $80\% \pm 5\%$. The average number of eggs laid per female is 89.7 ± 17.8 (Bhuiyan and Saifullah, 1997). The average egg stage duration is 4.1 ± 0.4 days. The length of the larval stage was calculated to be 32.1 ± 5.2 days and that of the pupal stage to be 9.9 ± 1.7 days. Female *Necrobia rufipes* have an average lifespan of 60.6 ± 39.5 days, whilst for males it is shorter (49.4 ± 18.2 days).

Clerids such as *Necrobia rufipes* begin to be attracted when volatile fatty acids and caseic breakdown products are released from the body (Turchetto, Lafisca and Constantini, 2001). Unpublished work by Bovingdon, recorded by Munro (1966), indicated that this species was attracted by the stearic and palmitic acids released during fungal growth. It tends to be found on bodies in association with cheese skipper flies (Piophilidae). Turchetto *et al*. confirmed this association when examining the strangled corpse of a young woman found in a cornfield in northern Italy, whose badly decomposed body had been damaged post mortem by a tractor. *Necrobia rufipes* was found along with the third larval instar of the piophilid *Stearibia nigriceps* Meigen, a species that is also a member of the British diptera. Richards and Goff (1997) cite *Necrobia rufipes* as an important forensic indicator species in Hawaii. In Peru, a study of succession of arthropods in July to October on corpses showed that *Necrobia rufipes* made up 0.45% of the total insects recovered (4405 specimens) (Iannacone, 2003).

In the dry stage *Necrobia rufipes* and *Dermestes maculatus* can be present on a body at the same time, although their interspecific competition has an effect on the population growth of both species (Odeyemi, 1997). At 20 °C, *Dermestes maculatus* will outcompete *Necrobia rufipes,* whereas at 32 °C both species are able to coexist on the same body. This suggests that *Necrobia rufipes* may be at the extreme of its environmental tolerance and tends to support the previous records of its success earlier in the decomposition sequence.

The presence of clerids may influence interpretation of the cause of death of the corpse. Members of this family, along with silphids and histerids have been found to cause damage to the cadaver skin and these marks at first sight resemble gunshot wounds. Such holes served as holes for breeding in, or resulted from feeding (Benecke, 2004). Therefore care should be taken in interpreting damage on well decomposed bodies where there is evidence of the presence of members of any of these three families.

11.5 Ecology of rove beetles (Staphylinidae)

Rove beetles are predators of fly colonisers feeding on the body and they feed on both the eggs and larvae. They arrive early on the corpse and are expected to be present when the corpse is in bloat. Chapman and Sankey (1955) recorded the following species of rove beetle on rabbit carcasses in the UK: *Anotylus* (=*Oxytelus) sculpteratus* Gravenhorst, *Philonthus laminatus* (Creutzer); *Philonthus fuscipennis* (Mannerheim) *Creophilus maxillosus*, *Tachinus rufipes* (Degeer); *Aleochara curtula* (Goeze). These carcasses were placed within 30–40 m of each other in shrubbery, under a plane tree or in thick meadow grass. Goff and Flynn (1991) recovered specimens of the same genus – *Philonthus* (adult *Philonthus longicornis* Stephens) from samples of sandy soil and leaf litter, from beneath where a body had lain at

Mokuleia, Oahu, Hawaii. Therefore their association with particular habitat conditions may be geographically variable.

The presence of Staphylinidae varies with season and location. In spring, Centeno, Maldonado and Oliva (2002) recorded Staphylinidae on an unsheltered corpse throughout the stages of decomposition. In summer, however, staphylinids were absent from the unsheltered corpse and only recorded in a sheltered corpse during the bloat stage. In contrast, in autumn, Staphylinidae were recorded in both the advanced decay and dry stages of decomposition on the unsheltered corpse. Their presence must be interpreted in the light of environmental conditions such as temperature and exposure to sunlight.

11.6 The ecology of dung beetles and related families

The superfamily Scarabaeoidea has been the topic of much discussion amongst taxonomists. Ratcliffe and Jameson (2004) have discussed the current state of taxonomy and their views on lineages within the classification have been taken into account in the following descriptions.

There are three families contained within the superfamily Scarabaeoidea that have forensic relevance. These are the Trogidae, Geotrupidae, and the Scarabaeidae which is also part of the Geotrupidae lineage.

11.6.1 Geotrupidae

Geotrupes species are exclusively dung and carrion feeders. Nuorteva (1977) collected *Geotrupes stercorosus* Scriba from a partially buried corpse in Finland. Whilst Gill (2005) concluded that specific Geotrupidae species had an association with particular soil types. She noted that Geotrupidae were typically found in areas with sandier soil types. The presence of *Geotrupes* sp. may therefore be valuable in indicating whether or not a body has been moved, if the soil is not of this type but the presence of *Geotrupes* is noted. The second family of interest to forensic entomologists, within the superfamily, is the Trogidae. These are carrion feeders at the later stages of decomposition (Figure 11.4).

11.6.2 Ecology of trogid beetles (Trogidae)

Trogid beetles are found at the dry stage of decomposition. Trogid larvae are easily recognisable, if present, as they have a typical ‘C’ shape. The larvae are reputed to thrive on skin, hair and the remnants of tissue which is dried onto the remaining skeletal bones. Trogid beetles have been recorded on dry tissue in various seasons.

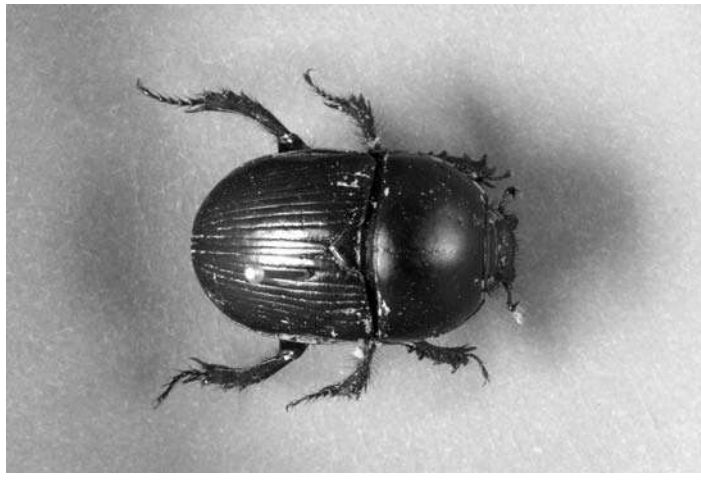

Figure 11.4 Geotrupid beetle

Tabor, Brewster and Fell (2004), studying succession on a pig in southwest Virginia, considered them spring colonisers on the dry stage of decomposition. However in Manitoba, Canada, Gill (2005) recorded *Trox unistratus* Beauvaris throughout the summer, autumn and spring. The family, therefore, seems to be specific to the later decomposition stage, but not to a particular season. Archer and Elgar (2003) noted that members of several Australian families of beetles including *Omorgus* sp., a member of the Trogidae, and the histerid *Saprinus* sp., left exoskeleton fragments by which evidence of their previous presence could be identified.

11.6.3 Ecology of dung beetles (Scarabaeidae)

Scarabaeidae or dung beetles have been known from Egyptian times in relation to dead bodies. Scarabaeidae inhabit tunnels that they construct beneath a corpse. Two of the most common genera of Scarabaeidae on bodies are *Onthophagus* and *Aphodius* (Payne and King, 1972). As with many other beetle species, because they are not immediately obvious on a corpse, so their presence can be missed as they hide.

In a study in an urban area, conducted in south-eastern Brazil dung beetles were the second most frequent coloniser of pig carcasses; the calliphorid *Chrysoma albicepes* was the major coloniser (Carvalho *et al*., 2000). Three species of dung beetle were considered by Carvalho *et al*., to be important forensic indicators for post mortem determination because they had been recovered from both human cadavers and pig carcasses in forested habitats near Campinas City, Brazil. *Deltochilum brasiliensis* Castelnau, and *Eurysternus parallelus* Castelnau, which were found on human cadavers. *Coprophanaeus (Megaphanaeus) ensifer* (Germar), were found with

Canthon sp., *and Scybalocanthon* sp., were found on both pig and human corpses. Despite this association, a corpse present in a suitable habitat rather than a particular stage of decomposition appears to be the deciding factor in whether or not Scarabaeidae colonise a body in any geographic region.

11.7 Ecology of ground beetles (Carabidae)

Carabids are predators of necrophagous flies and are most frequently active at night. Larvae of *Nebria*, *Notiophilus*, *Carabus* and *Pterostichus* species are frequently found on the soil surface (Luff, in Cooter and Barclay, 2006). There are few examples of the contribution of the ground beetles to succession on the corpse and Smith (1986) considered that they were of less importance as predators than other beetle families.

Carabids may be responsible for the degree of variation in the assemblage of insects responding to both season and stage of decomposition of the corpse. Only in the context of the local conditions can succession can be used to determine the time since death of a corpse at a crime scene. At the crime scene, attention should be given to examining the underside of the body for beetles that are hiding in soil during the day. The predatory effects of families such as carabids, which consume eggs or larvae, can cause a gap in the insect profile for a particular stage of succession and this should be considered when interpreting the evidence.

12

Investigations in an aquatic environment

In 2009, in the UK, 427 deaths were associated with water according to the Royal Society for the Prevention of Accidents (Royal Society for the Prevention of Accidents, www.rospa.com/leisuresafety/statistics/accidental-drownings-2005.aspx, accessed 4 November 2011). In general, of the people who die in water, 31% would be drowned in rivers, 15% at sea, 6% in lakes and reservoirs, 10% on canals, 4% in streams, 4% in ports and 4% in harbours. The rest relate to drowning in the context of dwellings (Department of Trade and Industry, 2001).

Forensic entomologists are frequently required to determine the amount of time between the discovery of the drowned corpse and the time it became submerged, in order to assist the investigations being carried out by pathologists. The cause of submergence is not necessarily an aspect of interest for the forensic entomologist but how long the body has been in water is of importance. This period between submergence and discovery is often called the post mortem submergence interval (PMSI). This terminology (the use of the word 'submergence') is particularly important as there is still discussion about the exact definition of death by drowning. The difficulty in diagnosing such a death is due to the variation in physical attributes expressed by the victims (Piette and DeLetter, 2006).

It is not just a matter of defining drowning on the basis that a liquid, most often water, is preventing gas exchange because the respiratory tract – nose to lungs – have become blocked by the fluid. The physical features expressed by victims of drowning vary. But in general it would be expected that the body hangs submerged in water with its face and limbs hanging downwards so the body forms an 'n' shape with the back uppermost under the water. Drowning is difficult to determine based on body condition and a number of techniques have been developed to investigate the condition. For example, a specific diagnosis using a bacteriological test has been developed to diagnose drowning (Lucci *et al.,* 2008) based on particular microbiological features. Other researchers have attempted to distinguish the condition (death as a result of drowning) in salt and freshwater on the basis of **alveolar macrophage** levels (Locali, de Almeida and de Oliveira, 2006). However no standard operating procedures appear, as yet, to exist to confirm that the death has been by drowning. The forensic entomologist, however, can potentially explore time since submergence

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

based upon the arthropods present on the submerged decomposing corpse and the rates of development to provide an estimate of the minimum time since submergence. Or they can use the fact that, on many occasions, the decomposing corpse will rise to the surface of the water and become infested by terrestrial species of insects, pinpointing the time when the body re-emerged.

12.1 Decomposition and submergence in water

Under water and within in the intertidal zone, the body will still exhibit five decomposition stages. However, distinctions in the decomposition process relate to whether, or not, gas in the gut has caused the body to rise to the surface, or whether the body is trapped and therefore remains submerged. Little work has been undertaken to examine and characterise the succession of organisms of importance for aquatic forensic entomology. However, the work of Payne and King (1972), Chin *et al*. (2008b) and Anderson (2010) provide some details of the sequence of colonisation on bodies which decompose in water. The sequence is outlined below:

Submerged fresh stage

Floating decay stage

Bloated deterioration stage

Sunken remains

Payne and King also included the category of floating remains, which was not included in the sequence that Chin *et al*. recorded. The reason for this observational difference may be a difference in water temperature and hence speed of decomposition. Local conditions will determine whether there is a specific assemblage of insects and **macroinvertebrate**s present when the body is submerged. If the body rises to the water surface this tends to be the point at which a body is noticed and recovered from the water. The period of time after death when this takes place will depend on the water temperature. At around 21 °C a body may resurface 24 hours later; at 16 °C a body may resurface between 48 and 72 hours, whilst at 10 °C it will take around 72 to 96 hours for re-emergence at the water surface. At 4 °C it will be considerably longer and could take a period greater than six days for the corpse to rise to the water surface. The time of resurfacing depends upon water depth and other features of the water body besides temperature, such as presence of clothing and speed of water movement.

The relationship between time of death and physical breakdown of the body has been investigated by Giertsen (1977). He cited Casper's dictum as a means of determining the length of the post mortem interval. This rule says that:

> at a tolerable similar average temperature, the degree of putrefaction present in a body lying in the open air for one week (month) corresponds to that found in a body

after lying in the water for two weeks (months), or lying in the earth in the usual manner for eight weeks (months).

The reason for this difference in decomposition is that the speed at which the body loses heat in water is much faster than the speed at which the body will lose heat in air. A naked corpse, submerged in water will cool twice as fast as the naked body on land. However the cooling rate will also depend on the body temperature at death (assuming death took place in the water), water temperature, and the effects of water currents. Decomposition is also impeded by the presence of clothing on the deceased.

Initially a number of physical changes can occur to a body submerged in water. For example regions of the body become wrinkly in a process called skin **maceration**, often called 'Washer woman hands'. In this condition there is thickening, wrinkling, and whitening of the skin, often starting in the fingers. The changes, occurring firstly

Box 12.1 Skin structure

The human skin is a tissue made up of cells in a number of layers. Over the majority of the body there are two distinct sections: the epidermis, and the dermis. Beneath the skin there is also a layer of connective tissue. This is called the superficial fascia and sits between the dermis and the covering of the muscles beneath. The dermis is anchored by fibres onto this superficial fascia.

The first layer, the epidermis – counting from the external skin surface – has four layers; on the palms of the hands and soles of the feet there are five layers. The epidermis is made up of a corny layer of rows of flat dead cells filled with keratin (the external layer), a clear layer (found only in epidermis of the palms of the hands and the soles of the feet), a granular layer, a spiny layer and the lowest layer which is called the basal layer. This layer is a single layer of cells that are cuboidal or columnar in shape. This layer of cells is capable of mitosis and therefore the source of skin renewal during life

The dermis is made of connective tissue comprising fibres of collagen and elastin. The dermis is thicker on the dorsal plane than the ventral plane of the body. The dermis contains nerves, glands, hair follicles, and blood vessels. The top fifth of the dermal depth is called the dermal papillary region. In this region the connective tissue is loose and the elastin fibres are fine. The dermal surface has finger like projections pushing into the epidermis. These projections are called dermal papillae and many have a good supply of blood provided by capillary loops.

The bottom four-fifths of the dermis is made up of bigger elastin fibres interlaced with collagenous fibres within a dense and irregularly arranged mass of connective tissue. Between the fibre spaces there is adipose tissue along with the glands, blood vessels and hair follicles and sweat gland ducts. This latter layer is called the reticular layer of the dermis.

at the fingertips, on the palms and backs of hands, soles and backs of feet, can subsequently occur at the elbows and knees. Frequently this can take place within one to two hours after submergence (Heaton *et al.,* 2010). It is the result of the epidermis detaching, the corny layer splitting and decomposition of the granular layer and the basal layer of the epidermis of the skin. Fibres can also rupture in the hypodermis, the layer beneath the skin epidermis.

If the body is in water for a long time the nails loosen and the skin peels from the hands. The time for this to happen also depends on temperature. For example in riverine conditions at 3.2 °C (winter temperatures) the nails can loosen after seven to eight weeks whereas at 18.6 °C they loosen after three days (Reh, 1969). Some forensic scientists have recorded contraction of the *erector pili* muscles associated with the hair follicles, possibly as a result of rigor, although this has also been recorded on bodies which have died in terrestrial environments too (Saukko and Knight, 2004).

The passage of the body into *rigor mortis* can be slowed down if the water temperature is low. However, the obverse can also take place – where a victim in the process of drowning has struggled a great deal this can lead to a more rapid onset of rigor mortis, which is strongly expressed and lasts a long time.

As with decomposing bodies on land, the corpse passes through hypostasis. This can occur on any region of the body as the corpse, if dead through drowning, may have moved because of the water currents, tidal movements or local water turbulence. Hypostasis is most often seen in the face, upper chest region, thighs, calves, and feet because of the position of the body in the water once it has sunk – the characteristic 'n' shape where the head and feet generally end up facing downwards.

Putrefaction of the body is slow because of a lack of bacterial and insect activity. This affects the rate at which the gas builds up in the gut and the body can begin to rise to the surface. Once it does this the rate of decomposition speeds up. Chemical changes also occur in the body. For example, after 13 days of submergence, ethanol levels of 104 mg ml^{-1} have been recorded in the tissue of rats drowned and then retained in water (Iribe, Ueno and Mukai, 1974).

The increase in speed of decomposition is also the result of exposure to the air and also to the presence of terrestrial insects, which supplement colonising aquatic macro-invertebrates such as stoneflies (Plecoptera) (Figure 12.1), May flies (Ephemeroptera) (Figure 12.2), midge larvae (Chironomidae) (Figure 12.4), Caddisflies (such as the Limnephilidae) (Figure 12.5 – also see the colour section) and other arthropods such as water snails (Figure 12.3), which feed on the body.

Factors that are considered to alter the rate of decay are when the body entered the water, the water temperature, the water pH and in particular acidity whether or not the body was clothed, the biomass including body fat and whether the body is consumed by scavengers such as mink, rats, fish such as pike or snapping turtles (see the colour section) (Hobischak and Anderson, 2002).

Some terrestrial species can be submerged when the body enters the water, particularly if drowning is the cause of death rather than submergence being a source of disposal. Where the victim is host to lice, fleas and ticks the degree to which

Figure 12.1 Stoneflies

these survive is an indicator of time since death. Fleas, if present on the body, will survive up to 24 hours post submergence (Simpson, 1985). According to Simpson it is possible to revive fleas up to this point and time to revival is a measure of the post mortem interval. Lice, in contrast will be dead if the body is submerged for longer than 12 hours (Mumcuoglu *et al*., 2004).

Figure 12.2 Ephemeroptera

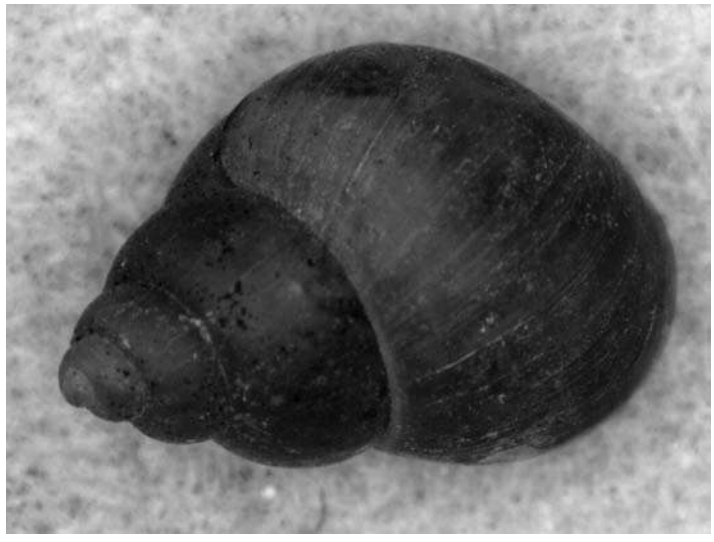

Figure 12.3 Molluscs feed as scrapers. *Source:* Reproduced by permission of Mr. Ian Ward

In addition to a knowledge of terrestrial arthropods, especially insects, it is necessary to have a good knowledge of the species present in the particular water body, even if this has to be achieved by subsequent experimentation. Such species include those in the crustacea as well as insect species. The collective name most suitable for the relevant group is the **macroinvertebrates**.

Figure 12.4 Nonbiting midges: an example of the Chironomidae

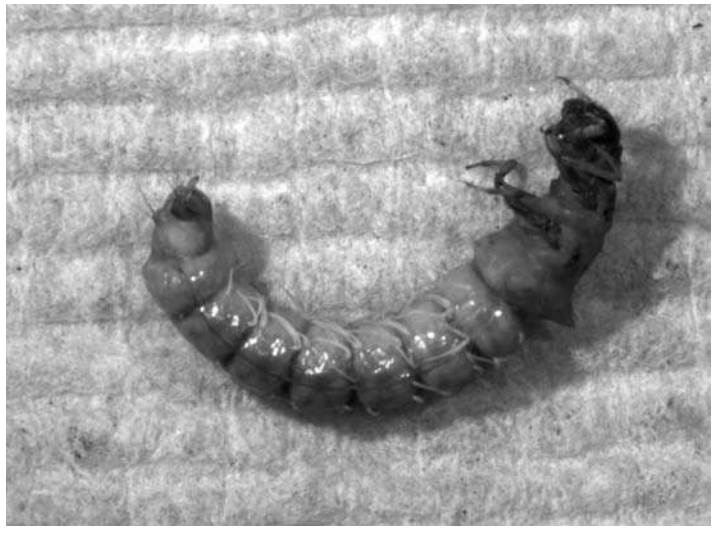

Figure 12.5 A Caddisfly larva

Keiper, Chapman, and Foote (1997) have pointed out that there may be differences in the macroinvertebrate assemblages for **riffles** and pools even in the same river. Hence, they suggest the development of different indices for calculating the post-mortem submerged interval depending on the particular conditions. This view is supported by work by MacDonnell and Anderson (1997) amongst others. Davis and Goff (2000) concluded that, in the intertidal zone, carcass decomposition resulting in loss of flesh was based mostly upon physical battering from the tidal cycle and wave action, along with bacterial decomposition. They found this to be true even though both marine and terrestrial scavengers were present at the sites.

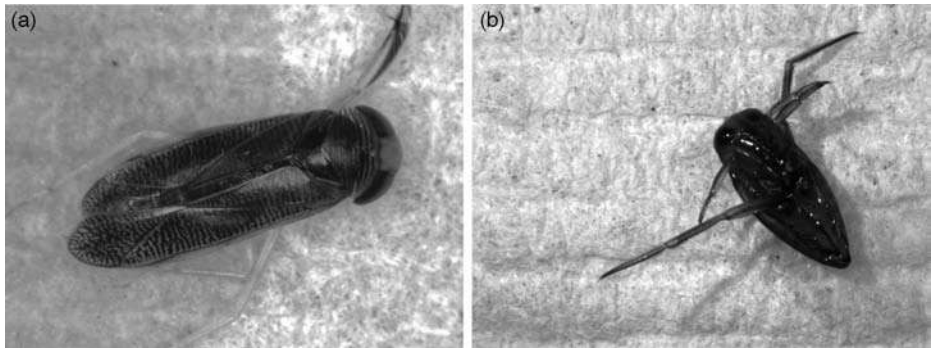

Figure 12.6 (a) Corixid bug (b) Notonecta nymph

12.2 The nature of the water bodies in which submergence may take place

As indicated earlier, submerged bodies can be found in a range of locations from the sea, lakes and rivers to garden ponds and bath tubs. In the latter the body is likely to be found relatively quickly as the water is associated with domestic residences. In lakes, rivers (Figure 12.7) and the sea, the nature of the water and its associated fauna and flora will differ. The features of lakes and rivers are considered in this section.

Water bodies such as ponds and lakes (**Lentic** water) are much more static water bodies than rivers (**Lotic** water bodies) and tend to be of greater depth. In winter ice may cover the surface of part of a lake (or pond) and the water is dense and a layer of 4 °C is located at the bottom, with cooler but less dense water at the top. In spring the surface water will warm up. Once the lake surface water reaches 4 °C, it will become denser and sink down the water column. Given a windy day the water in the lake will intermix for a sort time. As the weather warms up so the stratification of the lake water returns so that the surface water may reach a temperature of 18–20 °C whilst the lower layers are cooler and may be around 12–14 °C, depending upon the depth. It is this lower temperature that bodies submerged in the lake bottom will experience provided they are trapped in position and the water body water temperature is at some point in the year stratified. This temperature will affect the decomposition rate of the body. The nature of the water will also affect what is living in the water.

Aquatic plants can grow around the lake shore in a small depth of water, be anchored onto the bottom of the lake or pond, or float on the water surface. In the

Figure 12.7 The riffles and pools of a river

Figure 12.8 Dytiscid beetle – a predator

water plankton may be growing including algae which are capable of photosynthesising. If the water allows light to penetrate to depth, the algae may also be growing upon solid surfaces within the lake, including on the clothes. These in combination with other microbial organisms may form a biofilm. In lakes the range of macroinvertebrate families found includes **zooplankton** such as Cladocera of which *Daphnia pulex* Linnaeus is a member, crustacea, molluscs, insects such as chironomids, odonata and Gerridae, Dytiscidae (Figure 12.8 see also the colour section) and Gyrinidae and **oligochaete** worms.

Within lakes, the food web is based on either the bottom-dwelling species or on the micro-organisms depending upon how **eutrophic** the water body is. If the water body is poor in nutrients then the organisms living on the bottom provide the main source of energy, these include worms, shrimps and molluscs as well as large aquatic plants and bottom living algae. In water bodies richer in nutrients there will be a greater population of zooplankton such as protozoa combined with a high microbial load – bacteria and fungi which act as decomposers of any decaying plant material as well as consuming the plankton in the water. In addition the chironomid larvae including blood worms and oligochaete worms will feed on detritus. The predators that consume these creatures will include freshwater shrimps, water beetles and odonata amongst other creatures (see the colour section).

In rivers, what grows along the bank plays a role in the nature of the food present in the water body. This is because plant roots can pass down into the water and vegetation may overhang and drop into the water once it has died. It is therefore a source of organic material, which is a food source and makes a greater contribution

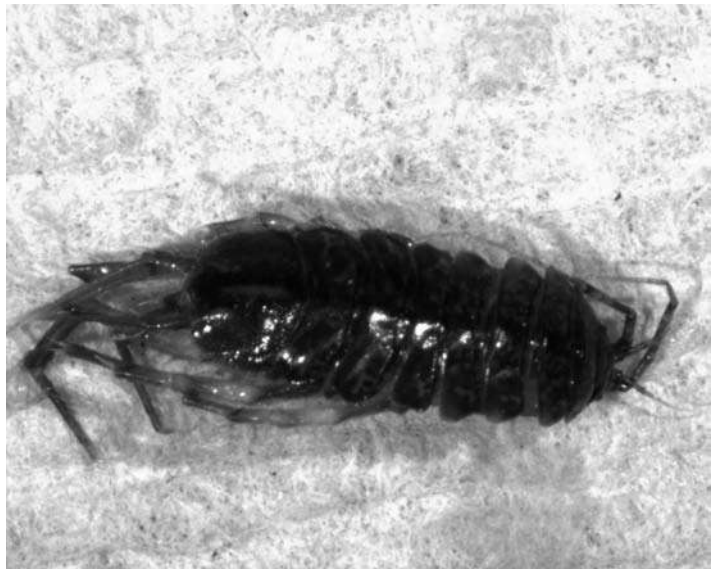

Figure 12.9 *Ascellus sp.* a shredder

to the ecology of the river than to a lake. A theory that relates the communities present based on their **functional feeding strategies** and the available energy sources at different points down an ever widening, moving water body such as a river is called the r**iver continuum concept** (Vannote *et al*.,1980). Such differences in macroinvertebrate assemblages, based on the nature of the available organic carbon, can be replicated at a localised site if a submerged decomposing body is present.

Micro-organisms provide a biofilm covering to the available surfaces and within these structures bacteria, fungi and **algae** coexist with the organic material. Micro-organisms break down the organic material, which is contributed to the flowing water as well as organic matter such as leaves, which naturally decay. Some creatures will feed upon the submerged leaves and assist in their breakdown and the release of fragments of organic matter into the water. In many streams, caddisflies are found alongside flies – which have an aquatic larval stage – beetles, and amphipods such as *Ascellus* sp. (Figure 12.9).

Such habitats are very well oxygenated, which facilitates the survival of May flies and stone flies. Along a river, the proportion of invertebrates will change depending upon whether there is a lot of organic matter in the form of vegetation and other organic matter through to locations where fine sediment dominates and the quantity of plankton is higher. A dead body provides a sudden influx of organic material and, as such. will alter the proportions of different organisms that can utilise the body and feed off it or off the organisms which are feeding on it. These may change as the corpse slowly decomposes.

12.3 Methods of establishing time since corpse submergence – indicator species

Water uptake in submerged macroinvertebrates means that the loss of body weight over time cannot be used as a means of determining post mortem interval as might be available for terrestrial corpses. As Haskell *et al.* (1989) point out, there are no obvious indicator species for determining post mortem submergence interval as might be available for terrestrial species. It is the developmental sequence and the biology of particular arthropods that are specific to a particular habitat which are most useful. However physical conditions such as body size, its position, water temperature, the exposure of the body to currents and the current speed, which determine rate of body decomposition, also determine the organisms that choose to colonise the body.

The macroinvertebrate families that have been most often found on decomposing bodies in fresh water are the chironomid midges (Chironomidae) and the net-spinning and cased caddis flies (Trichoptera). Crabs, lobsters and predatory and carrion-feeding fish such as sharks are attracted to bodies submerged in marine or brackish environments. Not only is the family and species required to determine the minimum time since submergence – it is also necessary to determine the abundance of the individual species because some may be present for the greater part of the year, so the presence and absence of particular species alone is insufficient to determine PMSI (Hobischak and Anderson, 2002).

The majority of work on forensic entomology in freshwater environments has drawn on either species presence or the responses of different feeding groups. Cummins and Merritt (1996) related food habitat, diet and the morphology of the mouthparts; they assigned macroinvertebrates to functional feeding classes. Five functional feeding groups were identified which are relevant to submerged post mortem investigation. These are **scrapers** or **grazers**, **collectors**, **filterers**, **shredders**, and **predators** (See SB12.2 on organic carbon for an explanation of these feeding types). Two subgroups can be assigned to collectors these are filterers and gathers (collector-filterers and collector-gatherers).

- *Scrapers.* This functional feeding group is made up of organisms that are plant feeding and those which feed on detritus (herbivores and detritivores). Examples of these types of feeders include mayflies such as the Heptageniidae and caddis flies (including the Glossomatidae) as well as molluscs such as water snails. If there are a large population of scrapers it indicates a large amount of organisms attached to the body which form food (**periphyton**). Interestingly the percentage of scrapers has been found to indicate a change in the nature of the habitat following oil spillage, which also results in an increase in both dead bodies and also abundant organic matter (Resh, 1994).
- *Shredders*: this group also comprises a large number of detritivores and herbivores. However these creatures feed on living and dead matter made up of coarse

particulate organic matter (CPOM). The group, however, is disturbed by water movements and so is less likely to be found where a body is trapped and the current is moved – perturbed. This group – shredders, includes many of the families of stone fly (Plecoptera) such as members of the American genus Pteronarcy (salmonflies) and the freshwater shrimps such as *Gammarus* spp (see the colour section). Tipulidae (crane flies or long horns) are members of the diptera. Their aquatic larvae feed as shredders. Members of the genus Tipula include species such as *Tipula maxima* Pod, which is found on the edges of streams and ponds in the larval stage but pupate on land. Other genera that can be found in fresh water from ponds, beneath stones or buried in sediment of flowing streams, include members of the *Dicranota* sp., and *Pedicia* sp.

- *Collectors.* This group comprises both gatherers and filterers. Both of these groups can be considered as generalist feeders. Collector-filterers include suspension feeders, which can be herbivores, carnivores, or detritivores. Such members include blackflies (Simulidae) and net-spinning caddis flies (Hydropsychidae). The food materials used by this group are the fine organic particulate matter (often termed FPOM). They can use filamentous algae to attach themselves to a particular site.
- *Gatherers.* This feeding subgroup is made up of animals that feed as detritivores and herbivores and are deposit feeders. Examples of these types of collector-gatherer feeders include the chironomids, some members of the Trichoptera (caddis flies) and the may flies (Ephemeroptera). Barbour *et al*. (1996) have pointed out that collector-gatherers are a dominant group where there is organic enrichment. This is a situation that would be found where a submerged body is breaking down under advanced decay.
- *Predators.* This feeding group is attracted by the presence of living organisms – the other feeding strategists, in association with the body (Figure 12 ab). Examples of such organisms include the dytiscid beetles, damselfly and dragonfly larvae, later stage caddis flies and several predatory beetle larvae.

Changes in the proportions of the different functional feeding groups, over time, has been found to be a valuable means of estimating the time since submergence (Merritt and Wallace, 2010). However, simply because a species in one habitat is a member of a particular feeding group may not necessarily hold true for it being present in another. The presence of a particular species is dependent on its habitat preferences. Equally food choice may alter over time or with differing circumstances. It has frequently been noted in the literature that herbivores and fruit eaters will include animal matter in their diet whilst those we ascribe to the predatory class may also include 'vegetable matter' in their diets (Buck *et al*., 2003).

Box 12.2 Organic carbon in the food web

Organic material in a water body comes either from within the water body or falls into it in the form of material of terrestrial origin such as leaves or wood and such unusual or intermittent sources as a human corpse. The dead bodies of aquatic species will also supplement this source of organic carbon – carbohydrates, fats and proteins.

In aquatic systems, particularly rivers and streams, organic carbon is captured from the sun by photosynthetic **phytoplankton** such as algae (micro-organisms) and aquatic plants, and is supplemented by the carbon released from dead organisms and as waste material. This material, in a variety of forms, is used as nutrients in a food or feeding web.

The initial breakdown of tissue provides coarse organic material (so called CPOM). Such material is initially colonised by micro-organisms, which use the material as food and release nutrients and smaller chunks of detritus from the coarse material. Solid surfaces such as leaves or the body are covered with large populations of micro-organisms which mass over the surface as a **biofilm**. Because of the breakdown of the surfaces they also release dissolved organic carbon (DOC) directly into the water, particularly where bacteria and fungi dominate such as will happen on the skin surface of a corpse, and when the gut microbial content is released into the local area. The surfaces of all the fragments of material will also leach dissolved organic carbon, increasing its availability.

Organisms that feed on the surface of the biofilm are called grazers. The breakdown products arising from this are available as large fragments. Macroinvertebrates called Shredders consume these large fragments. During feeding by this group consumed smaller fragments are produced. These are called fine particulate organic material (FPOM). Not only are the physical remnants a source of fine particulate matter – so are the waste products and faecal material from the macroinvertebrates. The organisms that consume FPOM are termed collectors. The nature of the collection of the fine organic material dictates the name of the group. Those that take up the fine particles from the bed of the water body are termed collector-gatherers; those that collect fine particulate material floating past in the water are called collector-filterers.

Each of these categories of feeders provides food for predators. Interestingly, some organisms will switch their feeding group as they pass through the life stages. Macroinvertebrates, which, when small, are collectors of the fine particulate matter can become predators once they are big enough to manipulate the other invertebrates and eat them.

The size of the populations of each of the feeding groups will depend upon the proportion of fine and coarse organic matter in the locality and also the size of the water body. Where there is a large amount of organic matter so the abundance of shredders will be large. However the availability of sunlight will dictate the abundance of the biofilm and hence the availability of grazers.

12.4 Attractants to the corpse

The submerged body produces cues that alert aquatic arthropods that it is there because of the release of different chemicals in the form of the dissolved organic carbon. Such materials can include proteins, amino acids, carbohydrates, fats and fatty acids. According to Zimmer-Faust (1987) a number of marine crustacean, like the spiny lobster or hermit crabs, for example, depend almost exclusively on chemoreception to determine the presence and appropriateness of potential food. The ratio of amino acids that diffuse out of the body and the quantities of ammonia that are generated by micro-organisms associated with the body provide an indication of its nutritional value for such organisms. Examples of the amino acids that have stimulatory effects as attractants causing orientation to the food include glycine and taurine. Concentrations of amino acids can be very low at around 10^{-11} mol l^{-1}. Adenosine triphosphate (ATP), the chemical energy source in respiration in humans, is an attractant for the spiny lobster, although for *Palaemonetes pugio* (Holthius) adenosine 5′-monophosphate (AMP) is an attractant (Carr and Thompson, 1983). The presence of ammonia, a breakdown product produced during active decay and postdecay because of protein breakdown, reduced the attractiveness of the feeding stimulants in some species such as the spiny lobster. However for some detritivores and scavengers it is an attractant.

12.5 Methods of culturing aquatic insects

The methods for culturing and maintaining the species of macroinvertebrates recovered from a corpse are discussed, along with the methods for culturing terrestrial species described in Chapter 8. In all instances, water quality and large sized tanks often with oxygenation are required.

12.6 Algae an alternative source of determining time since submergence

The body can provide a solid surface, which is rapidly colonised by periphyton such as algae where sunlight passes down through the water to provide a source of energy for photosynthesis. Over time the algae provide a food source for the scrapers and grazers that are found in the water.

Forensic entomologists recognise the advantage of a supplementary source of information by which to confirm their conclusions. Algae have been found to be a useful alternative source of information about time since submergence, particularly in the first month after the body has entered the water. Haefner, Wallace and Merritt

(2004) demonstrated the value of such organisms. This is especially true because some species of algae may colonise the body early in the decomposition, whilst others macroinvertebrates will arrive later. In all instances, because these are photosynthetic plants and hence use chlorophyll- α for food production the concentration of algae will also be reflected in the increase in concentration of chlorophyll-α. Haefner, Wallace and Merritt (2004) revealed a strong correlation with time since submergence. They did not consider that rain would influence the results by causing sloughing of the algae so this is a fairly reliable method of determining time since death.

13

The forensic entomologist in court

The final stage of the forensic entomological process is to summarise the findings and lay out the conclusions and opinions in the form of an expert's report. This is based upon the notes that you have produced as you have been carrying out the investigation. The report might have been requested for a number of purposes – including as a source of concise information on the topic, as whoever retained you will not be an expert in forensic entomology. It may also have been requested in order that the evidence may be assessed by members of the legal profession, either prosecution or defence, to determine its strength and value. The report may be used to assist in cross-examination in relation to an entomological problem that you have been asked to investigate such as the infestation of a building. This report could, at a later date, be part of proceedings in a civil court.

In each of these instances, the report has to have a logical pattern of explanation and clarity of information. If the report is sufficiently comprehensive and the conclusions are stated clearly enough, you may not need to attend court. It is therefore important that communication of the results of an investigation is clear and concise. All of the casework notes that you make as you go along should be well written and available if requested by the court. Information from the crime scene must be accurately collated and summarised and the information must be stored, together with the written records and preserved crime-scene specimens and any of those which were later preserved as the insect lifecycle progressed.

Whilst you might have carried out the investigation and written the report in response to instruction from a particular person, on all counts your responsibility is to the court if the matter is going to court – if not, then your opinion is likely to be treated confidentially. It is not to the 'party' who is instructing or paying you (Criminal Procedures Rules 33.2; Civil Procedure Rules (CPR) subsections 35 3.1. and 35.2) (http://www.justice.gov.uk/guidance/courts-and-tribunals/courts/procedure-rules/civil/pdf/practice_directions/pd_part35.pdf, accessed 3 November 2011). Nor is it your role to assist in 'proving someone guilty'. Suggestions that may assist in preparing the report in an objective manner are described in the following sections.

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

13.1 The expert's report

The style of report outlined below relates to the legal system which operates in England and Wales. It may differ in other countries.

13.1.1 Civil reports

In civil cases, the evidence used in court is generally the written report. The Civil Justice Council Protocol for the Instruction of Experts (CJPIE s13 summarising CPR 35 and PD35) (http://webarchive.nationalarchives.gov.uk/ + /http://www.justice.gov.uk/civil/procrules_fin/contents/form_section_images/practice_directions/pd35_pdf_eps/pd35_prot.pdf, accessed 3 November 2011) outlines 13 components that need to be included in a civil statement of witness. These include the need for professional objectivity, indication of disputed relevant facts, a summary of conclusions and the factual basis of the opinion expressed.

The report must also include a statement of the material instructions and information given both in writing and verbally upon which the report is based. If the opposing 'party ' wishes to ask a question relating to the report once it has been submitted, the question has to be presented in writing, within 28 days of the submission ('serving') of the expert's report.

At the end of the report a declaration is needed to indicate that the writer has complied with his or her duty to the court – including having read the 'Protocol for Instruction of Experts to give Evidence in Civil Claims' – and verifying that there are no conflicts of interest in relation to the case that remain undisclosed.

The mandatory wording of the Statement of Compliance for the Civil Court (Practice Direction for Civil Procedures Rules (PD) 35 2.2(9)) is as follows:

> I understand that my overriding duty is to assist the Court in matters within my expertise, and that this duty overrides any obligation to those instructing me or their clients. I confirm I have complied with that duty and will continue to do so, I am aware of the requirements set out in Part 35 of the Civil Procedure Rules and the accompanying Practice Direction, the Council Protocol for the Instructions of Experts to give Evidence in Civil Claims, and the Practice Direction for Pre-action conduct.

The wording of the Statement of Truth (PD 35 2.2(9) and Protocol for Experts 13.5) is:

> I confirm that I have made clear which facts and matters referred to in this report are within my own knowledge and which are not. Those that are within my own knowledge I confirm to be true. The opinions I have expressed represent my true and complete professional opinions on the matters to which they refer.

In criminal cases you must also make the following statement in the report, confirming that you know your duty is to the court:

> I understand my duty to the Court and I have complied with that duty and will continue to comply with that duty. (Criminal Procedure Rules 33.3(1) (I) and (j).)

Beneath these statements are the signature and the date the report is sent out.

Expert evidence is usually in the form of a written report. CPR 35.5 allows that, in response to that report, written questions can be asked of either a single joint expert or an expert instructed by the other side. In such a case (CPR 35.6) they can only be put once and must be submitted 28 days after the expert's report has been presented. In general, the purpose of such questions is for clarification. Such responses are considered as part of the expert's report for the civil court. If the expert does not answer the question two things can happen. The evidence from such an expert cannot be relied on in court and/or recovery of the costs of the expert cannot be recovered from anyone else.

13.1.2 Criminal reports

Section 33 of the Criminal Procedures (Amendment No. 2) Rules 2006 ('CPR 06') – 'Expert Evidence', an amendment of the Criminal Justice Act (CJ), considers expert evidence. The content of the expert report is outlined in Rule 33.3 in which 15 components are specified as part of the report content.

The criminal expert report structure has to follow a logical thread so that the nonexpert can understand it. Under the relevant Acts, the statement could potentially just be read aloud in court, unless it is directed that an oral account be given in person by the expert. The report must therefore be written very clearly and simply. Only with the agreement of the judge can expert witnesses be called to court to explain the contents of their report.

At the end of the report there is a Statement of Truth. This has a mandatory content, which is: 'I understand that my overriding duty is to the court and I have complied with, and will continue to comply with, that duty. This report is true to the best of my knowledge and belief, and I know that if it is introduced in evidence then it will be an offence wilfully to have stated in it anything that I knew to be false and did not believe to be true. (5 October 2009 (Criminal procedures update 9): Criminal procedures 33.3, Criminal procedures 27.2.)

A statement of compliance is also required. The wording for this is:

> I understand my duty as an expert witness to the court to provide independent assistance by way of objective unbiased opinion in relation to matters within my expertise. I will inform all parties and where appropriate the court in the event that my opinion changes on any material issues. (Criminal Procedures Rules 33.3(1) (i); R V Bowman para117).

It is also currently necessary, for criminal courts, where you are instructed by the prosecution, to add a statement that the guidance in the booklet *Disclosure: Expert's Evidence and Unused Material* (14 February 2006) has been read and that a list of unused material has been included in the report. A copy of the relevant declaration form is currently available at www.cps.gov.uk.

13.2 The content of the expert's report

An expert's report for both courts (civil and criminal) should have a number of sections and within it must clearly specify:

- report status (i.e. is it for evidence assessment, or to be submitted to the court?);
- to whom the report is addressed (according to the Civil Procedure Rules Practice Direction (PD) 35 Para 2, reports used for evidence should be addressed to the court, so indicate its name and location);
- the report's author (your name should be in full) and the fact that you are a forensic entomologist, and any assistance received to compile the report. In addition, indicate if you are employed in any other capacity such as a curator at a museum, or an academic, and the names and qualifications of anyone who assisted by carrying out investigations, measurements or analyses that you did not carry out personally;
- your professional details – i.e. name, professional address and contact details (telephone, fax and email);
- date – the day you sign and send out the report (sign all of the pages on that date);
- by whom you have been instructed to act as an expert witness, or whether you are acting as a joint single expert for a civil case – i.e. both the individual and the solicitors concerned, or the name of the particular police force;
- a court 'reference number' if this is relevant to the report style;
- the facts and information about the case that are relevant to you carrying out an investigation;
- a summary of the factual findings from the investigation, upon which the opinion is based;
- the range of opinions that could pertain as a result of the evidence and reasons for the specific conclusion reached (PD 35 2.2(6) Protocol para 13.12, 13.13);
- conclusions arising from the facts of the investigation that has been carried out;
- statements of truth, compliance, and disclosure.

There is currently no prescribed way of laying out the expert's report. However the comments below will assist you in preparing a clear readable expert's report, which

should address the requirements of the courts in England. Every report should have a cover sheet. For clarity a forensic entomology expert's report may be divided into sections under separate headings, starting by introducing yourself and ending with the expert's declarations followed by the annex.

The front cover sheet page (i) of the expert's report should comprise the first four points in the summary above: to whom the report is addressed; the name of the court; the instructing party and your name and contact details. This should be followed by (ii) a contents page and (iii) a glossary of technical words in alphabetical order. This should be included in the report to provide an explanation of the meaning of entomological terms.

In the text of the report, it is useful to embolden such technical words as this helps to speed up reading and assists the nonspecialists for whom you are writing, providing them with a ready source of information about meanings of the words in the glossary. The glossary of terms can be placed either after the contents page or included as part of the annex.

Next indicate your qualifications and experience (iv) to demonstrate the reason your opinion should be considered valid. This is a short summary of your academic qualifications, expertise, and experience in forensic entomology. A more comprehensive CV should form part of the annex.

The next heading (v) should be the Summary of Conclusions and your Opinion. This should comprise the conclusions about the results of the investigation and the opinion relating to the instructions you were given – for example that third instar larvae of a particular species were recovered from the dead body; that the person had last been seen alive 10 days earlier; that the time between reaching the specific instar and the eggs being laid was estimated as a minimum of 90 hours for this particular species and that the *minimum* time since death is therefore 90 hours.

Placing this summary of conclusions early in the report allows the barrister to gain a rapid overview of what your opinion is, having gained some idea of the breadth of your forensic experience, with respect to that conclusion. This will help support his/her questioning.

A short description of the background of the case (vi) – the matter upon which you have been asked to offer an opinion – should follow. This section is an outline of the relevant known facts of the case, which relate to the question being asked. The police, or person who is instructing you, will provide these details both verbally and in writing. All of this information should be included in this section.

Next come the instructions (vii) – what you have been asked to do. In this section you indicate what you were asked to do in terms of the legal case. The instructions you cite should include both those you received verbally and those that were written down. You should not stray outside these boundaries in terms of what you actually do.

This instruction section is followed by what could be described as a methodology (viii). You should provide a description of what you sampled to address the instructions; for example you might indicate that at a mortuary you sampled orifices

of the partially clothed body of a Caucasian female to recover insect larvae; or that you received photographs and specimens of 15 insects recovered from a hospital kitchen in Cholmondley and were being asked to confirm the identity of the specimens as cockroaches and to provide details of the lifecycle and the food consumed by such insects.

Descriptions of physical investigations at the locality or crime scene (ix) – what and where you sampled – follow. In this section you describe where and what you sampled on the day. In addition you refer the reader to the section where details of subsequent sampling are described in section (xi). Such samples would include the temperature recordings and soil-surveying techniques for any buried puparia; any other investigations that happened at the crime scene at the time you attended or subsequently should be included here. For example if the samples were collected in the mortuary (morgue) then this, too, is described in this section. Or for example, if this is a civil case, any investigations you subsequently undertook at a factory or urban dwelling or animal house should be cursorily mentioned in this section and explained in the later section (x).

The section on follow up investigations (x) describes any laboratory or additional investigations that you carried out, or which you requested to be undertaken. Such investigations might include additional temperature recordings taken for up to ten days at the crime scene after discovery of the body, or your request that the larval gut contents be examined to identify the source of the DNA. The section may also detail your approach to determining the different dimensions of the larvae from each sampling site to demonstrate age and so which part of the body was initially colonised. The conditions used for culturing eggs or larvae to eclosion, or through to the life stage found on the body, should also be summarised in this section. Specifying the identity of the specimens provides a logical link to help you to establish the duration of the post mortem interval using the estimated conditions at the crime scene, before the body was discovered.

Indicate in the meteorological data sources (xi) section the name of the organisation from which you sought information about temperatures between when the body was found and the time the individual was last seen. For example, did you use a local meteorological station or an amateur weather station? How far away from the crime scene were the weather details recorded? In a civil witness statement this section may not be relevant.

Under the heading Experimental Analysis of relevant Entomological Data (xii) you should state the method and references you used to investigate the ecology of the insects infesting the body, food, or the factory or domicile. (The actual reference list or copies of the papers should be placed in the annex.) For example, you may specify the titles and authors of the published papers that you used to confirm the duration of lifecycles of specific insects, and any taxonomy articles that helped you confirm the identity of the species of insect.

The next section is your deductions and conclusions about the time of death, or details about the implication of the presence of the insects you have been investigating.

The Conclusions section (xiii) – sometimes called Opinion – is where you link the issues you were instructed to address with the results you have recorded in the experimental and investigative sections of your report and describe your opinion of the implications of this evidence. It is a more comprehensive section than the summary you presented earlier in the report. You should indicate the limits of your investigation along with your estimate of the minimum time since death and an indication, where possible, of the level of accuracy of your findings.

The Experts' Declaration (xiv) is the set of declarations that indicate you know that your duty is to the court. You declare that you have disclosed any interest that you have in the case; that you have not strayed outside your area of competence; that your fees are independent of the outcome of the case and that you are aware that you recognise that you will be the subject of adverse public criticism by the judge if the court considers that you have not taken sufficient care to abide by the expected standards for being an expert.

It is valuable to indicate in this declaration the numbers of pages in the report (including the annex) in order to prevent the possibility of additions or removal of pages.

The Statement of Truth (xv) should be appropriate to the court to which the report is directed. The different formulae for use were specified earlier in the chapter.

There should be a signature and date (xvi). The report must be signed and dated by the author of the report. For the sake of continuity and integrity of evidence, it is good practice to sign and date each page (for a criminal case your signature should be witnessed) to prevent any question of pages being added or removed later.

Include in the Annex (xvii) material that helps explain additional aspects of the report – any supporting material such as a generalised lifecycle of the specific fly species that you have identified, photographs, diagrams and maps. These can be of the crime scene and of the body to indicate where the larvae were infesting the body, or if the investigation is for a civil prosecution they may be of the location and the substance in question. You should also include a list of any unused material from the investigation.

You may also choose to highlight pertinent sections from the academic references you used to draw your conclusions (a photocopy can be helpful) and provide an explanation, for example, of what physiological or thermal energy is to explain ADD and ADH.

Your actual calculations can be retained with your laboratory and crime scene records. They can be requested by the court if the need arises (Criminal Procedures Rule 33.4). So keeping a clear, readable laboratory and crime-scene notebook is essential (Table 13.1).

It is also useful to provide a timeline of the entomological activities you have undertaken to reach your conclusion. This helps you show when the samples were collected, when they reached the laboratory, your sampling regime for the life stages, when the adults emerged, or when you started to breed through to the life stage of the insect collected from the body.

If you have been assisted in undertaking the work, a list of your assistant's experience and qualifications (CV) should also be included in the annex.

Table 13.1 Contemporaneous notes checklist

Component	Content	Indicated (√)
1. Name of investigation/ examination	Title and page with appropriate sized margin	
2. Name of person making contemporaneous notes for the examination	Name indicated as being the examiner	
3. Date, Time and place	The day, date, time and crime scene location inc., GPS or map reference where appropriate	
4. Instructing officer or contact	Instructing party – indicate their name, rank and contact details (including professional address, telephone and email address	
5. Assistants names	Those working with you or conducting the assessments for you	
6. Background facts	Factual information provided so you cash interpret the best way to conduct your investigation of the matter	
7. Instructions	The written and verbal instructions which you were given in order to instruct you	
8. Materials provided	Any evidence or information such as meteorological data which was provided for you. Any samples (including item numbers) which were not collected by you	
9. Methodology	Your standard operating procedures (SOP) and an outline of any modification. You may just cite the method in a couple of sentences here and then place an exemplar of the SOP in the annex.	
10. Observations	The results of your sampling including your personal item numbers	
11. Activity time line	The time each activity starts. Or if laboratory work the time then stage was initiated)	
12. List of unused evidence – for disclosure	List any material which you examined but which was of insufficient quality to draw conclusions	
13. Check for accuracy	Read through your notes to check they say what you mean	
14. The quality of the written word and the notes	Check the English and grammar, including explaining any terminology used (or refer the reader to a glossary)	
15. Crossings out	Ensure that any amendments are made by ruling a line through the material to be excluded. Initial this change and then write the correct word(s)	
16. Rule across blank spaces	Ensure all blank spaces have a z-shaped line across them	

(*Continued*)

Table 13.1 (*Continued*)

Component	Content	Indicated ($\surd$)
17. Page numbers	Indicate the page number out of the total number	
18. Sign and date each page	Include the date and your signature at the bottom of each page of your contemporaneous notes	

13.2.1 Expert report presentation style (CPR 32 19.1)

The report should be double spaced on A4 paper (or foolscap in the USA) of a reasonable weight. The margins down each side of the page should be set at 3.5 cm so that comments can be written in them. Your report should be word processed on only one side of each page, using 14-point font so the text can be quickly and easily read.

Traditionally, in English courts, the font used is Times New Roman. The paragraphs should be numbered sequentially. This is traditional in a civil report or statement. (In a criminal report for forensic entomology, numbering the paragraphs may assist both you and the court when referring to aspects.) Dates should also be written in numbers according to the tradition of the country – i.e. in the UK this should be the day, then the month followed by the year (Table 13.2).

The report should be checked and double checked for accuracy of content and for spelling mistakes. Any inconsistencies and inaccuracies provide a target for questioning your work, or for necessitating your attendance at court. They will almost certainly be picked up, making your court experience all the more fraught!

For the sake of continuity and integrity of evidence it is good practice to sign and date each page (for a criminal case your signature should be witnessed), to prevent any question of pages being added or later removed (i.e. page 6 of 18 pages). This should include those pages in the annex. (Pages are numbered at the bottom centre of the page, for experts' reports.)

You should find out how many copies of the report are needed by the court and print out sufficient copies, ideally using a laser printer. This is better than photocopying them as it ensures that all the reports are presented professionally.

Each completed report should each be hole punched so it can fit into a lever-arch file, or become part of a court bundle. Ideally each report should be submitted bound between acetate sheets within a slip binder. This makes it easier to disassemble and for incorporation into the 'court bundle', which is often retained in a ring binder. It also means that you can handle the statement more easily if you need to refer to it in the 'witness box' and you drop pages less easily.

Should you be required to prepare a Statement of Witness for a criminal case rather than an expert's report, form MG/11 provides the structure required which conforms to Section 9 of the Criminal Justice Act 1967.

Table 13.2 Suggested headings for a forensic entomology expert's report

Page	Report Structure	Page Number
1	Title sheet *ref number, your name; date; institution name and address; name of the Court receiving the report; Instigators of the report.*	*Centre bottom state page out of total including the annex*
2	Contents page – *indicate both paragraph number and page number.*	*(sign each page)*
Paragraph 3	Introduction – *who you are; age, usually 'over 18' for an expert witness; your qualifications.*	
4	Summary Background *The nature of the case and the link to what you have been instructed to do i.e. entomological investigation.*	
5	Instructions – *what you were asked to do; by whom. Do not stray from these instructions.*	
6	Summary of Conclusions – *very short sentences to direct the reader's understanding of your facts and opinion.*	
7	Description of what you sampled at the Crime Scene - *for example if it was a crime scene with a dead body how you sampled for puparia; temperature assessments at the time; and specimens taken for additional analysis e.g. bullet residues or DNA.*	
8	Investigations at the Crime Scene – *further meteorological analysis in the 3–5 days after finding the body.*	
9	Follow-up Investigations or Research - *what other work was necessary i.e. growing up the larvae to identify species.*	
10	Meteorological Data sources *or research if for a civil case.*	
11	Conclusions/Opinion. *Clear explanation; indicate the limitations and confirm with a signature. Include a statement of truth here. For a criminal case include your statement of disclosure.*	
12	Annex – *Glossary; Life cycles; sampling and larval culturing methodology; photographs and sketches; time line; C.V.s.*	

A criminal statement is written following a similar outline to that of an expert report but, as it is intended for the criminal courts, there is a specific sequence of information at the start of the statement, which indicates who is writing the statement, their name, age and that they are making a truthful statement. The sequence of information is provided under set headings, which are:

Statement of. . .
Age if under 18
Occupation

This is followed by the Statement of Truth. The document is then signed and dated.

The body of the statement then follows. In addition to the information about the case, the body of the report is similar to the information required in an expert's report. A list of all unused case material that is in your possession must be incorporated in the statement. You are also required to indicate that you will inform all of the parties involved should you change your mind about any aspect of the material evidence.

A Statement of Witness for a case in the Civil Court has to conform to the Civil Procedures Rule 32 and the Practice Direction that relates to it. The first page of the civil statement of witness has the heading of the proceedings (PD 7.4, 20.7), indicating the names of the parties and whether they are defendants, claimants etc. The number of the statement in relation to the witness, the initials, and surname of the witness, the party on whose behalf the statement is made and the date are written at the top-right hand corner of the first page. In addition, the initials and number of any exhibits referred are also included. The rest of the statement format for the civil court is similar to that indicated for an expert's report. The Statement of Truth at the statement end is followed by the signature and date.

13.3 The forensic expert in the courtroom

A criminal court may wish to ask the forensic entomologist to attend in person to expand on the points in the report. This will certainly be the case in a mock courtroom role play. After you have taken the oath or affirmed you will first be asked to state your full name and address and to name your qualifications. Be prepared to explain the meaning of the acronyms for the qualifications that you hold.

The court may also be reassured to learn that you have been keeping up to date by regularly attending entomology and forensic entomology conferences, and contributing to learned journals. It should also be told about any membership of professional societies that you hold. You are then generally asked to summarise your findings and conclusions, after which you may be required to clarify further any points in your report either as **examination in chief** or during **cross examination**.

13.4 Communicating entomological facts in court

In a courtroom environment (mock or otherwise), you will be expected to explain your findings in a way understandable to people from a wide variety of backgrounds. Most members of the general public appear frightened by insects, expecting them to sting or bite, so their general understanding of the lifecycle of insects can be small. However, in the context of crime, the many crime-related television series have provided a useful background so that most people now know that maggots may be used to predict the time since the death of a person and the discovery of the body. Some are even aware of the concepts of complete and incomplete lifecycles.

Nonetheless, as part of the annex of report it is necessary to routinely provide details of such things as lifecycles for the particular species in question and the criteria for distinguishing maggot life stages (larval instars). It is also helpful to have ready explanations for the meanings of the words used or to use accurate but more familiar terminology in your explanations. As the expert witness in forensic entomology you should not use jargon, but sometimes the use of technical terms is unavoidable. The advice 'do not stray outside your expertise or be drawn to do this' is an important guide as you frame your responses, both in your mock court training and when acting as an expert 'for real'.

13.5 Physical evidence: its continuity and integrity

One of the crucial aspects of forensic science and especially forensic entomology is to ensure continuity of the evidence. This is also true where the life stages are cultured in order to answer questions about the post mortem interval. The crime scene itself may provide information, along with the insects inhabiting the body, so records, sketches, photographs and specimens should be taken and your sampling recorded using item numbers.

Some insect specimens only come to light after the body has been transported to the mortuary (morgue), either when the body bag is opened by other forensic scientists, or within the body on initial examination or dissection. This information must also be recorded if you, the forensic entomologist, are called to the mortuary, or later receive further specimens relating to the case.

Some forensic entomologists attend the scene in person, take samples, rear the specimens gathered from the body, and calculate the post mortem interval themselves so the evidence never leaves their possession. In this instance there are fewer problems of continuity and integrity of evidence provided that your actions and presence are logged both by you and in the scene investigating officer's log and that the two logs coincide.

However if a crime scene investigator collects the information then it is imperative that he follows a recognised protocol, records his actions faithfully and carries out the appropriate packaging procedures, separately sealing the live and preserved specimens into containers for each location, from each collecting site on the body.

These crime scene investigators (or police officers) must also ensure the integrity of the preserved samples and live cultures, keeping the cultures alive but below their base temperature, until the samples reach the forensic entomologist along with the relevant crime-scene notes.

If the entomological samples have been collected by the crime scene investigator, pathologist, or another forensic scientist, it is important that that information recorded about the contents of the packages of samples is very accurate. The samples of preserved larvae together with the live specimens from that site on the body should be packaged together and identified with an individual identity number (or, for example, a bar code), which is separate from those collected from a different location on the body.

On transporting and receiving the packages, the same records of contents, numbers and locations must be kept to ensure the integrity of the information. For example, to describe the contents of a package as three vials, each containing live larvae with liver plus three vials of preserved larval specimens collected from the corpse of J. G. Cowpit, leaves less room for challenge than solely describing the content of the package as six containers of maggots.

This integrity between the information relating to the samples and the notes about their collection is also important (Criminal Procedure and Investigation Act 1996 amendment). In every instance the specimens and sites must be photographed and these notes and photographs, along with any made subsequently in the laboratory, must all be catalogued. From this information an expert's report, or statement of witness can be generated, which will be considered accurate and comprehensive. Your laboratory notebook provides support material to demonstrate the continuity and integrity of the evidence if a question arises.

A further point to consider when presenting a report in court is the nature of the illustrations contained in your report. Photographs taken from the body, whilst illustrating where the samples were obtained, may also be quite disturbing if shown to members of the jury. When you assemble evidence to present to the court (mock or actual court) it is necessary to choose carefully the particular images that best illustrate the scientific point you wish to make. It may be helpful to give verbal warnings about the content of any photographs prior to showing them to the solicitor or barrister as well as in court. As Greenberg and Kunich (2002) also point out, in recounting the views of a Supreme Court Judge in Hawaii, the forensic entomologist should be aware that for those less familiar with corpse consumption by maggots, such pictures may influence their feelings about the individual accused of the crime. The use of black-and-white photographs may be less prejudicial.

13.6 The code of practice for experts*

A code of practice for experts was approved by the Master of the Rolls and Chairman of the Civil Justice Committee for use in civil claims and in 2006 by the Master of the

*Cited by permission of copy holder and identified as Pamplin 2011.

Rolls and President of the Queen's Bench Division for use in criminal proceedings. The code is that:

'1. Experts shall not do anything in the course of practicing as an Expert, in any manner which compromises or impairs or is likely to compromise or impair any of the following:

   (a) the Expert's independence, impartiality, objectivity, and integrity,
   (b) the Expert's duty to the Court or Tribunal,
   (c) the good repute of the Expert or of Experts generally,
   (d) the Expert's proper standard of work,
   (e) the Expert's duty to maintain confidentiality.

2. An Expert who is retained or employed in any contentious proceeding shall not enter into any arrangement which could compromise his impartiality nor make his fee dependent on the outcome of the case nor should he accept benefits other than his fee and expenses.

3. An Expert should not accept instructions in any matter where there is actual or potential conflict of interests. Notwithstanding this rule, if full disclosure is made to the judge or those appointing him, the Expert may in appropriate cases accept instructions when those concerned specifically acknowledge the disclosure. Should an actual or potential conflict occur after instructions have been accepted, the Expert shall immediately notify all concerned and in appropriate cases resign his appointment.

4. An Expert shall for the protection of his client maintain with a reputable insurer proper insurance for an adequate indemnity.

5. Experts shall not publicise their practices in any manner which may reasonably be regarded as being in bad taste. Publicity must not be inaccurate or misleading in any way.

6. An Expert shall comply with all appropriate Codes of Practice and Guidelines.' (Academy of Experts, 2005. Reproduced by kind permission of the Academy of Experts, www.academy-experts.org/codeofpractice.htm, accessed 3 November 2011.)

Currently the law has changed as the result of a decision in the Supreme Court arising from the case of Jones *v.* Kaney [2011] UKSC 13 and expert witness immunity in civil cases has been removed (Pamplin, 2011). This means that the expert is liable and can be sued by those who have instructed them if they are displeased with the outcome. For this reason it is important to have professional indemnity insurance and to ensure that not only is the expert's code adhered to but that any expert opinions that are offered are based in totality on the evidence that the expert has been retained to consider.

13.7 Use of single joint experts

The use of a single joint expert is encouraged in the civil court (CPR 35.7) and the aspects of advice in relation to instruction of experts is laid out in the Civil Procedures Rules Practice Direction 35 and the Civil Justice Council *Protocol for Instruction of Experts to give Evidence in Civil Claims* (June 2005). This does not prevent either of the parties from engaging their personal expert to advise them, however the cost of doing this cannot be recovered through the courts.

When you are instructed as a single joint expert it is important, in the first instance, that joint instructions are received. If you find that one party does not agree with the instructions and you receive separate instructions then these instructions should also have been copied to the opposing instructing party (CPR PD35.8). To ensure that you have been given all instructions from everyone it is good practice to specify the date when you expect to receive all of the instructions and to wait until that date is reached before you start work.

In all instances the key to the single joint expert's report is to outline the instructions and to seek to provide a report that clearly distinguishes the matters pertaining to the questions you are addressing and to highlight the factual evidence on all counts. The overall style of the report can remain the same as that outlined above (CPR PD35.10). On completion of the work and the report addressing all the questions it is sent simultaneously to all parties as well as the court.

13.8 Practical assignment – writing an expert report using the post mortem calculations generated from Chapter 9

You have been instructed by the police to prepare an expert report on the estimate of the post mortem interval (Assignment Chapter 9) for the body of a female found in the Pleasure Gardens, Corton-on-Sea. The trial for murder (R. *v.* Jennings) will be heard on 27 November. The suspect is one Arnold Jennings, also of Corton-on-Sea, who was arrested in May. For the purposes of the assignment, images taken by the police photographer will be submitted separately and are not be included in your report. Use the format described in this chapter to prepare your report.

13.9 Further reading on presentation in court

Civil Justice Council. 2005. *Protocol for the Instruction of Experts to Give Evidence in Court*. Institute of Expert Witnesses and Academy of Experts: London.

Greenberg B. and Kunich J.C. 2002 *Entomology and the Law: Flies as Forensic Indicators*. Cambridge University Press: Cambridge, pp. 249–283.

Hart A. J., Hall M. J. R. and Whitaker A. P. 2010 The use of forensic entomology to assist the criminal justice system. *The Barrister Magazine,* http://www.barristermagazine.com/services/free-monthly-newsletter.html (accessed 31 October 2011).
Wall W. 2009. *Forensic Science in Court: The Role of the Expert Witness*. Wiley-Blackwell: Chichester.

13.10 Web site addresses

Academy of Experts: www.academy-experts.org (accessed 31 October 2011).
Civil procedure rules, practice directions, and protocols are located at the web site of the Ministry of Justice: http://www.justice.gov.uk/guidance/courts-and-tribunals/courts/procedure-rules/index.htm (accessed 31 October 2011).
Expert Witness Institute: www.ewi.org.uk (accessed 31 October 2011).

Appendices

Appendix 1: Post mortem interval – Excel example for review exercise in Chapter 9

Species	Met data temperatures	Corrected crime scene temperatures	Base temperature °C	Degree days	∑DD i.e. ADD
A	B	C	D	E	F
Calliphora vomitoria Linnaeus	15	16.3	3	13.3	
	15	16.3	3	13.3	26.6
	14	15.4	3	12.4	39
	14	15.4	3	12.4	51.4
	14	15.4	3	12.4	63.8
	15.5	16.75	3	13.75	77.55
	15	16.3	3	13.3	90.85
	14	15.4	3	12.4	103.25
	13.5	14.95	3	11.95	115.2
	12	13.6	3	10.6	125.8
	12.5	14.05	3	11.05	136.85
	13	14.5	3	11.5	148.35
	13	14.5	3	11.5	159.85
	12.5	14.05	3	11.05	170.9

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

Appendix 2: Answer to chapter 9 review technique exercise

Calculation of the post mortem interval for the body at the Pleasure Gardens, Corton-on-Sea

The temperatures in the data are low so it is appropriate to use the experimental lifecycle estimates on experimental growth of *Calliphora vomitoria* at 12.5 °C (Greenberg and Kunich, 2002). From their figures the data for *Calliphora vomitoria* are:

Egg stage: 64.8 h average minimum duration.
LI stage: 55.2 h average minimum duration.
L2 stage: 60.0 h average minimum duration.

We do not know how long the larvae have been in the second stage so the minimum duration will be to at least the end of the first instar if the second stage had newly hatched. The egg stage (64.8 h) and the first larval instar minimum duration (55.2 h) estimates were therefore used in the calculation since this would ensure that the conclusions were based on demonstrable fact, i.e. minimum age of larva:

Total duration in hours (64.8 + 55.2): 120 h.
Experimental constant temperature used: 12.5 °C.
Base temperature chosen: 3 °C.
$\text{ADH} = \text{time}_{\text{hours}} \times (\text{temperature} - \text{base temperature})$
$\text{ADH} = 120 \times (12.5 - 3)$
$\text{ADH} = 120 \times 9.5$
$\text{ADH} = 1140\ ^{\circ}\text{H}$ (ADD = ADH/24)
$\text{ADD} = 47.5\ ^{\circ}\text{D}$

From Table A1 we can count back using the sum of the actual degree days that accumulated to estimate the minimum time since death. Each cell in column F represents the sum of the amount of physiological energy generated per day (°D) which has accumulated. So by counting the cells we find that the estimated post mortem interval is four days because the ADD we are looking for is a value of 47.5 ADD. The table value of relevance in the final column will be after 39 ADD and within the day giving a total of 51.4 ADD – i.e. the values relating to time within the day four days previous to the 20 April when the body was found. The figure of 47.5 ADD is more than the values of 39 ADD for the end of day 3 but under the value of 51.4 ADD to the start of day 5. So the estimated post mortem interval is a minimum of four days (47.5 ADD would have been achieved at some point within day 4) and a minimum time of death would be sometime on 17 April.

Appendix 3: Crime scene protocol for forensic entomology

FORENSIC ENTOMOLOGY
QUESTIONS TO BE ASKED AT THE CRIME SCENE

Location of the Crime Scene _____ **Date and Time Body Found** _____ **Name of Victim if known** _____ **Date and Time last seen** _____

Who is the collector of the specimens? _____
Date of collection _____
Who is the person instructing you to investigate the scene?
_____

What is the Scene of Death Like? i.e. is it rural or urban; **Indoors** are the windows open or closed. **Outdoors**; in shade or full sunlight; what is the vegetation like? Is the body buried or on the soil surface? (Photographs are valuable)
_____
_____
_____
_____

What is the position of the Body (Sketch)?

Is the Body Clothed? If so describe the nature and condition of the clothing or wrappings?

What is the State of Body Decomposition?

What is the Temperature: on the Body surface _____; 0.31m above the body surface _____; 1.1 m above the body surface _____; **Beneath the body** _____; Soil **temperature** 10 cm _____and 20 cm below the surface _____

Glossary

Abdomen – the third and last division of the body of an insect.

Acalypterate – a classification group describing flies with small or vestigial squamae, *Drosophila* sp. are an example of such flies.

Accumulated degree hours – cumulative sum of temperatures per hour. The figures reflect the physiological energy required by an insect to develop over the period of time stated at the temperatures recorded. The units are expressed in °H.

Acrostichal – hairs found between the rows of dorso-central bristles on the top of the thorax.

Adecticous – insects (or arthropods) that have immoveable (nonarticulated) mouth-parts. This is frequently true of the pupae and the mandibles may be reduced. Two types are known: those with appendages that do not attach to the rest of the pupal body as in most of the Brachycera (called exarate adecticous pupae) and those where the appendages are firmly stuck to the pupal body as is found in the Nematocera (called obtect adecticous pupae).

Adephaga – a suborder of Coleoptera (beetles) where the hind coxa are fixed to the metasternum and the first abdominal sternite is completely divided by the hind coxae.

Adipocere – grave wax, found on bodies in the first weeks or months after death because decomposition is taking place under damp conditions.

Algae – this is a collective name for simple plants that do not belong to the higher plant groups. Filamentous algae form long chins of cells which are initially fixed onto the substrate. An example is the Blanket weed *Enteromorpha* sp.

Allochthonous material – organic food materials that falls into the water and forms a source of energy for the organisms in the water body e.g., wood, leaves and even corpses.

Alveolus – an air sac that forms part of the lungs and in which gas exchange takes place in humans and other mammals (gives rise to the word alveolar).

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Amplify – enhance the number of sections of DNA between primers. Usually related to PCR.

Anaerobic – needs the absence of (or not dependent upon the presence of) oxygen.

Anal – positioned in the direction of the anus. Can refer to veins 6 and 7.

Antenna – A structure on the head that detects odours. It is made up of a number of segments. The first two have names. The scape – first antennal segment; the pedicel – the second antennal segment.

Appendages – limbs, for example a leg or wing that is attached to the body by a joint.

Arcadian Forest – these are temperate broadleaf and mixed forest habitats. They are found on hills, mountains and plateaus in New England and the northeast USA and in the Maritime Provinces of eastern Canada.

Archostemata – a suborder in the classification of the Coleoptera in which the larvae are mainly wood feeders.

Arista – bristle-like projection from the third antennal segment it can be feathery or with long or short hairs (pubescent).

Bacteria – enucleate (prokaryotic) micro-organisms, which in this instance are involved in decomposition.

Base temperature – the minimum temperature for development of the particular insect species.

Basicosta – the second epaulette or plate-like structure on the lead vein of the membranous wing. In *Calliphora* species the colour difference may distinguish species.

Biofilm – surface coating colonies of prokaryotic cells such as bacteria.

Brachycera – the classification group (suborder) in which the Calliphoridae are now placed, alongside such flies as the horse flies (Tabinidae) within the Diptera.

Bucca – the jowls on a dipteran head.

Caecum – a structure, which ends in a blind end or in a sac, for example a region of the insect mid-gut.

Calypter – flap of tissue (often coloured white and with black hairs). There are usually two flaps at the base of each wing and these (amongst other features) give the calyptrates their distinctive name. These flaps can also be called squamae.

Campodeiform – elongated and flattened with clearly developed legs and antennae.

Cephalopharyngeal skeleton – the internal head skeleton at the anterior end of, for example, the Calliphorid maggot, which makes up the first part of the alimentary canal and comprises the mouth hooks and an oral sclerite.

Cerci – (singular cercus) sensory appendages located at the tip of the abdomen.

Chlorophyll-α – the photosynthetic pigment, which is directly involved in the light-reaction process of photosynthesis.

Chromosome – a single DNA molecule, which is a component of the genetic makeup of the organism and morphologically apparent during cell division.

Cocaine – white crystalline alkaloid ($C_{17}H_{21}NO_4$); a narcotic.

Coleoptera – the order of insects comprising the beetles, where the front elytra are hardened or leathery and cover the membranous second pair of wings.

Collectors – this functional feeding group is made up of organisms that feed by collecting small floating particles. The material can be collected as fine fragments from sediment surfaces or can be collected in suspension from the water body. Collectors can be split into two groups: the collector filterers and the collector gatherers.

Compound eye – a collection of individual visual components (ommatidia) that combine to make a faceted eye structure characteristic of the adult.

Correction factor – in the context of PMI calculation, the formula that allows the interpretation of the crime scene temperatures from those of the local meteorological station.

Coxae – (singular coxa) the proximal segments of the insect legs – i.e. at the base of the legs, where they join to the body.

Cross examination – questioning by opposing lawyers, which takes place in the court proceedings once you have been questioned by the lawyer who called you as a witness.

Decomposition – organic matter breakdown into its constituents, in the case of the corpse by cell lysis and also micro-organisms.

Dermal – to do with the skin, where the insects in the case of myiasis are consuming the skin tissue.

Diapause – delayed development often induced in the offspring as a result of environmental conditions experienced by the mother rather than those experienced by the larva.

Diptera – the order comprising the flies.

Distal – distant from the body.

Dorsal – upper, top surface.

Dorsocentral – usually referring to bristles on the surface of the thorax. They are found to the sides of the acrostichal bristles.

Dorsum – the upper top surface and a term often used when referring to the thorax.

Electrophoresis – where an electric current is applied to a gel loaded with molecules such as nucleic acids, to separate them.

Elytra – (singular elytron) the modified, hardened front wings of the coleopteran. The elytra cover the hind, membranous wings that are used for flight where present.

Examination-in-chief – initial questioning by the lawyer who called you as a witness.

Exarate – having free appendages. This may, for example, relate to the pupal stage of the lifecycle.

Exoskeleton – the hardened, cuticular, external skeleton to which muscles are attached.

Eutrophic – a water body with high nutrient status, turbid water and high microbial loads generating a large amount of organic compounds (high primary production of chemical energy).

Exuvia – (plural exuviae) outer insect cuticle 'skin' shed at the end of each stadium.

Facultative – optional behaviour where an organism is able to adapt its behaviour to take advantage of a particular situation or food source.

Family – a classification division – i.e. genera with similar features are grouped into families. *Lucilia sericata,* for example, is in the family Calliphoridae.

Filiform – threadlike; often used to describe the structure of the antennae.

Filterers – a functional feeding group that is made up of animals which filter small fragments of food. Blackfly larvae are an example of filter feeders. Filterers comprise a subgroup of the collectors – i.e. collector-filterers.

Frass – solid insect, most often larval, waste products from digestion (another name for insect 'faeces').

Frons – the area between the antennae, eyes, and running to the top of the head of the fly.

Functional feeding groups – groups generated by classifying organisms based upon their feeding strategies; for example shredders, collectors, predators, scrapers.

Furuncular – relating to boils.

Gena – the cheek of the insect (the region below and behind the insect eye).

Gendarmerie – a police force; often French or from a French-speaking country.

Gene – inherited genetic material that is found in a fixed position or locus, on a chromosome. It is part of the 'programming' for the production of a polypeptide.

Genome – the genetic composition of an organism.

Grazers – these feed on solid surfaces from the micro-organisms attached. They are also termed scrapers.

Haltere – reduced hind wings used for balancing. They are sometimes described as resembling drum sticks as they have a knob at the distal region.

Head – the initial division of the three into which the insect body is split.

Hemimetabolus – insects where immature stages go through moults gradually becoming increasingly like the adults. Their wings develop externally.

Heteroplasmy – two or more different mitochondrial genomes in the same species.

Holarctic – the zoogeographical region which includes the northern regions of the earth. It has two sections one called the Nearctic region (North American) and the other the Palearctic region (Eurasia). The terms are used to describe insect distributions.

Holometabolus – insects that undergo complete metamorphosis.

Hypopleuron – a plate above the hind coxa and posterior to the sternopleuron (a plate (pleuron) above the middle coxa). In *Calliphora* sp. there will be a crescent of bristles, which are useful aids to identification.

Imago – the adult stage of an insect (plural imagines).

Instar – the stage between two successive splittings of the outer layers of the larval cuticle (exoskeleton) from the inner layers (endoskeleton).

ISO9000 – a suite of standards issued by the International Organisation for Standardisation to ensure quality in management systems. In this instance the context is the forensic science laboratory and the effectiveness of the standards applications are assessed externally.

Isoelectric focusing – a protein separation method using electrophoresis based on the isoelectric point of the proteins – i.e. the pH at which it has no net charge.

KAAD – a solution for killing larval specimens containing ethanol, formaldehyde and kerosene in the proportion of 10:2:1.

Kahle's solution – a solution for preserving dead larvae and for killing and preserving adult insects.

Larvae – (singular larva) collective term for the immature stages that emerge from the egg and prior to pupation.

Lateral – on or at the sides.

Lentic – standing water as is present in pools, lakes and ponds.

Lipofuscin – a yellow brown, granular residue, laid down around the nucleus arising from the digestive action of lysosomes.

Lotic – running water such as is found in streams and rivers.

Lysis – the breakdown or splitting of cells by enzymes. This takes place spontaneously after death.

Maceration – a process of soaking a specimen in a solution to soften it by breaking down parts of the tissue. The softened material will breakdown further if force is then applied to it by wave action or when using pairs of mounted needles when preparing insect spiracles for mounting.

Macroinvertebrates – a group of aquatic animals which are visible to the naked eye but not extensively large. They include aquatic insects as well as crustacea, molluscs, mites, worms and spiders.

Macrophage – cells that encircle microorganisms (act phagocitically) and remove them from an environment.

Mandibles – regions of the insect mouthparts; the jawlike structures used for biting in, for example, the Coleoptera.

Metabolism – the sum of the biochemical processes that take place in a living cell or organism.

Meron – the posterior lobe of the pleuron where the coxa joins to the side of the insect body.

Mesothorax – the second segment of the thorax.

Metamorphosis – the specific change in body form. The term can be applied, for example, to the transition between pupa and adult.

Metasternum – the under (ventral) side of the mesothorax.

Micropyle – the hole in the egg coating that facilitates fertilisation.

Mitochondria – (singular mitochondrion) a rod-like organelle found in the cell cytoplasm in which energy is produced. Mitochondria contain circular DNA that can be duplicated. It originates from the maternal parent.

Monophyletic – descended from a single ancestor.

Myiasis – injury or secondary infection caused by larvae, usually fly larvae (Diptera), feeding on living human and animal tissue.

Myxophaga – a suborder of the Coleoptera, which includes the Calyptomeridae. This suborder is of little forensic note.

Nearctic – in the context of insect distribution this indicates a region comprising North America, areas north of the Tropic of Cancer and Greenland.

Neotropical – a region that includes Central and South America including southern Mexico together with the West Indies – i.e. south of the Tropic of Cancer.

Notopleuron – the plate (pleurite) on the side of the fly body just at the end of the transverse suture.

Notum – a thoracic top surface of a segment (tergite).

Oligochaetes – a group of worms of varying morphology from the Class comprising earthworms, leeches, etc. The majority of oligochaetes feed by swallowing mud and removing suitable food particles. They move using a looping movement of body contraction and expansion. Some oligochaetes are reddish or brown in colour.

Omnivores – organisms that can feed on a wide range of food materials; in this context they feed on the corpse and the insects present on it.

Opiates – a narcotic drug that contains opium, or an alkaloid of opium.

Order – a component of the classification system. There are 29 orders of insects of which the Diptera and Coleoptera are two.

Orient – countries which are east of the Mediterranean.

Ovoviviparous – where fertilised eggs develop within the female and they hatch instantly when they are laid although this can take place within the female reproductive tract.

Palaearctic – in the context of insect distribution, this indicates a distribution throughout most of Europe, Africa north of the Sahara and the majority of Asia north of the Himalayas.

PCR – Polymerase chain reaction. The cycles of denaturing the DNA, annealing the primer and extending with the enzyme DNA polymerase, which increase the amount of the target sequence of DNA.

Pedicel – the second antennal segment (working from the head outwards).

Periphyton – microfauna (algae and diatoms) attached to sites as parts of rooted aquatic plants or the body surface and or clothing of submerged bodies.

Pharate adults – where the adult once developed is retained within a pupa for a period prior to emergence.

Pheromone – a chemical that is released and engenders a particular communication pattern in other members of the same species. Examples include chemicals that are sex attractants.

Phototaxis – directional response to light. For example *Calliphora* sp. and *Musca* sp., show negative phototaxis and crawl away from light in the feeding stage.

Phoresy – the transport of one animal by another; for example a mite by another organism such as a beetle or dragonfly.

Pleuron – the side plates of the body, where the legs are joined to the sides of the body.

Polyphaga – a suborder of the Coleoptera characterised by moveable hind coxae, with respect to the metasternum and an incomplete division of the first abdominal sternite.

Posterior callus – region on the posterior region of the top surface (dorsal surface) of the thorax in flies with calypters. It can also be called the postalar callus.

Predator – an organism that consumes live animals as its source of food – for example the Staphylinidae and the Dytiscidae are predators.

Primers – a short DNA (or RNA) sequence, which is paired with one of the DNA strands from the test organism. An available 3' hydroxyl group provides an anchor point for DNA replication to start.

Probative value – significance in terms of providing evidence or proof.

Prognathous – where the head points forward – i.e. is horizontal and the mouthparts point to the front.

Prolegs – appendages on the abdominal region of the insect body.

Pronotum – first part of the upper plate of the thorax.

Prothorax – the first segment of the thorax of the insect body.

Proximal – close to the body.

Pteridines – pigment colours that are often found in the insect cuticle, as eye pigments, and also in wing scales. They are minor excretory products and also function as co-factors for growth and development enzymes.

Ptilinum – a balloon-like sac pushed out from the head to force open the puparium and assist the emergence of the adult fly.

Ptilinal suture – the retracted ptilinum once the fly has emerged and the body has hardened. The ptilinal suture is characteristic of flies of the Schizophora.

Pubescence – covered in very short, fine 'hairs'; extensions of the cuticle.

Pulvilli – (singular pulvillus) the two pads between the claws at the end of the tarsae.

Puparium – the final coat of the larval instar, which becomes hardened and 'tanned'. Inside this casing, the pupa develops into the adult stage in the Cyclorrapha.

Pyrosequencing – a method in which the makeup of a single strand of DNA is determined using enzymes, including one that is chemoluminescent, and the detection of pyrophosphate manufacture, as the complementary strand is built up nucleotide by nucleotide in the sequence.

RAPD – Random Amplification of Polymorphic DNA. Variable bands of DNA produced on a gel after PCR amplification.

RFLP – Restriction fragment length polymorphisms. Base changes at sites as a result of restriction digestion, resulting in different-length DNA fragments.

Riffles – areas of rivers and streams in which the water is turbulent and flows rapidly (although some areas may be less rapid where the water flows around impendences such as rocks, or even bodies).

River continuum concept – this is a theory that relates to the changes in proportions of functional feeder groups as one passes from headwaters, where a lot of material such as vegetation and other matter falls into the water, through to wider water bodies where there is more light and hence a greater concentration of periphyton as the speed of the water slows on its way to the sea. In the head waters shredders predominate; downstream collector, gatherers feed off the fine particulate matter generated by, amongst other things, the shredders; in the river middle where the algae are abundant, scrapers are also abundant. This concept can be translated into the sequence of functional feeders that can feed on a submerged body.

RNA – ribonucleic acid found in the form of nuclear RNA, transfer RNA, and messenger RNA

Saponification – hydrolysis of fatty acid esters under alkaline conditions. Substances such as grave wax can be generated by this reaction in the context of body decomposition.

Scape – first antennal segment closest to the head.

Sclerotised – made up of a 'tanned protein' (sclerotin), which makes a hard, horny, outer layer of the cuticle. The structure could also be described as chitinised.

Scrapers – this functional feeding group, also known as grazers, feed on algae and diatoms that colonise fixed surfaces. These can include the skin surface or clothing of submerged organisms.

Scutum – the middle division of the top surface of a thoracic segment.

SEM/EDAX – scanning electron microscopy and energy dispersive spectroscopy. These combined techniques allow the microstructure of solid materials to be visualised and a quantitative elemental analysis to be made.

Sequester – to set aside in, for example, fat cells; it is a means of storage or protection.

Shredders – this group feeds on plant material, living or dead including wood, leaves and stems. They have mouthparts suitably modified in order to shred the plant tissue.

Single nucleotide polymorphisms (SNPs) – variation in a single nucleotide of the shared DNA sequence of a population or individual, relative to other members of the species.

Species – a group of individual organisms that can interbreed. Their offspring are fertile and resemble the parents.

Spiracle – the opening to the outside that is the end of the insect trachael system.

Squamae – (singular squama) flaps of wing tissue (often coloured white and with black hairs), there are usually two flaps at the base of each wing and these (amongst other features) give the Calyptrates their distinctive name (also known as calypters).

Stadium – (plural stadia) the stage of morphological development between two moultings, for example L1 and L2.

Sterna – ventral plates of the segments (plural; the individual plate is termed a sternite).

Sternum – the ventral segment of the insect body. A division of the sternum is called a sternite.

Subdermal – below the skin.

Surstyli – sclerotised organs, which are in pairs and together make up the lower parts of the male genitalia.

Suture – a 'seam' visible on the surface of the insect body, which indicates where two plates join. For example the transverse suture running across the mesonotum in the Diptera.

Synanthropic – associated with humans and their activities.

Taxon – (plural Taxa). These are overarching terms for groups to which organisms are assigned, based upon principles of taxonomy – for example, phylum, class, genus, and species – the top (upper or dorsal) plates of a segment, for instance, of the abdomen. An individual plate of any upper surface of the insect body is called a tergite.

Terrestrial – living or growing on the land, rather than in fresh or sea water.

Tessellated – chequered. This is a common description of the abdomen in flesh flies.

Thanatosis – appearing to be lifeless insect carcasses; 'playing' dead'.

Tibia – the leg segment between the femur and the tarsus.

Tubercles – small rounded projections. The projections on the posterior segment of dipteran larvae for example of the families Muscidae and Calliphoridae.

Urogomphi – small hardened structure at the end of the beetle larva abdomen. They are fixed and unjointed.

Ventral – from below; underneath.

Vertex – the top of the head.

Vestigial – structures that have, over time, become degenerate or reduced.

Vibrissae – large bristles located at the sides of the mouth in some fly species.

Vitellogenesis – oocyte growth by laying down egg yolk in the vitellarium of the ovarioles in the ovary.

Viviparous – females that lay live young rather than eggs. The Sarcophagidae are an example of dipteral, which are viviparous.

Zooplankton – unicellular organisms that feed on primary producers or their products; some zooplankton are capable of active swimming.

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Index

Accumulated degree days (ADD), 41, 121, 124, 127–129, 131
Accumulated degree hours (ADH), 59, 121, 122, 125–129, 133, 142
Adipocere, 33, 37, 38
Adventive species
  feeding relationships, 5
Algae, post mortem interval determinant
  Chlorophyll-α, 183
Allozymes, 23–24
American Food and Drug Administration, 3
Ants
  Pharaoh's Ant*s (Monomorium pharaonis)*, 2
Arson *see* Insects and burning
Arthropoda
  Chelicerata, 43
  Crustacea, 43, 174, 177, 182
  Hexapoda, 43, 48
  Myriapoda, 43

Basal (base) temperature
  calculation, 129
  linear approximation method, 123
Beetles, *see* Coleoptera
  Head shape *see* Coleoptera
Bergeret, M, 8
Blood spatter
  fly species, 14, 17, 18, 24, 29, 52, 55, 60, 71, 115, 137, 139, 150, 155, 190
Blowflies (*see also* Calliphoridae and Diptera)
  activity and temperature, 141, 145
  black blowfly *see Phormia regina*
  cold resistance, 141
  competition between species, 140
  daily activity pattern, 141
  distribution and climate change, 142
  greenbottles *see also Lucilia* sp.
Body
  sampling strategy, 106–108
    boiling water for fixing larvae, 107
    eggs, 106–107
    larvae, 107–108
    meteorological equipment, 109–110
    puparia, 108
Bone beetles *see* Cleridae
Burning *see also* Decomposition of the body
  degree of burning, 39
  speed of colonisation, 38, 40
Burying Beetles *see* Silphidae

Caddis flies *see* Trichoptera
Calliphoridae
  Calliphora erythrocephala, 60, 141
  Calliphora vicina, 9, 17, 23, 25, 34, 36, 53, 55, 60, 61, 79, 81, 114, 116, 122, 123, 128, 131, 132, 140, 141, 145, 155
  Calliphora vomitoria, 7, 17, 34, 36, 37, 53, 60–62, 79, 114, 123, 133, 138, 140, 141, 202

Forensic Entomology: An Introduction, Second Edition. Dorothy Gennard.

Calliphoridae (*Continued*)
characteristics, 61
Chrysomya albiceps, 64, 79, 116, 123, 143
Chrysomya megacephala, 12, 20, 26, 27, 64, 79, 140, 152
Chrysomya pinguis, 20
Chrysomya rufifacies, 20, 21, 27, 64
Cochliomyia macellaria, 17, 20, 64, 116, 143
Cynomya mortuorum, 64, 80, 144
habitat preference, 77
forests, 145
open habitats, 142
Lucilia ampullacea, 144
Lucilia caesar, 62, 79, 144, 145, 153
Lucilia cuprina, 78, 79, 141, 145, 152, 153
Lucilia illustris, 62, 79, 144, 144, 145, 152
Lucilia richardsi, 63, 144, 145
Lucilia sericata, 17–19, 26, 36, 49, 56, 60–63, 113, 114, 116, 123, 132, 144–146, 152, 153, 155
Protophormia (=Phormia) regina, 23, 64, 79, 114, 123, 140, 142, 152
Protophormia terraenovae, 19, 57, 63, 64, 79, 112, 114, 123, 143, 153
Carabidae
characteristics, 96
predators, 88, 168
Carrion beetles *see* Silphidae
Casper's dictum, 170
Cerambycidae, 1
Cheese skippers *see also* Piophilidae
Chequered beetles *see* Cleridae
Chironomidae
bloodworms, 73, 177
larvae, 71, 172
life cycle, 73
Pharate adults, 74, 136
Civil Justice Council Protocol for the Instruction of Experts, 185
Civil Procedures Rules, 185, 194, 198
Cleridae, *see also* Bone beetles
Characteristics, 88
life cycle, 164
Necrobia ruficollis, 97
Necrobia rufipes, 2, 94, 97, 156, 164–165
Necrobia violacea, 97
post mortem feeding damage resembles gunshot wounding, 165
Clothes moths, 40, 106, 151, 177
Clown beetles *see* Histeridae
Coleoptera
Adephaga, 88, 89, 96
Carabidae, 27, 87, 88, 96, 99, 157, 168
Dermestidae, 2, 87–89, 93, 100, 117, 118, 157, 159, 161, 163, 164
Dytiscidae, 88, 99, 177
Elytra, 82–84, 91, 94, 96, 100
Gyrinidae, 88, 99, 177
hypognathous head, 82
mandibles, 82
Polyphaga, 88, 89
prognathous head, 82
Contamination, 15, 101, 150
bleach, 15
crop excision, 16
freezing, 15
samples, 15, 101
Copra Beetle *see Nebria rufipes*
Criminal Procedures Rules
Disclosure, 184, 187
Expert's declaration
Statement of compliance, 185, 186
Statement of truth – civil court, 186, 190, 193, 194
Culturing insects,
Diet, 113, 115
laboratory, 111, 112
storage in transit, 107, 111
Cuticular hydrocarbons (*see also* Molecular Identification of insects)
Phormia regina, 23, 64, 79, 114, 123, 140, 142, 152
Cytochrome C *see also* Mitochondria,
COI, 13, 15, 17–22, 97
COII, 13, 17, 19–21, 97
geographic variation, 13, 18
Sub-units I and II, 13

Decomposition of the body
buried bodies, 34–36, 41, 147, 150
colonisers, 36
depth, 36
exhumation, 35

burned bodies, 39
hanging bodies, 39
on land, 34–35
anaerobic fermentation, 34
decomposition in confined spaces, 38
vehicles, 38
effects of wrappings, 40
fatty acids, 37, 38
Grave wax (adipocere), 33, 37
mummification, 32, 33
nutrient-rich islands, 30
post mortem submerged interval, 175
stages of decomposition, 30–36
time of decomposition in relation to temperature formula, 29
in water, 29
algae, 73
Decomposition Volatiles
ammonia, 37
2-butanone, 37
decomposition odour differences between species, 37
dimethyl disulphide, 37
dimethyl trisulphide, 37
ethanol, 37
nonanal, 37
octane, 37
2-propanone, 37
Volatile detection of buried bodies, 41
LABRADOR, 41
Dermestidae, *see also* Coleoptera
characteristics, 88, 159
Dermestes frischii, 159
Dermestes lardarius, 93, 117, 159, 162
Dermestes maculatus, 93, 94, 117, 159–163, 165
Frass as a PMI indicator, 160
pheromones, 161
reproduction, 161
Diapause, 115, 141
maternal induction, 141
Diptera (true flies),
Calliphoridae, 2, 14, 34, 36, 38, 39, 49, 52, 60, 69, 77, 140, 142, 147, 151
Fannidae, 36, 52, 68
Muscidae, 2, 36, 37, 48, 52, 67, 68, 146, 147
Phoridae, 2, 36, 51, 67, 149, 151
Piophilidae, 24, 52, 66, 67, 148, 150, 151, 153, 156, 165
Sarcophagidae, 2, 20, 21, 36, 52, 55, 64, 65, 78, 140, 147, 148, 151
Stratiomyidae, 69, 70, 116, 147, 151
Tipulidae, 71, 180
Trichoceridae, 50, 70, 71, 150, 151. *see also* winter gnats
DNA
barcoding, 22–23
barcoding for Life (BOLD-IDS), 22
DNA concentration, 16
magnetic fields, 41
extraction, 15, 16
Chelex®, 16
DNAzol®, 16
DNeasy® tissue kits, 16
human identity, gut contents, 15
insect identification, 7
Drosophilidae
Drosophila melanogaster, 3
Drosophila yakuba, 14, 18
Dung flies *see* Spheroceridae
Drugs
amitriptyline, 24–26
aspirin, 26
Arthropod use for extraction, 10, 27–28
barbiturates, 25
phenobarbital, 25
benzodiazepines, 25
diazepam, 24
oxazepam, 25
triazolam, 25
cannabis 27
cocaine, 25, 27
opiates
morphine, 24–26
predatory insects value for recovering drugs, 27
steroids
hydrocortisone, 26
variation in insect tolerance, 26
victim's previous drugs, 26

Eburia quadrigeminata see Longhorn Beetles
Eclosion – egg, 26, 55, 189
Egg laying
  Ephemeroptera, 73, 172, 173, 180
    Mayflies, 73, 179
  life cycles, 117, 144
  night, 148
  and temperature, 40, 118, 142, 144
Entomological equipment
  ethyl acetate, 15, 101
  forceps, 75, 76, 101
  labels, 75, 76, 101, 106–107
  sampling jars, 101
Ethyl acetate *see also* preservation of insects
European Association for Forensic Entomology (EAFE), 9–11
Exhumations and drugs, 24, 35 (*see also* diazepam, amitriptyline, morphine)
Expert's Report
  Code of practice for experts – Civil Justice Council, 185, 198
  presenting in court
    cross examination, 194
    examination in chief, 194
  purpose, 186, 198
  structure, 186
Expert witness testimony, 10, 186, 187, 195, 197–198
Explosives, *see also* landmine detection
  Arthropods, 27, 28
  Stealth Insect sensor project, 28
    TNT (trinitrotoluene), 28

Fannidae, 36, 52, 68
  Fannia canicularis, 69, 153
Feeding relationships
  collectors, 113, 179–181
  grazers, 179, 181, 182
  necrophages, 4, 5, 89, 99, 134, 143
  omnivores, 6
  opportunists, 6, 7, 100
  predators, 5, 27, 34, 36, 74, 88, 89, 91, 94, 105, 134, 143, 165, 168, 177, 179–181
  scrapers, 174, 179, 182
  shredders, 178–18
Fleas, 172, 173
Fleshflies *see* Sarcophagidae
Flight
  distance, 145
  weather, 146
    rain, 141
    wind, 141
Food infestations *see* Urban forensic entomology
Frass, 160
Functional feeding groups
  aquatic forensic entomology indicators, 17, 170
  collectors, 113, 179–181
  filterers, 179–181
  predators, 5, 27, 34, 36, 74, 88, 89, 91, 94, 105, 134, 143, 165, 168, 177, 179–181
  scrapers, 174, 179, 182

GenBank, database, 14, 15, 20, 21
Ground beetles *see* Carabidae

Habitat, blowfly distribution, 144
Hanging, 39, 169
Health and safety, 112
Hide (skin) beetles *see* Dermestidae
Histeridae
  Arcinops pumilo, 163
  characteristics, 88
    night feeder, 163
  indicator species, 164, 165, 179
History of forensic entomology
  Chinese, 6
  La faune des cadavres: Application de l'entomologie à la médicine légale, 8
  Linnaeus, 7
  Mégnin, 8
  Redi, 7
  Ruxton, Buck, 9

Identifying life stages, 26, 29
Insect classification
  beetles (Coleoptera), 88

flies (Diptera), 35
acalypterate, 52
Brachycera, 49– 51
calyptrate, 52
Nematocera, 49, 51, 70, 71
Insect damage
gunshot, 165
Insect growth
effects of refrigeration, 143
linear relationship, 59, 122
Insect morphology
abdomen, 34, 42, 43, 60, 61, 63–65, 68, 72–74, 80, 87, 97, 99, 100, 135, 158, 163
sternites, 63, 82, 88, 90, 96, 161
tergites, 82
antenna, 13, 43, 47, 48, 51, 52, 62, 67, 69, 71, 96, 99
of beetles, 83
of flies, 12, 56
legs, 35, 42, 43, 46, 63, 64, 66, 67, 70, 72–74, 83, 85, 88, 89, 91, 92, 96–100, 106, 135, 139, 158, 164
leg bristles, 45
ptilinum, 52, 58
tarsal formulae, 96
thorax, 15, 42–44, 51, 52, 60, 61, 64, 65, 67, 68, 72–74, 80, 82, 83, 88, 89, 94, 99, 100, 163
scutellum, 83
wing structure, 47
vein names, 46
Insects and burning, 39
oviposition, 39, 40, 55, 77, 115–117, 140, 141, 143, 147
Isomegalen – diagrams, 130, 132
Isomorphen – diagrams, 130, 132
ITS (Internal Transcribed Spacer region), 16, 20, 195

Kahles solution, 16, 101, 102, 113

Landmine detection, 28
bees, 28
Latrine flies *see* Fannidae
Lice
survival in water, 172, 173
Longhorn beetles, 1

Macroinvertebrates, 1, 108, 109, 119, 120, 170, 174, 179, 181–183
Chironomidae, 17, 71, 72, 171, 174, 179
Crustacea, 43, 174, 177, 182
Maggot mass, 107, 108, 128, 130, 143, 146
effect on post mortem interval calculation, 128, 130
heat shock proteins, 141
Marchenko, 122, 125
Mayflies. *see* Ephemeroptera
Midges–non biting *see also* Chironomidae
Mites, 1, 6, 43, 106, 135, 136
Mitochondria, 12
Cytochrome C, 12, 13
Mitochondrial DNA, 12–14, 17, 64, 97
Cytochrome Oxidase (COI), 13, 14, 17, 18, 20, 22, 41, 97
haplotype, 13, 18
Molecular identification of insects,
allozymes, 23
base pairs, 12–15, 20–22, 97
Blast searching, 15
C-banding, 12
cuticular hydrocarbons, 23
GenBank, 14, 15, 20, 21
Lipofuscins, 24
Mitochondrial DNA (mtDNA), 12–14, 17, 64, 97
ND4 (Nicotinamide Adenine Dinucleotide Dehydrogenase), 20
nuclear material, 17, 19
SNP (Single nucleotide polymorphisms), 22
validity of methodologies, 21–23
voucher specimens, 15, 22
Mummification, 32, 33
Muscidae, 2, 36, 37, 48, 52, 67, 68, 146, 147
characteristics, 36, 67, 68
Hydrotaea, 35,. 37, 68, 137, 146, 147
Hydrotaea carpensis, 146
Hydrotaea dentipes, 38, 146
Hydrotaea ignava, 146, 147
Musca autumnalis, 37, 68
Musca domestica, 67, 68, 146, 152–154
Ophyra, 19, 35, 146, 147

Myiasis, 78, 145, 147, 151–154 *see also* physical abuse
biological, 152
Piophilidae, 24, 52, 66, 67, 148, 150, 151, 153, 156, 165
Piophila casei, 66, 67, 114, 148, 153
Wound, 78, 152

Nanotechnology, 28
National Research Council of America, 9
Mandatory Standards, 9
Standardised Protocols, 9, 22
Necrobia rufipes, 2, 94, 97, 156, 164, 165
Necrophages, 4, 5, 89, 99, 134, 143
Nitidulidae, 35, 87, 89, 94, 96, 100, 117, 157, 163
characteristics, 96
dry stage indicators, 163
North American Forensic Entomology Association (NAFEA), 9, 11
North American Board of Forensic Entomology, 9
Nucleotide Bases, 13, 20
adenine, 13, 18, 20
cytosine, 13
guanine, 13
thymine, 13

Odours and corpse location
butyric acid, 30
caseic acid, 34
colonisation, 8, 29, 34, 36, 38, 39, 40, 103, 120, 132, 134, 139, 140, 150, 153, 154, 156, 164, 170
insect signalling, 158, 161
Office of the Forensic Science Regulator (UK), 9, 10
advice and guidance, 9
criminal justice system, 10
ISO9000, 11
quality assurance, 11
standards monitoring, 11
United Kingdom Accreditation Service (UKAS), 11
Omnivores, 6, 74
Opportunists, 6, 7, 100 *see also* Adventive
Oviposition *see* egg laying
and decomposition, 39, 40
locations on corpse, 8, 37

Phaenicia sericata see Lucilia sericata
Pharaoh's Ants *(Monomorium pharaonis)*, 2
Phoresy, 135
Phoridae
characteristics, 67
Conicera tibialis, 150
Megaselia giraudii, 150
Megaselia rufipes, 150
Megaselia scalaris, 150, 152
Triphleba hyalinata, 150
Physical abuse, indications, 152
Physiological energy budget *see also* basal temperature
Piophilidae
characteristics, 67, 150
Piophila casei, 66, 67, 114, 148, 153
Stearibia nigriceps, 165
'Playing dead' *see* Thanatosis
Plecoptera, 73, 74, 180
Perlidae, 74
Perlodidae, 74
predators, 74
Poisons (*see also* drugs)
agricultural chemicals, 27
organophosphates, 27
heavy metals, 24, 25, 27
arsenic, 24
mercury, 24, 27
suicide, 25, 27, 38, 39, 144
PCR (Polymerase chain reaction), 14, 16 (*see also* mitochondrial DNA)
Calliphoridae, 2, 14, 34, 36, 38, 39, 49, 52, 60, 69, 77, 140, 142, 147, 151
late-PCR, 21
PCR-RFLP, 17
non-biting midges, 17, 71
primers, 14, 16, 17, 18, 21
Post mortem interval (PMI), 17, 26, 29, 33, 39, 40, 50, 53, 58, 59, 106, 121, 122, 126, 128–130, 132, 133, 137, 138, 142, 146, 148, 150, 157, 163, 170, 173, 179, 189, 195, 198, 201, 202 *see also* time since death

estimating, 33, 122, 130, 132, 148, 198, 202
factors affecting, 40, 50
insect succession for PMI determination, 38, 132, 133, 136, 163
isomegalen diagrams, 130, 132
isomorphen diagrams, 130, 132
larval length for PMI determination, 40
linear approximation method, 123
movement of the corpse, 134
sources of error, 128–130
Post mortem submergence interval (PMSI) *see* Time since submergence
Practice Direction, 184, 185, 187, 194, 198
Civil Court, 186, 194,198
Predators, 5, 27, 34, 74, 88, 89, 91, 94, 105, 134, 136, 143, 165, 168, 177, 179, 180, 181
Preservation of insects
ethyl acetate, 15, 101
Kahle's solution, 16, 101, 102, 113
packaging and labelling, 102, 103, 153, 195
Professional Entomological Associations
European Association for Forensic Entomology, 9, 10
North American Forensic Entomology Association, 9
Psychodidae, 2, 24, 151
causal agent of myiasis, 152, 153
Pupa
adecticous, 87
exarate, 87
obtect, 87
Pyrosequencing, 16, 20, 21
automation, 16, 21
Sarcophagidae, 20, 21,

RAPD (Random amplified polymorphic DNA), 16–18, 97, 98
disadvantages, 18
false positives, 18
geographic variation, 18
RFLP(Restriction fragment length polymorphism), 16, 17, 20, 97
Rivers *see* Water Bodies
RNA analysis, 16
cytoplasmic RNA, 19
16S, 19
sedimentation, 19
messenger RNA (mRNA), 19, 41
ribosomal RNA (rRNA), 13, 19, 20
transfer RNA (tRNA), 13, 19
tRNA-leucine genes, 17, 19
Rove beetles *see* Staphylinidae
Ruxton, Buck, 9

Sampling the crime scene, 101–110
adults, 104–106
cars, 102, 104
eggs, 106–107
larvae, 107–108
night, 101
pupae and puparia, 108
soil, 105, 106, 108, 110
temperature, 102–104, 107–110
Sap-feeding Beetles *see* Nitidulidae
Sarcophagidae, 2, 20, 21, 36, 52, 55, 64–65, 78, 140, 147, 148, 151 *see* fleshflies
characteristics, 20, 65, 78
Neobellieria (=*Sarcophaga*) *bullata*, 148
Sarcophaga (Robineauella) caerulescens, 148
Sarcophaga africa (=haemorrhoidalis), 148
Sarcophaga carnaria, 2, 7
Sarcophaga cooleyi, 148
Sarcophaga scoparia, 148
viviparous, 55, 147
Scarabaeoidae, 166
classification, 166
Geotrupidae, 166
Scarabaeidae, 167
Trogidae, 166–167
forensic indicators, 166
can move bodies by burrowing beneath, 165
Scuttle flies *see* Phoridae
Searching the crime scene, 106
Sepsidae, 52, 66, 150
characteristics, 66

Sheep strike *see* myiasis
Silphidae, 89–90, 97, 100, 117, 133, 157–158
  characteristics, 157
  classification, 157
  Hyponecrodes erythrura, 156
  Microspores sp., 117
  Necrophila(=Silpha) americana, 87, 157
  Nicrophorinae, 157, 158
  Nicrophorus defodiens, 158
  Nicrophorus humator, 35, 90, 157, 158
  Nicrophorus orbicollis, 158
  Nicrophorus vespillo, 157, 158
  Nicrophorus vespilloides, 90, 118, 157, 158
  Oiceoptoma thoracicum, 97
  Oxyletrum discicollis, 157
  preference for corpse size, 157
  preference for soil type, 158
  semiochemicals, 158
  Thanatophilus sinuata, 157
Simulidae, 71, 72, 120, 180
  blackflies, 180
Skin beetles *see* hide beetles
Sphaeroceridae, 52, 69, 147
  associated with advanced decay, 147
  characteristics
    Leptocera caenosa, 147
    Minilimosina parvula, 147
    Telomerina eburnea, 147
Staphylinidae, 34, 88, 89–91, 100, 157, 165, 166
  characteristics, 90
  Creophilus maxillosus, 27, 91, 165
  Oxyletrum discicollis, 157
  Philonthus longicornis, 91, 165
  predators of insects feeding on the corpse, 91, 165
Statement of compliance, 185, 186
Statement of truth, 186, 190, 193, 194
  purpose, 186
  structure, 186, 192
Stoneflies, 74, 172, 173, *see also* Plecoptera
  carnivorous larvae, 74
  lifecycle, 74
Stored Product pests, 1, 2, 159, 164
  Dermestes maculatus see Dermestidae
  Dermestes lardarius see Dermestidae
  Plodia interpunctella, 3
  Tribolium castaneum, 3
  Tribolium confusum, 3
Stratiomyidae, 69–70, 116, 147, 151
  aquatic larvae, 69
  Hermetia illucens, 69, 70, 116, 147
  soldier flies, 69, 147
Suicide, 25, *27*, 38, 39, 144 *see also* poisons
  hanging, 39
Sung Tz'u, 6
Succession of insects, 8, 29, 40, 132, 133, 135
  buried corpses, 133
    Conicera tibialis, 150
  exposed corpses, 8, 40, 132, 133
  PMI determination, 136
  rural habitats, 156, 157, 160
  urban habitats, 131, 135

Tarsal formulae *see* insect morphology
Temperature
  recording at the crime scene, 7, 102–104, 107–110
  recording in the laboratory, 12, 15
  meteorological stations, 110
Thanatosis (playing dead), 91, 93, 160, 163
Thermal units *see* physiological energy budgets
Time since death, 7, 19, 24–26, 28, 29, 39–41, 43, 109, 111, 122, 132, 134, 135, 136, 138–140, 146–148, 151, 154, 163, 168, 173, 183, 188, 190, 202 *see also* post mortem interval
  calculations, 147, 148
  correction factors, 121, 131, 138
  sources of error, 128–130
    maggot mass, 128, 130
    soil temperature, 130
Time since submergence, 169, 170, 179, 180, 182, 183
Tipulidae, 71–72, 180, *see also* Nematocera
  Dicranota spp., 72
  Pedicia spp., 72
  Tipula, 72, 180
Trichoptera, 73, 74, 179, 180
  Caddis flies, 74, 179, 180

Trichoceridae (winter gnats), 50, 70, 71, 150, 151
  mating behaviour, 140
  member of the Nematocera, 50, 70, 71
  Trichocera annulata, 151
  winter coloniser useful for PMI determination, 133, 134
Trogidae, 89, 91–92, 157, 166–167
  dry stage indicators, 93, 166, 167
  seasonal indicators, 167
  stored product pests, 1, 2, 159, 164

Urban forensic entomology, 3, 136, 147
  compost heaps, 3
  Environmental Health, 3
  longhorn beetles, 1
  structural timber, 1
Urogenital myiasis, 153

  Fannia canicularis, 69, 153
  Musca domestica, 67, 68, 146, 152–154
  Sylvicola fenestralis, 153

Water bodies, 108, 110, 176, 177
  lakes, 74, 169, 176–178
  ponds, 109, 119, 176, 180
  river continuum concept, 178
  rivers, 9, 72, 74, 169, 175–178, 181
    riffles, 119, 175, 176
Wildlife trusts, 109, 134
Winter gnats *see* Trichoceridae
Wing veins, 46, 51, 67–69, 71
  Costa, 46, 62, 64
  Medial, 46
  Radial, 46, 71
  Subcosta, 46
Woolly bears, *see also* Dermestidae

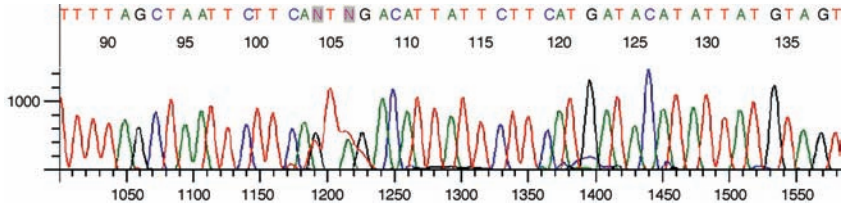

Plate 2.1 Electropherogram

Plate 3.1 Decomposition of a human body

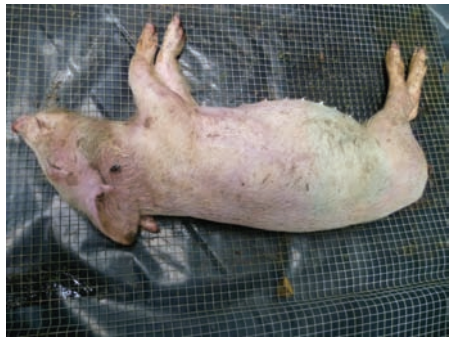

Plate 3.2 Fresh stage of decomposition

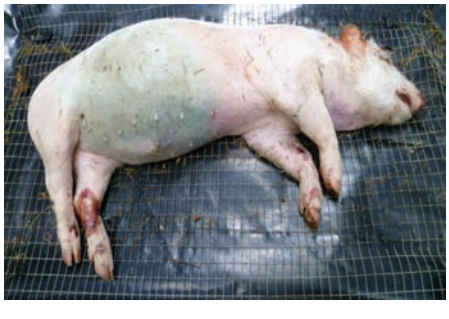

Plate 3.3 Bloat

Plate 3.4 Active stage of decay

Plate 3.5 Advanced stage of decay

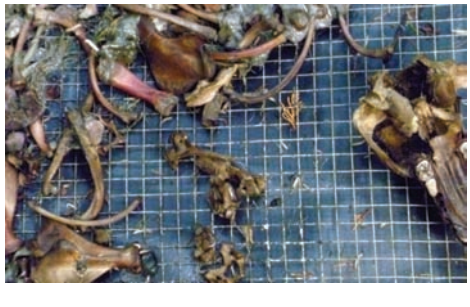

Plate 3.6 Skeletal remains

Plate 3.7 A cadaveric island surrounding the body where vegetation has died away

Plate 4.1 A Tabanid larva

Plate 4.2 Emerging calliphorid with inflated ptilinum

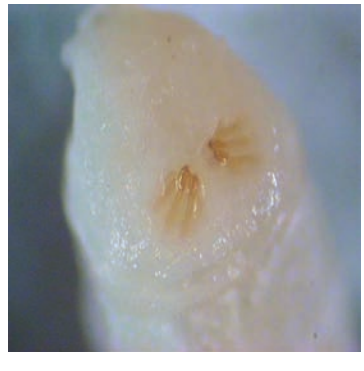

Plate 4.3 Transition from larval stage L2 to larval stage L3

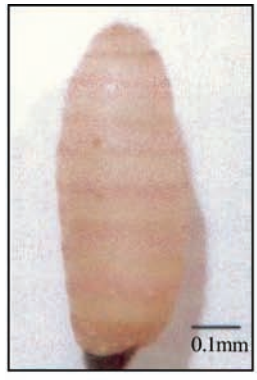

Plate 1: 0 Hour puparium

Plate 2: 3 Hour puparium

Plate 3: 6 Hour puparium

Plate 4: 9 Hour puparium

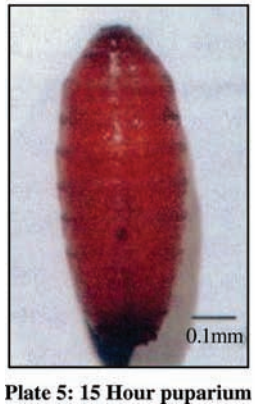

Plate 5: 15 Hour puparium

Plate 6: 25 Hour puparium

Plate 4.4 Puparial colour changes in *Calliphora vomitoria* Linnaeus within the first 25 hours of pupariation

Plate 4.5 A pair of Piophilids taken ‘in cop’

Plate 4.6 Head of *Piophila casei* Linnaeus

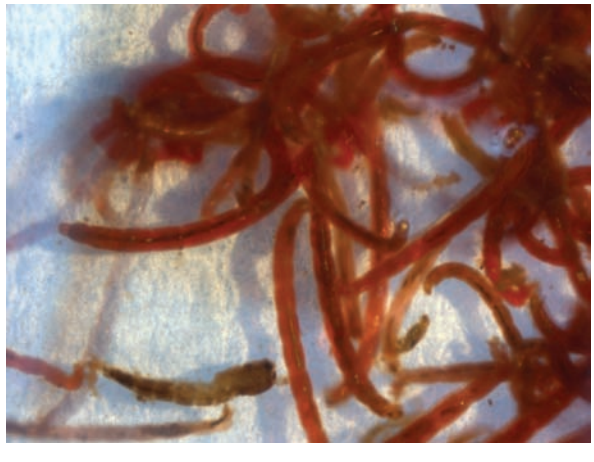

Plate 4.7 The Chironomidae

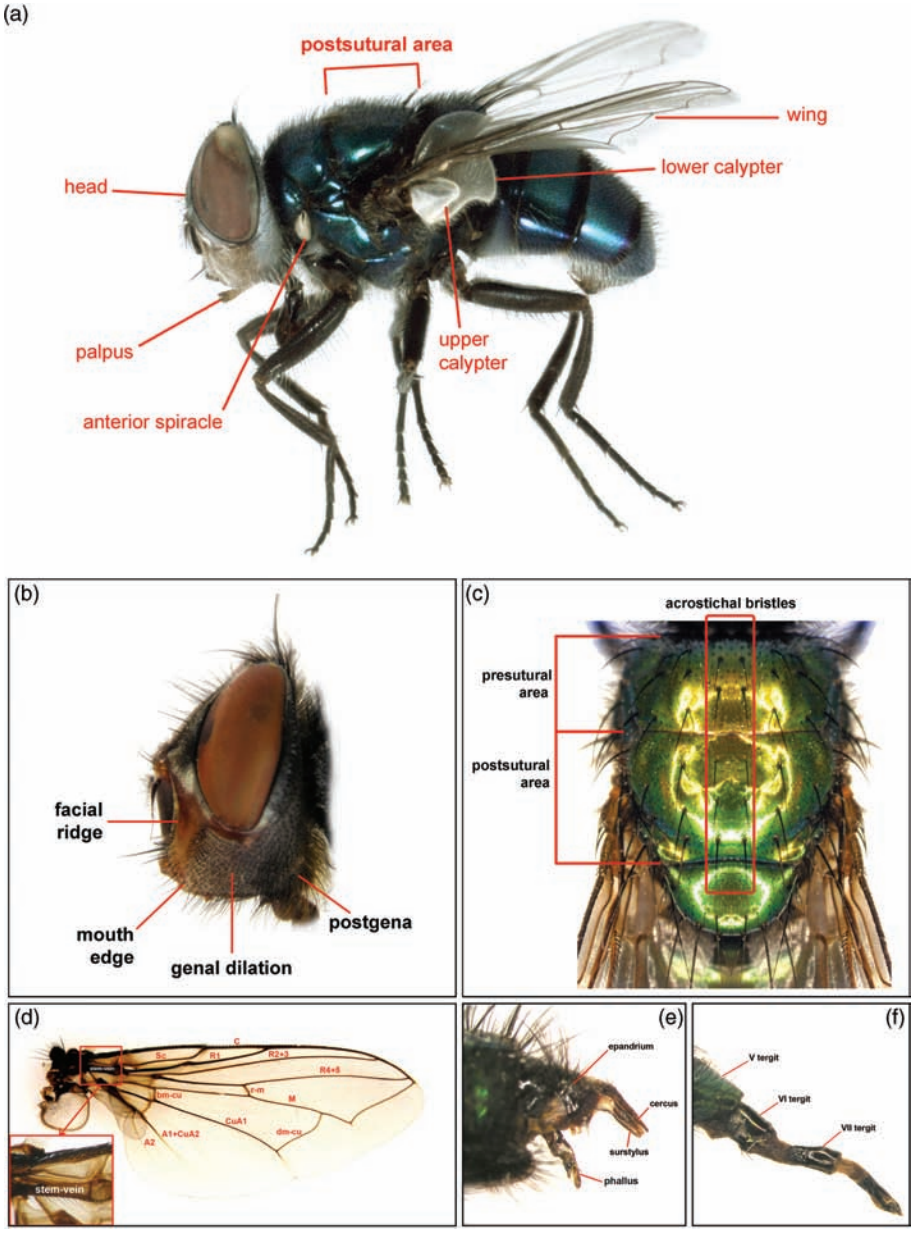

Plate 5.1A–F General morphology of blowflies. A – habitus, *Chrysomya albiceps*, male; B – head profile, *Calliphora uralensis*; C – thorax, dorsal view, *Lucilia sericata*; D – wing, *Protophormia terraenovae*; E – male terminalia, *Lucilia bufonivora*; F – ovipositior, *Lucilia illustris*. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

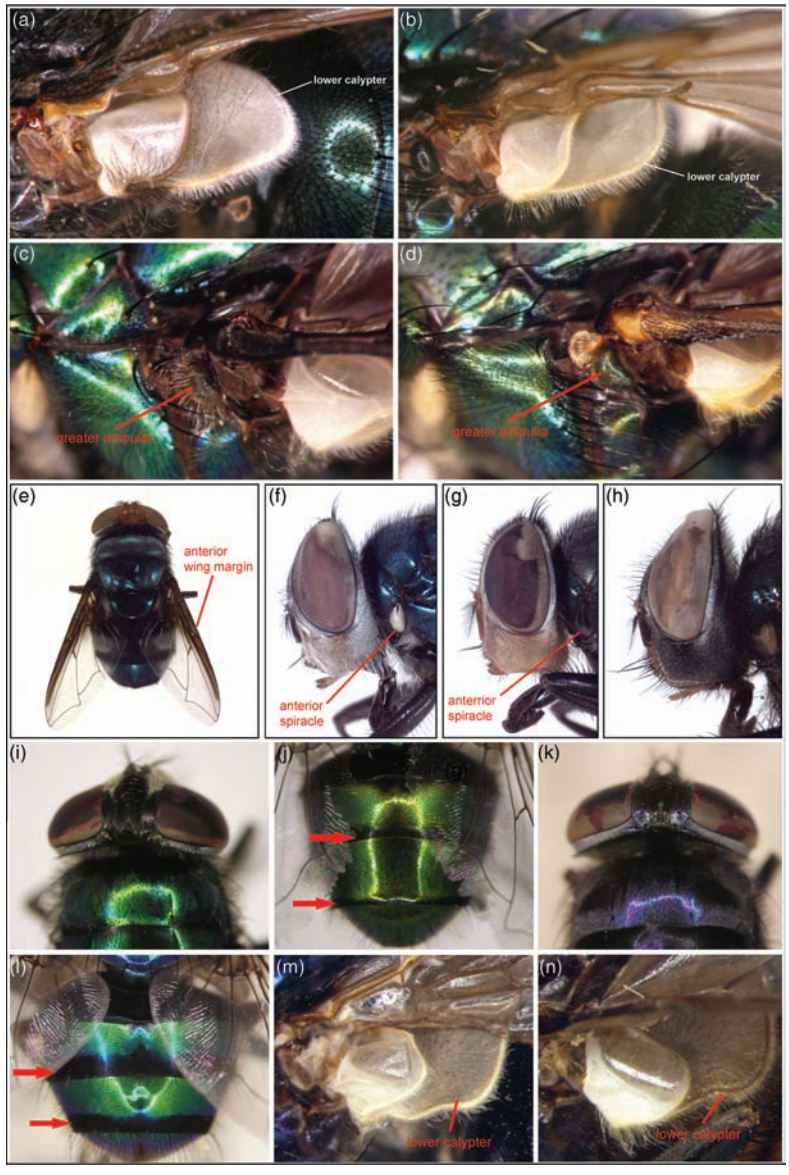

Plate 5.2A–N Details of morphology of blowflies. A – calypters, *Cynomya mortuorum*; B – calypters, *Lucilia caesar*; C – greater ampulla, *Chrysomya albiceps*; D – greater ampulla, *Cochliomyia hominivorax*; E – habitus, dorsal view, *Chrysomya marginalis*; F – head profile, *Chrysomya albiceps*; G – head profile, *Chrysomya megacephala*; H – head profile, *Phormia regina*; I – anterior part of thorax, *Chrysomya albiceps*; J – abdomen, *Chrysomya albiceps*; K – anterior part of thorax, *Chrysomya putoria*; L – abdomen, *Chrysomya putoria*; M – calypters, *Chrysomya bezziana*; N – calypters, *Chrysomya megacephala*. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

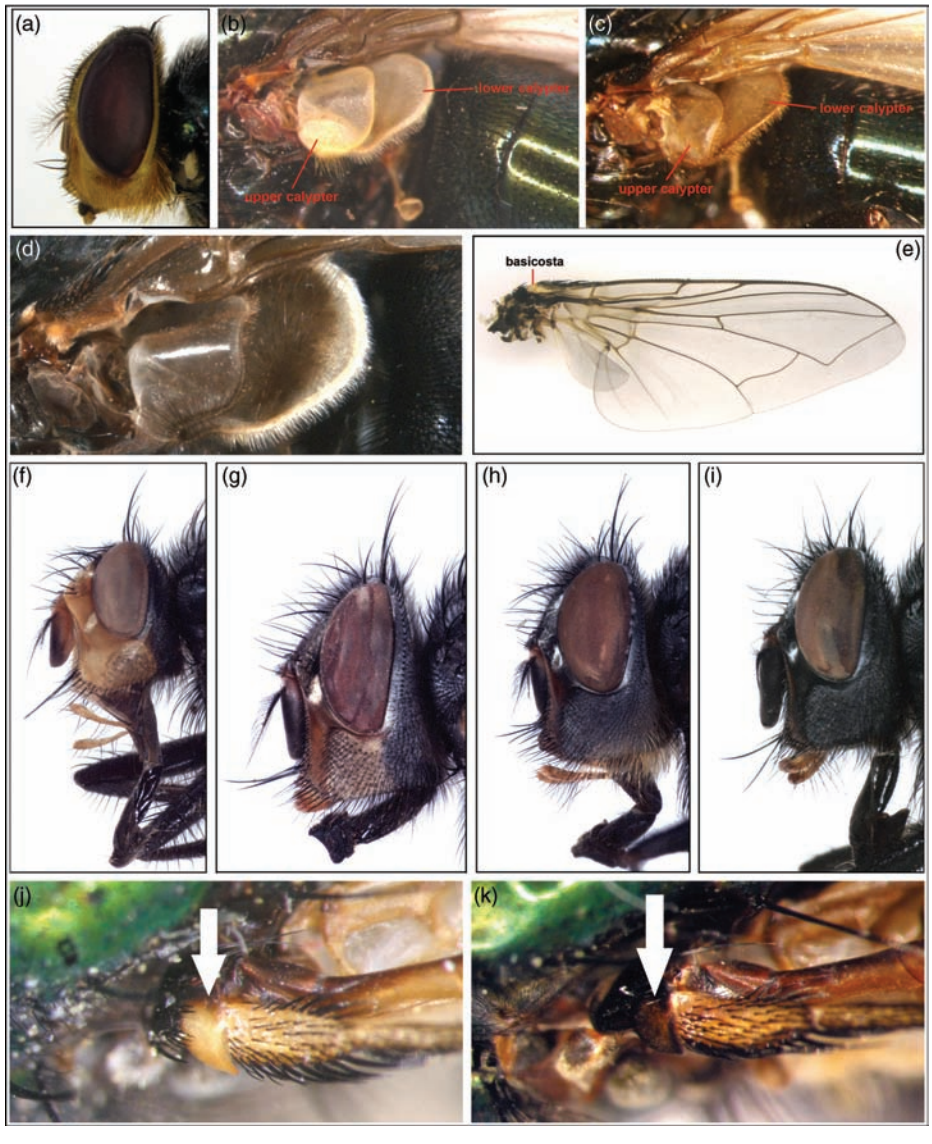

Plate 5.3A–K Details of morphology of blowflies. A – head profile, *Cochliomyia hominivorax*; B – calypters, *Phormia regina*; C – calypters, *Protophormia terraenovae*; D – calypters, *Calliphora vicina*; E – wing, *Lucilia sericata*; F – head profile, *Cynomya mortuorum*; G – head profile, *Calliphora vicina*; H – head profile, *Calliphora vomitoria*; I – head profile, *Calliphora loewi*; J – basicosta, *Lucilia sericata*; K – basicosta, *Lucilia caesar. Source:* Reproduced by permission of Dr. Krzysztof Szpila

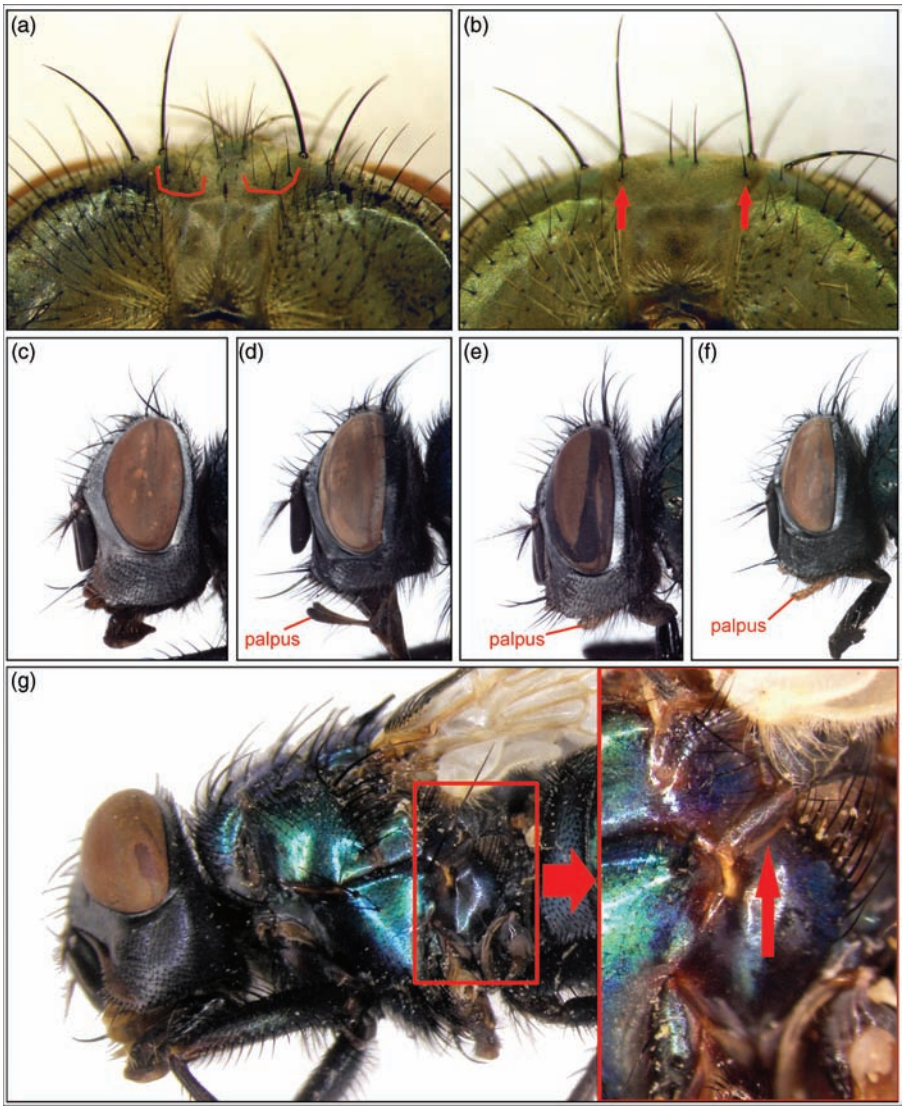

Plate 5.4A–G A – occipital area, *Lucilia sericata*; B – occipital area *Lucilia cuprina*; C – head profile, *Lucilia sericata*; D – head profile, *Lucilia silvarum*; E – head profile, *Lucilia caesar*; F – head profile, *Lucilia illustris*; G – coxopleural streak, *Lucilia caesar.* *Source:* Reproduced by permission of Dr. Krzysztof Szpila

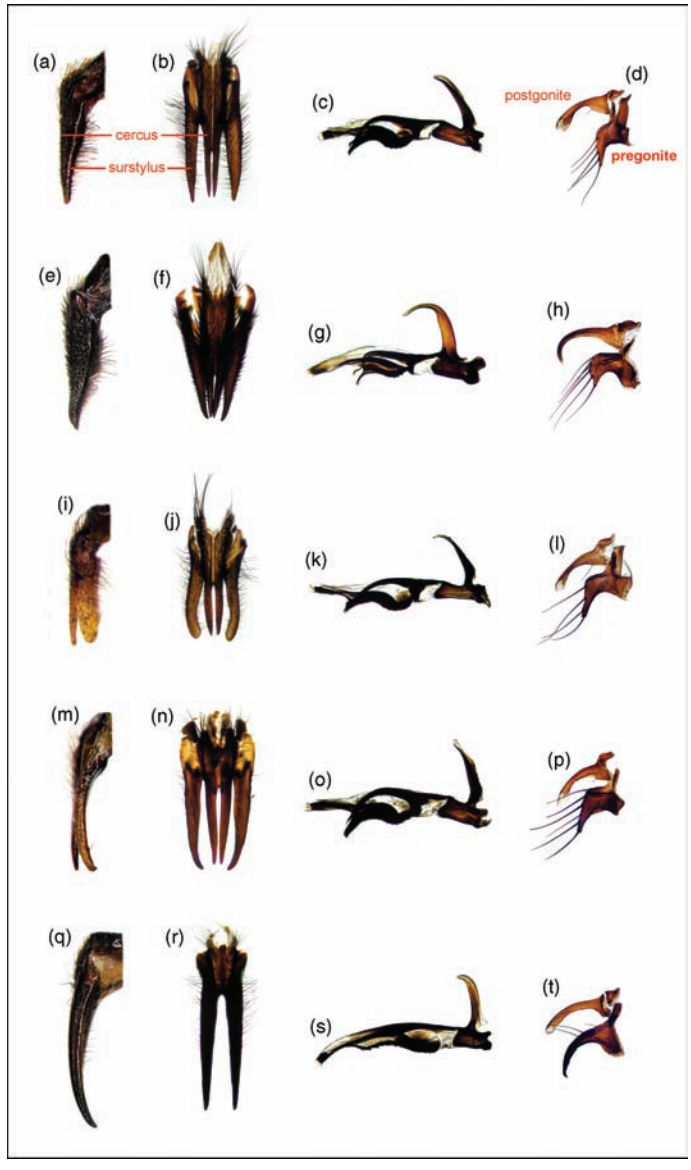

Plate 5.5A–T Male genitalia of Calliphorinae. *Calliphora loewi*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Calliphora subalpina*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite, lateral view. *Calliphora vicina*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Calliphora vomitoria*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view. *Cynomya mortuorum*: Q – cerci and surstyli, lateral view; R – cerci and surstyli, dorsal view; S – phallus, lateral view; T – pregonite and postgonite, lateral view. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

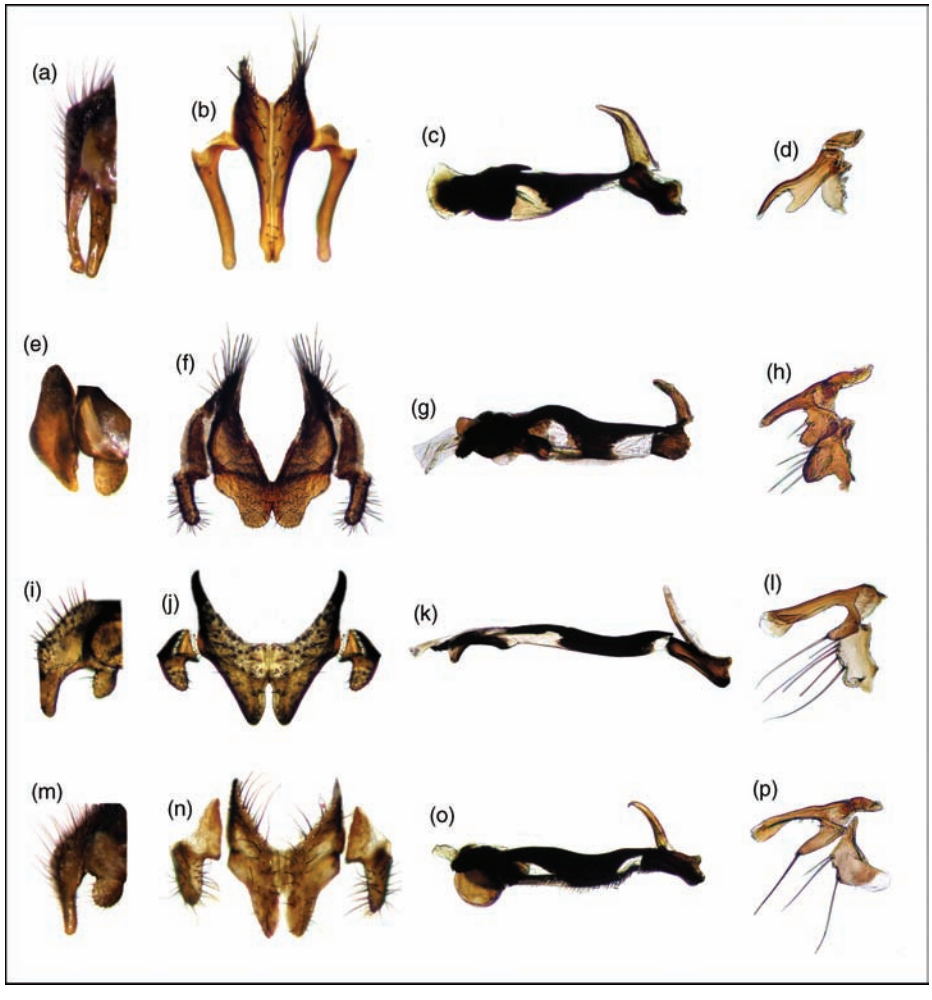

Plate 5.6A–P Male genitalia of Chrysomyinae. *Chrysomya albiceps*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Chrysomya bezziana*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite, lateral view. *Chrysomya marginalis*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Chrysomya megacephala*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

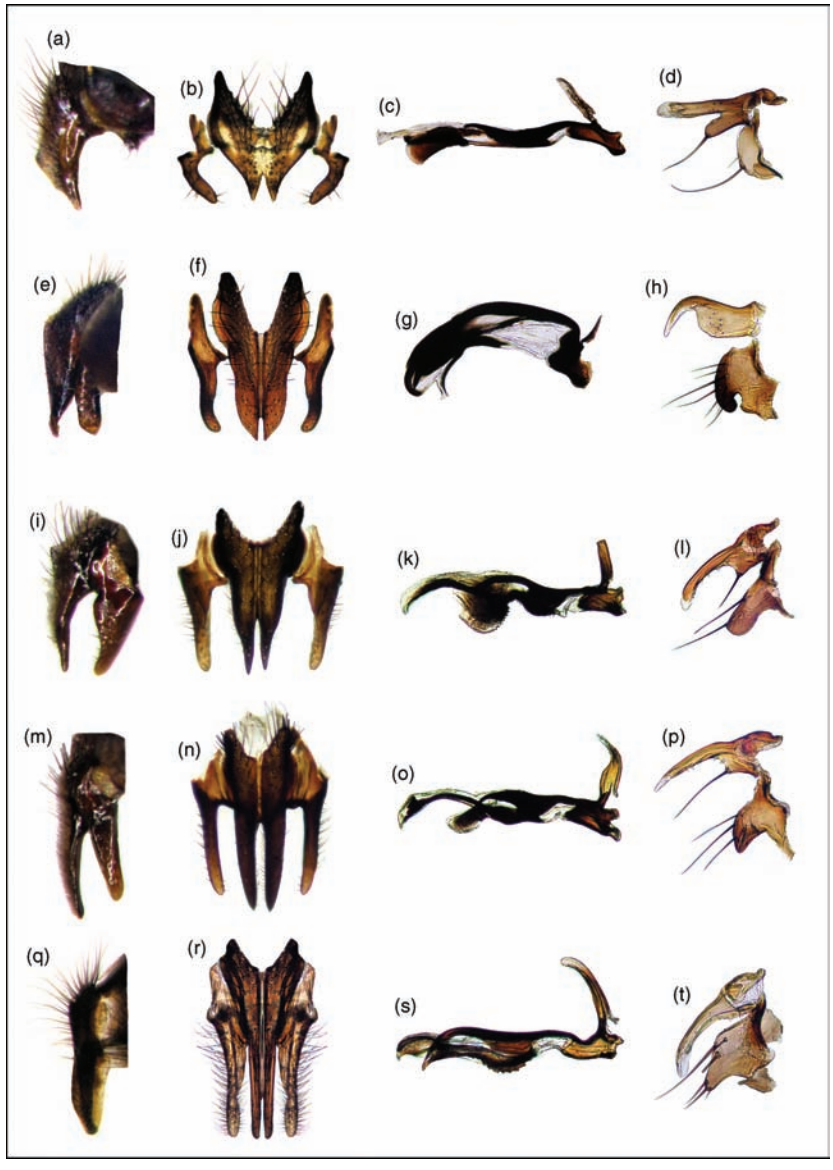

Plate 5.7A–T Male genitalia of Chrysomyinae and Luciliinae. *Chrysomya putoria*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Cochliomyia hominivorax*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – pregonite and postgonite, lateral view. *Phormia regina*: I – cerci and surstyli, lateral view; J – cerci and surstyli, dorsal view; K – phallus, lateral view; L – pregonite and postgonite, lateral view. *Protophormia terraenovae*: M – cerci and surstyli, lateral view; N – cerci and surstyli, dorsal view; O – phallus, lateral view; P – pregonite and postgonite, lateral view. *Lucilia cuprina*: Q – cerci and surstyli, lateral view; R – cerci and surstyli, dorsal view; S – phallus, lateral view; T – pregonite and postgonite, lateral view. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

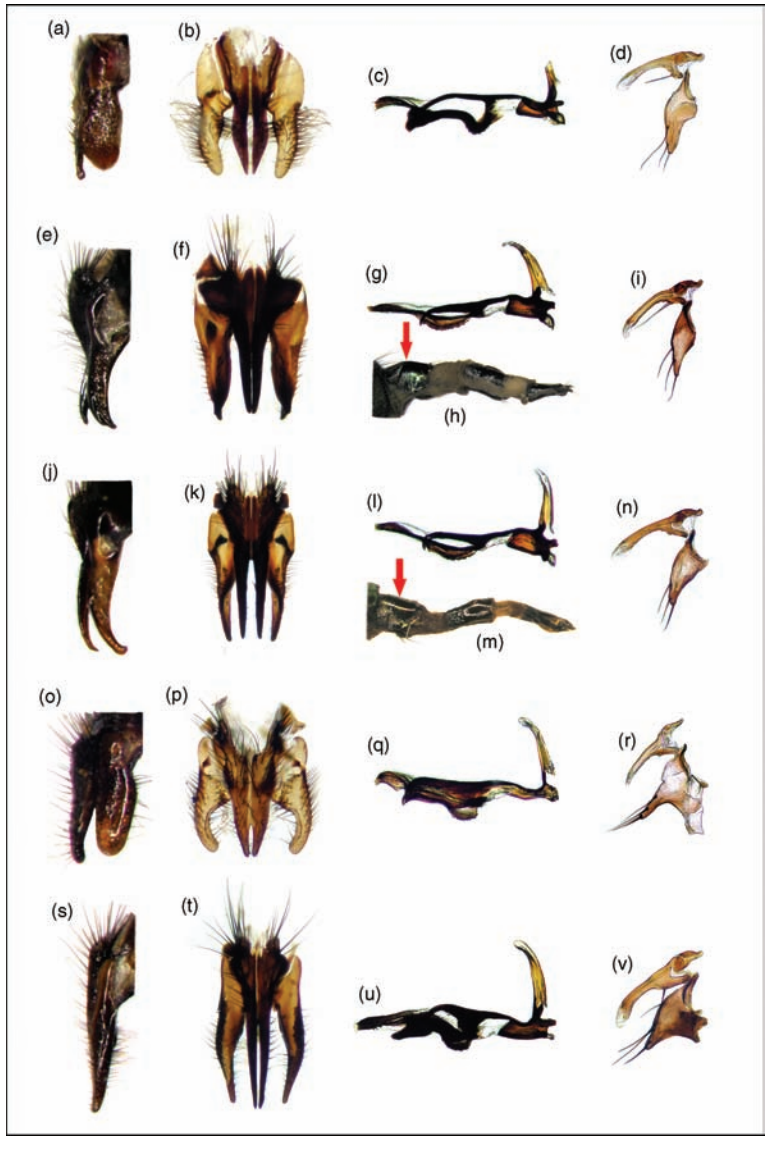

Plate 5.8A–V Terminalia of Luciliinae. *Lucilia ampullacea*: A – cerci and surstyli, lateral view; B – cerci and surstyli, dorsal view; C – phallus, lateral view; D – pregonite and postgonite, lateral view. *Lucilia caesar*: E – cerci and surstyli, lateral view; F – cerci and surstyli, dorsal view; G – phallus, lateral view; H – ovipositor, lateral view; I – pregonite and postgonite, lateral view. *Lucilia illustris*: J – cerci and surstyli, lateral view; K – cerci and surstyli, dorsal view; L – phallus, lateral view; M – ovipositor, lateral view; N – pregonite and postgonite, lateral view. *Lucilia sericata*: O – cerci and surstyli, lateral view; P – cerci and surstyli, dorsal view; Q – phallus, lateral view; R – pregonite and postgonite, lateral view. *Lucilia silvarum*: S – cerci and surstyli, lateral view; T – cerci and surstyli, dorsal view; U – phallus, lateral view; V – pregonite and postgonite, lateral view. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

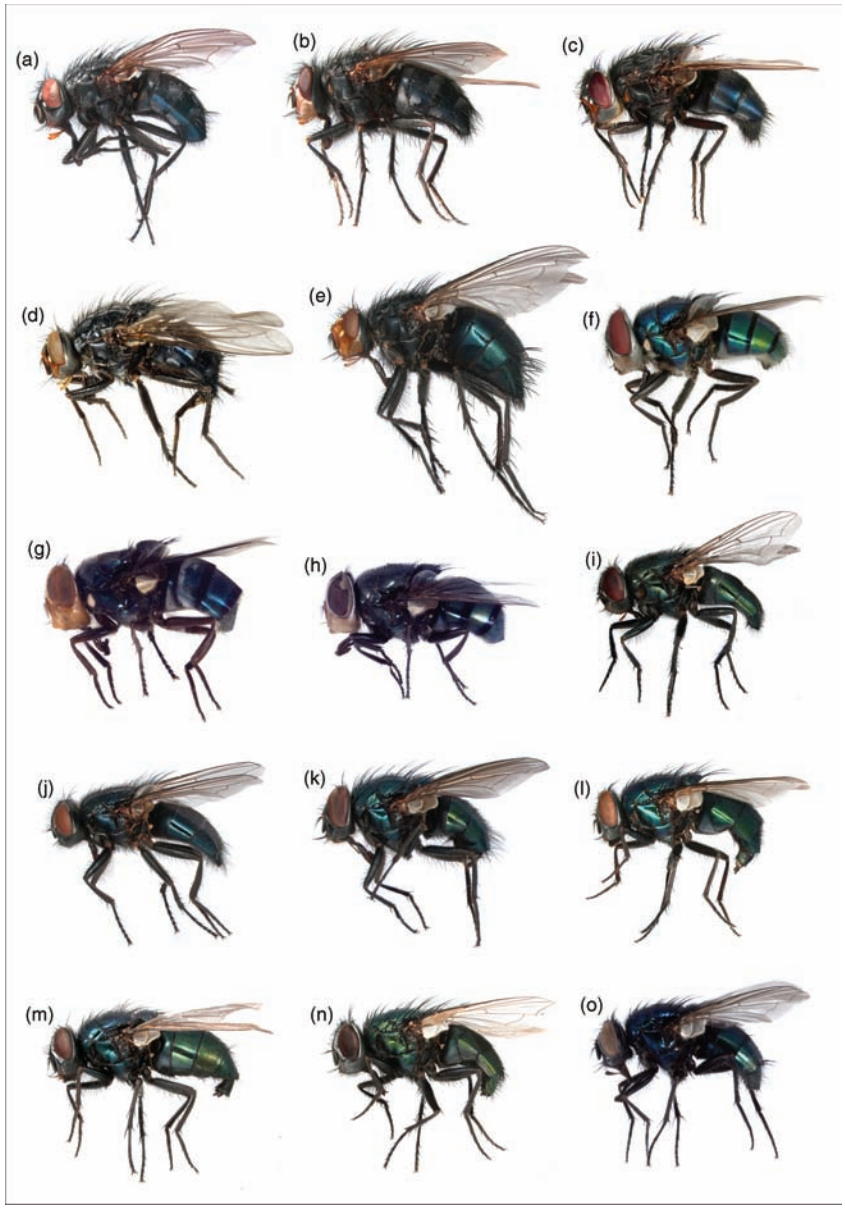

Plate 5.9A–O European and Mediterranean blowflies of forensic importance, general appearance (A-C, E, F, I-N phot. by T. Klejdysz). A – *Calliphora loewi*, B – *Calliphora vicina*, C – *Calliphora vomitoria*, D – *Calliphora subalpina*, E – *Cynomya mortuorum*, F – *Chrysomya albiceps*, G – *Chrysomya marginalis*, H – *Chrysomya megacephala*, I – *Phormia regina*, J – *Protophormia terraenovae*, K – *Lucilia ampullacea*, L – *Lucilia caesar*, M – *Lucilia illustris*, N – *Lucilia sericata*, O – *Lucilia silvarum*. *Source:* Reproduced by permission of Dr. Krzysztof Szpila

Plate 6.1 *Dermestes lardarius* Linnaeus

Plate 6.2 *Dermestes maculatus* DeGeer

Plate 6.3 A clerid beetle

Plate 6.4 *Nicrophorus humator* Gleditsch

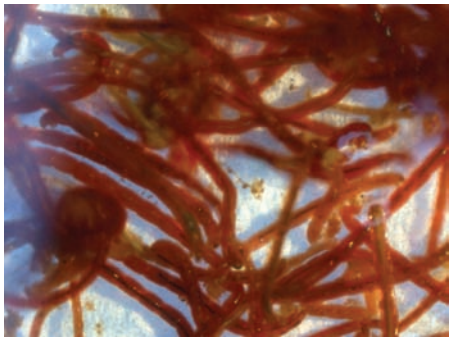

Plate 12.1 Chironomidae – blood worms

Plate 12.2 Caddisfly – cased

Plate 12.3 River with bankside vegetation

Plate 12.4 Hydrophilid larva

Plate 12.5 Hydrophilid beetle

Plate 12.6 Dytiscid beetle larva

Plate 12.7 *Gammarus* sp., - an example of a shredder

Plate 12.8 Culicid pupa

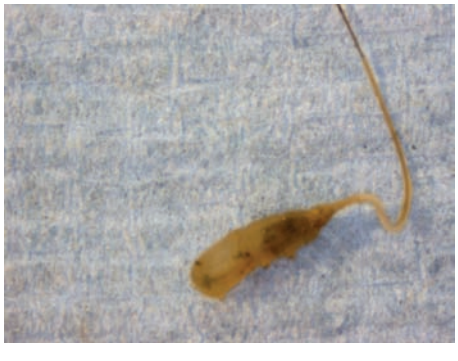

Plate 12.9 Rat-tailed larva (Syrphidae)

Plate 12.10 Turtle – a scavenger

Plate 12.11 Gyrinidae larva (whirligig beetles)