Systematics and Thermobiology of Carrion-breeding Blowflies (Diptera: Calliphoridae)

by

James Frederick Wallman, B.Sc. (Hons)

A thesis submitted for the degree of Doctor of Philosophy within the University of Adelaide

Department of Environmental Biology

April, 1999

Volume I: Text and References

Contents

Abstract

The systematics and thermobiology of carrion-breeding blowflies from southern Australia are investigated, with particular emphasis on their forensic application.

Nine forms of carrion-breeding *Calliphora* were initially analysed using allozyme electrophoresis. All were found to be distinct species, although the results suggest that *Calliphora* in its current form may not be monophyletic. Allozyme electrophoresis was also shown to be a useful tool in the identification of blowfly larvae.

The third instar larvae of eight carrion-breeding *Calliphora* species were described and examined for morphological characters to further aid in their identification. A number of such characters were found, although significant morphological distinctness exists only at the species-group level. A key was produced to identify these larvae from their morphology. A key is also given to both sexes of the adults of all 15 known or suspected carrion-breeding species of *Calliphora* from southern Australia.

Experiments on thermogenesis in blowfly larvae showed that they generate heat from the earliest stage of development. Their heat production is more accurately determined by comparing temperatures in infested carcasses with temperatures in uninfested control carcasses of similar mass, rather than with ambient air temperatures, although the amount of heat produced is influenced by the temperature of the ambient air. It is also influenced by the size of the larval aggregation and the size of the carcass. Also, solar radiation may profoundly influence larvae, especially in small carcasses. Exposure to gradually increased heat appears more damaging to larvae than a more rapid increase.

Experiments on adult blowflies were carried out using a summer-adapted and a winter-adapted species to examine their activity across a range of ambient temperatures after they had been subjected to various temperatures at three stages during development and as adults. Different effects were observed for each species and at each stage. Adult activity in response to carrion odour was also measured. Only females increased their activity when exposed to the odour. The increase occurred in both protein-deprived and gravid individuals.

Taken overall, given the level of existing knowledge, the results emphasise the limitations of the forensic application of blowflies, particularly for the estimation of time since death.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying.

James Frederick Wallman

23 April, 1999

Acknowledgements

I am indebted to my supervisor and friend, Dr Derek Duckhouse, for providing invaluable guidance and encouragement throughout this study. I also thank Derek and his wife, Sylvia, for their warm hospitality and for many other kindnesses.

Dr Dick Norris of the CSIRO in Canberra generously acquainted me with the carrion-breeding blowflies of southern Australia and hosted my visits to the Australian National Insect Collection on several occasions.

I am very grateful to the Department of Zoology at the University of Adelaide (now the Department of Environmental Biology) for providing the facilities and administrative support to enable me to undertake my research. I thank particularly the chairmen of the Department during the period of my studies, Em. Prof. Bill Williams, Dr Alan Butler and Prof. Russ Baudinette, for ensuring I was always supported with teaching work. I thank also David Williams and Piers Brissenden for their administrative help, Terry MacKenzie and Phil Kempster for technical assistance and Drs Keith Walker and Roger Seymour for their helpful advice on statistical and physiological matters, respectively.

I am grateful to Mark Adams of the Evolutionary Biology Unit of the South Australian Museum for kindly allowing me the use of the Unit's facilities to undertake the biochemical taxonomic work. I am indebted to him for training me in the techniques of allozyme electrophoresis and assisting with the interpretation of results and with analysis. Malcolm Krieg and Bridgette Winton are thanked for their technical assistance with this work.

I also thank the following: George Hassan of Chapman's Smallgoods at Tailem Bend for provision of pig carcasses; Phil Leppard of the Department of

Statistics at the University of Adelaide for assistance with statistical analysis of the data in Chapter 5; Peter Hudson for assistance with map production; and Dr David Cook, Western Australian Department of Agriculture, for furnishing me with live specimens of *Calliphora varifrons*.

Finally, my special and heartfelt thanks to my friend, Mike, to my brother, Tim, and to my parents, Pat and Neill, for their encouragement, support and assistance in so many ways.

Chapter 1

General Introduction

1.1 Preamble

Blowflies, Diptera of the family Calliphoridae, are world-wide in their distribution, although the family as currently understood is probably not monophyletic (Rognes 1997). Many species breed in carrion, but some are parasites of earthworms (Norris 1991). Carrion-breeding blowflies are among the most familiar of insects, yet many aspects of their biology remain unexplored or unclear. This is undesirable in view of their considerable importance to the environment and to human affairs. They are important ecologically because their larvae are the main agents of decomposition of dead animals (Putman 1983). They have considerable applied economic and medical importance because of their involvement in myiasis and in the mechanical transmission of disease (Hall and Wall 1995; Crosskey and Lane 1993). Additionally, since their larvae may infest dead bodies, they are sometimes useful forensically in estimating time since death (Catts and Goff 1992). In Australia, carrion-breeding blowflies are also conspicuous nuisance pests, especially because of their habit of entering buildings in response to food odours (Norris 1969).

1.2 Ecological and Forensic Importance of Carrion-breeding Blowflies

This thesis primarily investigates aspects of the biology of these carrion-breeding blowflies that are important forensically. The relevant literature is referred to and discussed more fully in the appropriate places in the thesis.

However, I begin with an introductory account of the ecological and forensic significance of these flies.

1.2.1 Ecological

Because of their great abundance and ubiquity, carrion-breeding blowflies are the most important insects in carcasses. They invade them often only minutes after death to lay, depending on the blowfly species involved, either eggs or first instar larvae (larvae may be sheathed). By feeding on the decomposing tissues the larvae considerably reduce the time taken for nutrients to be released and thus made available to other components of ecosystems (Putman 1983).

Different groups of species utilise carcasses at different times during their decomposition, and may be labelled primary, secondary, or tertiary, depending on the timing of their arrival (Fuller 1934; Norris 1959). The activity of each group renders the carrion unsuitable for continued occupation by itself, but more suitable for the next group which was unable to use the carcass in its previous form. Thus the succession of species is dynamic and interactive; each group does not colonise and leave the carcass in passive response to its changing state; it is itself responsible for the change. In general, blowflies are primary and secondary invaders.

1.2.2 Forensic

Forensic entomology is the scientific study of insects involved in matters pertaining to the law. It is best known for its application to the investigation of crimes, especially violent crimes such as murder (Catts and Goff 1992).

Most commonly, forensic entomology uses insects to estimate the period that has elapsed since death (also known as the postmortem interval). An estimate of the time of death may help in identifying the body and in assessing the

truthfulness of the statements of suspects. Pathological methods for determining the postmortem interval become increasingly inaccurate over time, and after about 72 hours insects are often the only means of determining it with any precision, e.g. Kashyap and Pillay (1989).

There are two main methods for estimating time since death entomologically - determination of the stage of faunal succession in the body, and determination of the age of the blowfly larvae in and around it.

Stage of Faunal Succession in Carcasses

Since the composition of the insect fauna in a carcass changes successionally, it is possible in principle to determine the stage in the succession which the faunal assemblage represents and thus to estimate the time that has elapsed since the death of the animal.

As already mentioned, blowflies are the commonest component of this assemblage. An array of other flies and beetles and soil mites of various families will also frequently be present (Smith 1986).

Age of Blowflies in Carcasses

The second method for determining time since death is to identify and determine the age of particular insects, usually larval blowflies, in the carcass.

The task here is to identify the blowfly species and then to infer the minimum period that they could have been present, judging from their stage of development.

The time since death may equal the estimated minimum age of the larvae, since blowflies often arrive at bodies within a few hours or even minutes after death. However, oviposition can occur *before* death if a wounded or soiled person is lying unconscious or helpless for some time before dying, e.g. Goff *et al.* (1991).

Conversely, oviposition may have been delayed. There are two possible reasons for this. The first is that the body was protected from infestation, perhaps because it was inside a building or car, or because it was wrapped in material of some sort, or covered with soil and/or vegetation. The second is that the climatic conditions were unsuitable for it (Catts 1992). Oviposition is in any case unlikely at night, although possible in certain circumstances (Greenberg 1990).

1.3 Species of Carrion-breeding *Calliphora* in Southern Australia

There are many endemic Australian blowflies, especially in the genus *Calliphora*. In fact, Australia has the richest *Calliphora* fauna of any continent (K. R. Norris, Division of Entomology, CSIRO, pers. comm.). This thesis deals mainly with *Calliphora* species.

In southern Australia there are 10 known endemic species or probable species and one introduced species of carrion-breeding *Calliphora*. All of these are dealt with systematically in Chapters 2 and/or 3 but their taxonomy and distributions are introduced below. Subsequently, aspects of the biology of representative species are dealt with in Chapters 4 and 5.

Calliphora albifrontalis Malloch occurs throughout south-western Australia. *Calliphora stygia* (Fabricius) is found throughout the south-east of the continent and has been introduced into New Zealand (Dear 1985). The two occur sympatrically on the Eyre, southern Yorke and Fleurieu Peninsulas, and on Kangaroo Island. They are recognised as sister species, and were assigned by Hardy (1937) to the *C. stygia* species-group (*C. albifrontalis* was referred to by Hardy as *C. australis* Boisduval). Kurahashi (1971, 1989) placed them in the subgenus *Neocalliphora* Brauer & Bergenstamm. It should be noted that the establishment by Kurahashi of this and other subgenera of *Calliphora* has not

been embraced by other workers on Australasian Calliphoridae (Dear 1985; Norris 1994).

Calliphora ochracea Schiner also was placed in *Neocalliphora* by Kurahashi (1971, 1989). It is known from Queensland, New South Wales and Victoria. Hardy (1937) placed it in the *C. ochracea*-group. Fuller (1931) showed that this fly readily oviposits on beef covered with rabbit fur in the laboratory and that its larvae develop fully in decomposing beef. However, it was not until the work of Palmer (1980) that it was documented as breeding in carrion in the wild.

Calliphora dubia (Macquart) is distributed across south-western and central-southern Australia, with *Calliphora augur* (Fabricius) replacing it in the south-east. These two are sympatric throughout south-eastern South Australia and on Kangaroo Island. They are sister species, placed in the *C. augur*-group by Hardy (1937) (C. *dubia* was referred to by him as *C. nociva* Hardy), and are now assigned to the subgenus *Paracalliphora* Townsend (Kurahashi 1971, 1989).

Calliphora hilli hilli Patton is a south-east Australian form, *Calliphora varifrons* Malloch is a south-west Australian form and *Calliphora hilli fallax* Hardy is found in eastern Victoria, northern and eastern New South Wales and southern and eastern Queensland. *C. hilli hilli* has also been introduced into New Zealand (Dear 1985). For reasons presented in the next chapter, *C. hilli fallax* is clearly a full species, subsequently referred to as *C. fallax*. Three other subspecies are recognised from morphological analysis - *C. hilli kermadecensis*, *C. hilli tahitiensis* and *C. hilli tasmanensis* (Kurahashi 1989) - all of which are outside the scope of this thesis.

Hardy (1937) placed *C. hilli hilli* (referred to by him as *C. rufipes* Macquart), *C. varifrons* (referred to by him as 'sp. (from Western Australia)') and *C. fallax* in the *C. rufipes*-group, but since the type of *Calliphora rufipes* is a fly quite

unlike *C. hilli hilli* (Norris 1973) this species is not relevant here and *C. hilli hilli* and related species should be referred to as the *C. hilli*-group. The *hilli*-group has been allocated, with the *augur*-group, to *Paracalliphora* (Kurahashi 1971, 1989).

Calliphora sp. nov. is undescribed and known only from a few localities scattered across south-eastern South Australia, and from Seaford in Victoria. It was first recognised by K. R. Norris, who kindly brought it to my attention.

Calliphora maritima Norris is restricted to certain littoral habitats along the southern coast of the continent, where it is frequently sympatric with some of the other species discussed above.

Calliphora vicina Robineau-Desvoidy was introduced from Europe. In Australia it is found only in urban regions. It has been placed in a third subgenus, *Calliphora s. str.* (Kurahashi 1971, 1989) and a separate species-group, the *C. erythrocephala* [*vicina*]-group (Hardy 1937).

Four other species that may be carrion-breeders are included in the key to adults in Chapter 3 and also referred to on pages 58 and 59.

1.4 Other Important Carrion-breeding Flies in the Present Study

Aside from the species of *Calliphora* introduced above, several other important carrion-breeding flies were encountered in this study. Two of these, *Lucilia sericata* (Meigen) and *Chrysomya rufifacies* (Macquart), are also blowflies. The cosmopolitan *L. sericata*, like *Calliphora vicina*, was introduced from Europe and occurs in all states. *Ch. rufifacies* also occurs in all states. It is widespread in the Australasian and Oriental regions and has recently begun to invade the Americas (Baumgartner and Greenberg 1984).

An important tertiary species is the muscid *Hydrotaea rostrata* (Robineau-Desvoidy) whose larvae breed in carrion but also in dung (Fuller 1934). It is widespread in Australia.

1.5 Aims and Rationale of the Present Study

The material in this thesis contributes to an understanding of both the evolution and ecology of carrion-breeding blowflies, but it is their forensic application which has been emphasised. Thus the thesis is primarily concerned with the species and biological problems that have particular relevance to the use of blowflies in forensic investigations in southern Australia. Specifically, the topics relate to three key questions that must be addressed where blowflies might indicate the time of death.

1. What is the identity of the flies concerned?

This is obviously a question of fundamental importance because the biology of different species may vary markedly, particularly in response to temperature (O'Flynn 1983).

The taxonomy of Australian *Calliphora* species is poorly resolved; many species remain to be described, and phylogenetic relationships among named forms are disputed (Norris 1994). In the first part of the thesis (Chapters 2 and 3) I therefore investigate the systematics of carrion-breeding *Calliphora* species from southern Australia. The aim was to finally determine the taxonomic status of the various forms and to develop reliable means of identifying their adults and third instar larvae. Both molecular and morphological approaches were adopted.

2. What is the thermal environment of larvae in a carcass?

This is a complex issue; the dynamics of larval activity in a decomposing carcass are influenced by many factors. Nevertheless, the aim of the work described in Chapter 4 was to help clarify this question by making detailed measurements of temperatures among larvae feeding in carcasses of different sizes, at different times of year. These studies focused on the phenomenon of larval thermogenesis. However, I also investigated the role of the sun and of bacteria in influencing carcass temperature.

3. What is the delay before adult blowflies locate an exposed corpse?

In Chapter 5 I aimed to help answer this question by studying the effect of temperature on adult activity. The main intention here was to investigate the effect of a blowfly's thermal history on its subsequent activity. I was also interested to learn how activity might be influenced by carrion odour.

The thesis concludes in Chapter 6 by summarising the results and examining what they suggest about the applicability of blowflies to forensic science.

Chapter 2

Molecular Systematics and Identification of *Calliphora* Species

2.1 Introduction

The previous chapter outlined the importance of carrion-breeding blowflies. Although there are a number of reasons why it is desirable to have the means to identify all species reliably, such an aim is particularly necessary from a forensic perspective and is only achievable following a thorough investigation of the systematic status of these flies.

The morphology of the carrion-breeding *Calliphora* species is difficult and the differences between species are subtle. To avoid future uncertainty it was decided to initially employ molecular analysis to clarify the taxonomy of this group of flies. This was also applied to the larvae of select species to determine their similarity to the adults for the purposes of identification.

This chapter comprises two parts. The first part examines the molecular systematics of the group. The second part focuses on the application of molecular methods to the identification of larvae.

2.2 Molecular Systematics

2.2.1 Introduction

This section deals with the analysis of nine of the forms of native southern Australian *Calliphora* detailed in Section 1.2, using the methods of molecular systematics - *C. stygia*, *C. albifrontalis*, *C. augur*, *C. dubia*, *C. varifrons*, *C. hilli hilli*, *C. fallax*, *C. varifrons*, *C.* sp. nov., and *C. maritima*. *C. ochracea* was omitted because at the time of the molecular work I was not aware that it had been confirmed by Palmer (1980) as a carrion-breeder. *Calliphora vicina* is analysed in the second part of this chapter.

The taxonomic status of the nine forms has been open to doubt for two reasons. The first is historical, based on the morphological similarity existing within the groups. For example, *C. dubia* and *C. augur* have variously been regarded as subspecies (Kurahashi 1971) and full species (Norris 1973; Kurahashi 1989). Also, *C. hilli* and *C. fallax* have been treated as subspecies of *C. hilli* by Kurahashi (1989), but this is not supported by their distributions, which overlap in New South Wales and Victoria, each form maintaining its morphological distinctness in the area of overlap. The second source of taxonomic uncertainty comes from the possible existence of unrecognised sibling or 'cryptic' species. The high incidence of sibling species in many animal groups and the need for vigilance in this regard has been stressed repeatedly in the literature (e.g. Richardson *et al.* 1986; Baverstock 1988; Donnellan *et al.* 1993). Thus, a number of systematic issues remain unresolved in the carrion-breeding *Calliphora*, even though they have been the subject of several morphological studies. In cases such as this, a molecular genetic approach is warranted in order to obtain an independent view of the systematic framework underlying the morphological data.

Initially, allozyme electrophoresis was employed to assess species boundaries among the nine forms. The technique permits rapid assessment of genetic

relatedness between individuals and populations and is usually the first choice for an initial examination of species boundaries within a group (Richardson *et al.* 1986; Avise 1994; Hillis *et al.* 1996). The allozyme data were then further examined for any obvious population trends within species, as well as for their phylogenetic implications.

Since *C. maritima* and especially *C.* sp. nov. are closer morphologically to members of the *hilli*-group than to those of the other two groups, they were considered initially to be part of this species-group.

2.2.2 Materials and Methods

Collection of Specimens

Blowflies were collected between 1991 and 1995 from the 19 southern Australian locations shown in Fig. 2.1. They were captured at carrion baits with an insect net and preliminarily identified in the field using a hand lens. Identification was confirmed later using a dissecting microscope. Each fly was placed in an Eppendorf tube and coded according to its morphological form and locality (Table 2.1). The flies were then frozen alive in liquid nitrogen and stored at -80°C to await allozyme analysis.

Two individuals of *Onesia tibialis* (Macquart) were inadvertently included among the flies analysed. Although this species was initially thought to be irrelevant to this study due to its different generic status and biology (like many blowflies it is a parasite of earthworms (Norris 1991)) it has been included in the results because it throws light on the ranking and phylogenetic relationships of the species-groups and subgenera of *Calliphora*.

Allozyme Analyses

Whole specimens were homogenised using a sonicator in an equal volume of cold homogenising solution (Richardson *et al.* 1986), then centrifuged at 10,000*g* for 10 minutes. The supernatants were stored at -20°C as separate 5-10 microlitre portions inside glass capillary tubes until required for electrophoresis.

Allozyme electrophoresis was performed on cellulose acetate gels ('Cellogel') using the procedures outlined in Richardson *et al.* (1986). In summary, it was conducted in a coldroom (4-8°C), where a draughtsman's pen was used to transfer half a microlitre of supernatant from each specimen onto 'slots' on a gel. The gel was then subjected to a potential of 200 volts for 1-3 hours, depending on the locus under investigation and the running buffer used. At the conclusion of an electrophoretic 'run', gels were stained with enzyme-specific histochemical stains. The enzyme systems that gave sufficient resolution and activity to allow the assignment of genotypes are given in Table 2.2, along with the electrophoretic methods employed for each. The nomenclature used for loci and allozymes follows Adams *et al.* (1987).

Gels were scored as isozymic bands appeared, using photocopies of the gradual appearance of bands to aid this process. Interpretations of gels were made with reference to the quarternary structure of the enzyme in question as well as to any other known factors such as the presence of sub-banding, electrophoretic artifacts etc. Secondary 'line-up' gels were conducted to verify initial allozyme typings.

The analyses were conducted in two parts. In an initial overview study, 1-5 individuals of each morphological form from each locality were analysed for the full range of allozyme markers available. This identified three distinct groups of taxa, consistent with the morphological groups described in Section 1.3. Subsequently, additional individuals of *C. albifrontalis* and *C. stygia* from the *stygia*-group and of *C. augur* and *C. dubia* from the *augur*-group were

genotyped at those loci found to be polymorphic in the overview study and suitable for retyping on the older, frozen homogenates. Given the complexity of the *hilli*-group, the additional specimens run from within this group were typed at all loci and incorporated into the overview study.

Statistical Analyses

The genetic distances between populations and taxa in the overview study were calculated as percent fixed differences (%FD) and displayed visually on a phenetic dendrogram constructed using the unweighted pair group method of analysis (UPGMA; Sneath and Sokal 1973). The genetic affinities of individuals within each of the *stygia*-, *augur*-, and *hilli*-groups were then explored using Principal Co-ordinates Analysis (PCoA), as implemented by the statistical package PATN (Pattern Analysis Package; Belbin 1987). Each of the genetic distance matrices used for PCoA was calculated using Rogers' genetic distance (Rogers' R; Rogers 1972). Statistical comparisons of allele frequencies were carried out by pooling alleles into two classes and using a 2 x 2 contingency table, to which was then applied either Yates correction for continuity or, where an expected value was less than five, the Fisher exact test (Zar 1984). The genetic distance matrix was used to assess phylogenetic relationships according to the distance Wagner method (Farris 1972) as implemented by the computer program WAGPROC (Swofford 1981).

2.2.3 Results

Overview Study

Thirty-five presumptive loci were detected among the 27 enzyme systems displaying interpretable zymograms. Of these, four loci (*Fum*, *Hk-2*, *Mdh-1* and *Tpi-1*) were invariant for all animals screened. The allozyme profiles at the remaining 31 loci for the 92 specimens included in the overview study are

presented in Table 2.3. It was assumed that all 31 loci have autosomal modes of inheritance.

Genetic relationships among the 34 populations sampled are shown diagrammatically in Fig. 2.2. As populations are only represented by small samples it is possible for them to display the occasional fixed difference due to sampling error, rather than because such a difference exists in reality. The dendrogram is therefore presented only as an overview of the broad genetic affinities among the various forms of blowfly. Lineages which consist of two or more taxa displaying genetic distances of less than 10%FD were then examined in more detail to assess the implications of the allozyme data for species boundaries within the lineage.

Five major lineages are present at levels of genetic distance above 10%FD within the *Calliphora* under study. These correspond to the *stygia*-group (*C. stygia* and *C. albifrontalis)*, the earthworm parasite *O. tibialis*, the *augur*-group (*C. augur* and *C. dubia)*, the single taxon *C. maritima* (hereafter not considered as a member of the *hilli*-group), and the *hilli*-group itself (*C. hilli hilli*, *C.* sp. nov., *C. varifrons* and *C. fallax*). The five lineages are genetically quite distinct and cluster at levels of genetic divergence ranging from 25-68%FD. The *stygia*-group is the most distinct lineage. Indeed it is genetically more divergent from the other species of carrion fly than is the non-carrion species *O. tibialis* (68%FD versus 52%FD). The other three lineages show varying degrees of genetic relatedness, with the *augur*-group being the most distinct at 40%FD and *C. maritima* clustering with the *hilli*-group at 25%FD.

Fig. 2.2 also reveals two main points of interest concerning within-lineage genetic affinities. First, the forms in each group are genetically similar, with genetic distances ranging from 3-7%FD (equivalent to fixed differences at one or two loci of the 35 examined). Second, no populations of the same form display any fixed differences from one another. It should be noted, however,

that when the dendrogram was repeatedly reconstructed by shuffling the order of populations in the genetic distance matrix, on some occasions three populations clustered with other forms which are morphologically very similar. The populations concerned are HI-I, which occasionally clustered with the other populations of *C.* sp. nov., SN-I with the other populations of *C. hilli hilli*, and AL-E with the other populations of *C. stygia*. These anomalies point to the need for a more detailed approach to the assessment of species boundaries within the *stygia*, *dubia*, and *hilli* species-groups. This was achieved using the multivariate technique of PCoA to explore the genetic similarities of individuals within each of these lineages, allowing within-population heterogeneity to be taken into account in assessing the significance of between-population differences.

Species Boundaries Within the C. stygia*-group*

Thirty additional specimens of *C. stygia* and 20 of *C. albifrontalis* were screened for seven polymorphic enzyme markers, encoding eight loci. Table 2.4 presents the allelic profiles of these specimens, integrated with those of the 18 specimens included in the overview study. A plot of the PCoA scores for the total of 68 specimens within the first two dimensions is shown in Figure 2.3. While considerable heterogeneity is expressed within the second dimension for both *C. stygia* and *C. albifrontalis*, nevertheless all flies except one cluster into two discrete groups within the first dimension. The single exception, *C. albifrontalis* AL-E2, falls between the *C. stygia* and *C. albifrontalis* clusters and is quite distinct from the other two *C. albifrontalis* sampled from locality E. No obvious geographic trends were evident from the PCoA analysis within either *C. stygia* or *C. albifrontalis*.

Table 2.5 summarises the allele frequency data for the three clusters revealed by PCoA analysis. *C. albifrontalis* and *C. stygia* show fixed differences at *Mpi* and *PepD-2*, and a major difference in allele frequency at *PepB*. As the two taxa were collected in sympatry at five locations (localities A, B, C, E and K, Table

2.1), this provides convincing evidence that they are distinct biological species. The allozyme profile of AL-E2 is consistent with it being an F_1 hybrid between *C. albifrontalis* and *C. stygia*. It is heterozygous for diagnostic allozymes at *Mpi* and *PepD-2*, is heterozygous for the near-diagnostic allozymes at *PepB*, and carries genotypes at the other loci which are in accord with a hybrid origin. Unfortunately, as whole animals were used for electrophoresis, it was not possible to carry out a further detailed anatomical assessment of this hybrid to assess its fertility, or to reassess its morphological identification.

Species Boundaries Within the C. augur*-group*

The allozyme profiles of nine *C. augur* and 25 *C. dubia* at eight loci are shown in Table 2.6. These comprise the individuals used in the overview study plus additional specimens screened for the seven polymorphic enzymes found to be the most informative in that study. Interestingly, in most cases these enzymes are the same as those used for the *stygia*-group, suggesting that they may encode loci that are fast-evolving in *Calliphora* and therefore likely to be both polymorphic within a species, and able to distinguish recently-diverged species.

The data in Table 2.6 were used in a PCoA analysis for all individuals of the *augur*-group. The results are presented in Fig. 2.4. Here, within the first dimension all individuals cluster into one of two discrete groups corresponding to the morphospecies *C. dubia* and *C. augur*, i.e. every specimen falls into the same group as that indicated by prior morphological identification. As with the *stygia*-group, both taxa exhibit considerable heterogeneity within the second dimension (reflecting the large number of alleles at several loci), but none of this variation has any obvious geographic basis.

The allozyme frequencies displayed by the two species at these eight loci are summarised in Table 2.7. Apart from a single fixed difference at *PepD-2*, these taxa share alleles at all other loci, with only *Gda* showing some measure of genetic divergence (frequency of Gda^h = 78% in *C. augur* versus 40% in *C.*

dubia, with *Gda*^{k} being the only rare allele among 11 others to be found in both taxa; see Table 2.7). Despite this high degree of genetic similarity, the sympatric occurrence of both species at two locations (A and G) without any evidence of genetic exchange at the *PepD-2* locus strongly supports their recognition as distinct biological species.

Species Boundaries Within the C. hilli-*group*

During the overview study it became apparent that the *hilli*-group forms were very similar genetically. It was therefore necessary to screen a considerable number of the geographically-widespread *C. hilli hilli*, plus every available specimen of the other taxa (a total of 51 specimens) for all 35 loci. The allozyme profiles of the 51 specimens are displayed in Table 2.3. The results of a PCoA analysis, based on the genetic distance matrix for the 26 loci polymorphic within the group, are presented in Fig. 2.5.

Fig. 2.5 reveals that the blowflies of the *hilli*-group cluster into four more or less discrete PCoA groupings. The first dimension separates the Western Australian *C. varifrons* from the other species to the east, and the rare *C.* sp. nov. from *C. fallax*. These last two are split from the widespread *C. hilli hilli* by the second dimension. As *C. varifrons* and *C. fallax* are allopatrically separated from all other taxa in the group, this analysis alone is not sufficient to verify their specific status.

The rare form *C.* sp. nov. deserves further consideration. *C.* sp. nov. and *C. hilli hilli* were collected in sympatry from localities G and I, with *C.* sp. nov. individuals showing a greater extent of genetic divergence from *C. hilli hilli* than any *C. hilli hilli* individuals do from one another, even though they were collected as far apart as Canberra and the Fleurieu Peninsula (Fig. 2.1 and Table 2.1), a distance of roughly 1,000 km. This supports the recognition of *C.* sp. nov. as a distinct species.

The allozyme frequencies displayed by the four taxa of the *hilli*-group are presented in Table 2.8. Fixed differences or near fixed differences (i.e. where the alternate allele is present in the other taxon at frequencies of less than 5%) exist between *C. fallax* and all other taxa (*Me-1* and *Mpi*), and between *C. varifrons* and all other taxa (*Dia* and *Gda*). These two allopatric taxa are therefore genetically as distinct from the other members of the *hilli*-group as are the sister species within the *stygia*- and *augur*-groups. However, *C. hilli hilli* and *C.* sp. nov. are not distinguishable by means of fixed differences. Their recognition as distinct gene pools in sympatry is based mainly on major differences in allozyme frequency at three loci. The allozymes Gda^{g}, $Hk\text{-}1^{b}$, and $Me\text{-}1^{d}$ are all found at high frequency in the specimens of *C.* sp. nov. sampled in this study (at frequencies of between 88% and 94%; Table 2.7), whereas they are rare in *C. hilli hilli* (8%, 38%, and 13% respectively). The combination of the partial discrimination at each of these three loci thus supports the morphological assessment that the two taxa represent distinct but closely-related species.

Population Genetic Trends Within Species

The small sample sizes per locality in this study preclude any detailed analysis of population substructuring within species. Nevertheless, allozyme data gathered primarily for taxonomic purposes may contain useful population genetic information. The PCoA analyses in Figs 2.3-2.5 were therefore examined to determine whether, for widespread taxa, individuals from the same broad geographic region tend to cluster together to the exclusion of other regions. Any such tendency can then be assessed in more detail by a comparison of pooled allele frequencies for the regions concerned.

As mentioned earlier, no obvious geographic trends are evident from the PCoA analysis within *C. stygia, C. albifrontalis*, *C. augur*, or *C. dubia*, each of which was sampled both on Kangaroo Island and at sites on mainland South Australia (Fig. 2.1 and Table 2.1). Within the *hilli*-group, only *C. hilli hilli* itself was sufficiently common and widespread to permit such an assessment. Fig. 2.5

reveals that all of the animals collected on Kangaroo Island (localities A-D) fall into the top half of the *C. hilli hilli* cluster. A comparison of allele frequencies between a Kangaroo Island sample set (A-D pooled) and a mainland sample set (G, I-K, R pooled) suggests that the Kangaroo Island population is genetically distinct (calculated directly from Table 2.3). Significant differences in allele frequency are present at two loci ($\chi^2 = 4.50$, $P < 0.05$ for $Acyc^e$ vs. $Acyc^{rest}$; $\chi^2 = 6.88$, $P < 0.01$ for $Hk\text{-}1^b$ versus $Hk\text{-}1^{rest}$), with one remaining significant if the Canberra site (R) is excluded from the analysis ($\chi^2 = 5.28$, $P < 0.05$ for *Hk-1*).

In view of the existence of a Kangaroo Island versus mainland split in *C. hilli hilli*, the other species with similar distributions were also examined for significant differences in allele frequency between these two regions. Sample sizes greater than 10 were available for both Kangaroo Island and mainland collections of *C. stygia* and *C. albifrontalis* (Table 2.3). In both cases the existence of a discrete Kangaroo Island subpopulation is supported by one locus: *Pep-B* for *C. stygia* (Fisher exact $P < 0.001$ for $Pep\text{-}B^c$ versus $Pep\text{-}B^{rest}$) and *Pgm* for C. *albifrontalis* (Fisher exact $P < 0.01$ for Pgm^b versus Pgm^{rest}). Thus the allozyme data suggest that a degree of genetic divergence exists between Kangaroo Island and mainland South Australian populations of *C. hilli hilli*, *C. stygia*, and *C. albifrontalis*. Unfortunately, the Kangaroo Island sample sizes of five or less for *C. augur* and *C. dubia* do not allow allele frequency differences of the magnitude found in the other taxa to be properly tested for levels of significance. Nonetheless, in each species, differences in allele frequency of comparable magnitude apparently exist between Kangaroo Island and mainland sample sets at one locus ($\Delta p = 30\%$ for $Gldh^c$ in *C. augur* and $\Delta p = 33\%$ for Gda^f in *C. dubia*), suggesting that these taxa may be similarly substructured.

Phylogenetic Implications of the Allozyme Data

It might have been expected that the species of *Onesia* included in this study would have provided an ideal outgroup for phylogenetic as opposed to phenetic

analysis of the allozyme data. However, one of the most striking features of the UPGMA dendrogram in Fig. 2.2 is that the carrion-breeding *Calliphora* species do not all cluster together to the exclusion of *O. tibialis*. The *stygia*-group forms a genetic lineage which is as separate from all other carrion-breeding *Calliphora* as from the single representative species of the genus *Onesia*. Consequently it would not be appropriate to use *O. tibialis* as an outgroup. Thus, although in principle it is desirable to analyse the character state data phylogenetically, under established principles of maximum parsimony (Swofford *et al.* 1996), this is not possible here, for two reasons. First, without the inclusion in the analysis of an unquestioned outgroup, there is no way to properly root the phylogenetic network thus obtained. Second, even if such an outgroup could have been recognised and included later, it would not have provided phylogenetically useful information because the maximum genetic distances within the ingroup (~70%FD) are already at or beyond the level at which allozyme data can be informative phylogenetically (Richardson *et al.* 1986; Avise 1994).

There is no simple solution to this dilemma. Nevertheless, UPGMA dendrograms will provide realistic reconstructions of phylogenetic relationships provided that rates of molecular evolution are constant within the group under study (Avise 1994). In addition, they often provide quite accurate trees during computer simulations, even when rates of molecular evolution are not constant (Nei 1987). Moreover, an analysis of the genetic distance between species data using the distance Wagner method, an algorithm which does not assume constant rates of evolution, gave a tree of essentially identical topology when rooted at the midpoint of the longest branch. The dendrogram presented in Fig. 2.2 is therefore a reasonable initial hypothesis for evolutionary relationships among these carrion-breeding blowflies.

A series of phylogenetic inferences can be drawn from this dendrogram. First, the existence of the three species-groups recognised by Hardy (1937) is

supported by the allozyme data. Second, the assumption based on morphological criteria that *C.* sp. nov. belongs with the *hilli*-group appears to be valid. Third, since *C. maritima* diverges to a markedly greater degree from the other species in the *hilli*-group than these species diverge from each other, treatment as a separate group is warranted. This hypothesis also supports the recognition by Kurahashi (1971, 1989) of two distinct subgenera within the Australian carrion-breeding blowflies, one containing the *hilli*- and *augur*-groups (*Paracalliphora*), and the other embracing the *stygia*-group (*Neocalliphora*).

Several alternative inferences can be drawn from the fact that the genetic distance data reveal a closer relationship between *O. tibialis* and *Paracalliphora* than between *Paracalliphora* and *Neocalliphora*. This might indicate that *Calliphora* is paraphyletic, and that either *Paracalliphora* or *Neocalliphora* belong in a separate genus. Alternatively, *Onesia* is a sub-group of *Calliphora*, or this particular species is a true *Calliphora* which has been incorrectly assigned to *Onesia*. However, the allozyme data themselves cannot distinguish between these alternatives.

2.2.4 Discussion

As far as can be determined from the literature, this is the first time that allozyme electrophoresis has been employed in the taxonomic study of Calliphoridae. Nevertheless, other workers on Diptera have adopted the same technique to elucidate systematic relationships in Culicidae (Foley *et al.* 1994), Ceratopogonidae (Holbrook and Tabachnick 1995), Psychodidae (Dujardin *et al.* 1996), Cecidomyiidae (Hawkins *et al.* 1986), Sciomyzidae (Manguin 1990), Tephritidae (Steck 1991), Drosophilidae (Parkash *et al.* 1994) and Muscidae (Loeschcke *et al.* 1994).

The species boundaries demonstrated by the data indicate that all nine forms of blowfly examined are separate species. No cryptic species were detectable among the individuals sampled. Therefore both morphological and molecular evidence complementarily resolve the systematic uncertainties which have overshadowed these important insects.

The presence of an apparent hybrid between *C. stygia* and *C. albifrontalis* in one of the populations sampled suggests that hybridisation might also occur between other pairs of sympatric sister species in nature. Monzu (1977) showed the potential for this in laboratory studies in which he produced crosses between what were assumed to be *C. stygia* with *C. albifrontalis*, *C. dubia* with *C. augur*, and *C. hilli hilli* with *C. varifrons*. In direct crosses (no choice) all of these pairs produced F_1 progeny. In addition, F_2 progeny were produced by self-crossing of the F_1 offspring of *C. stygia* x *C. albifrontalis* and *C. hilli hilli* x *C. varifrons*. Mate-choice experiments conducted by Monzu also produced hybridisation in all species pairs, but at much lower levels. It is unlikely that the hybrid collected in this study differed more than minutely in its external morphology from *C. albifrontalis*, otherwise it would have been detected during preliminary identification. However, morphological intermediacy between *C. stygia* and *C. albifrontalis* may have been apparent in the terminalia. Monzu did not comment in his work on the morphological features of hybrids.

The population trends within species appear to establish the genetic distinctness of the Kangaroo Island populations of *C. hilli hilli*, *C. stygia* and *C. albifrontalis*. This suggests that Kangaroo Island has been isolated from the mainland for long enough (about 10,000 years (Griffin and McGaskill 1986)) for the effects of genetic drift and/or selection to be noticeable. Studies by various authors on calliphorids show differences between their dispersive capacities (Norris 1965). The maximum flight records for species studied here were 14.5 km for *C. stygia* and 13 km for *C. augur* (Gurney and Woodhill 1926). Since the minimum distance between Kangaroo Island and the mainland is

approximately 14 km, this suggests that the island is sufficiently close for genetic exchange to occur occasionally, thus reflecting the results of the present study.

As shown in Fig. 2.2, *O. tibialis* falls between the *stygia*-group and the *dubia*- and *hilli*-groups. This could be interpreted as supporting the views of Bezzi (1927), Hardy (1930, 1937, 1940, 1947), and later Norris (1994), that *Onesia* holds only subgeneric status within *Calliphora*. Malloch (1927, 1932) failed to see grounds for either generic or subgeneric status, choosing not to recognise *Onesia* at all. *O. tibialis* was therefore classified by Bezzi, Hardy and Malloch as a *Calliphora*. Prior to the reappraisal of Kurahashi (1989), its last taxonomic treatment had been by Hardy (1937), who placed it in the *tibialis* species-group of *Calliphora*. Kurahashi (1970) gave reasons for considering *Onesia* a valid genus, but not for his transference (Kurahashi 1989) of *tibialis* from *Calliphora* to *Onesia*.

There is morphological evidence that *Calliphora* as recognised here may be paraphyletic. In *Paracalliphora* and three other subgenera of *Calliphora* erected by Kurahashi (*Australocalliphora* and *Papuocalliphora* (Kurahashi 1971) and *Oceanocalliphora* (Kurahashi 1972)), the ovipositor is considerably shorter than that of other *Calliphora* species. In particular, ST8 is very short (Kurahashi 1971, 1972), which is similar to *Onesia* and to *Bellardia*, another genus of earthworm parasites (Rognes 1991). These four subgenera are either viviparous or ovoviviparous, having a uterus with a pair of lateral incubatory pouches (Kurahashi 1971, 1972; Rognes 1991). All other subgenera of *Calliphora* (including *Neocalliphora*) are more primitive in having an ST8 of full length, a cylindrical uterus without lateral incubatory pouches and unsclerotised lateral sacs (Rognes 1991). *Paracalliphora*, *Australocalliphora*, *Papuocalliphora* and *Oceanocalliphora* may therefore belong in a separate genus, while *Neocalliphora* and the remaining subgenera of *Calliphora*

comprise *Calliphora s. str.* Rognes (1991) agrees that *Paracalliphora* may not belong in *Calliphora*, but does not specify where it should be placed.

The results of this study greatly improve the basis for identification of the common species of carrion-breeding *Calliphora* in southern Australia. Future work should aim to clarify the evolutionary affinities of *Calliphora* and *Onesia* by examining both molecular and morphological characters in a wider range of species. This may contribute to a resolution of the phylogenetic uncertainties which currently beset the higher-level systematics of the Calliphoridae (Pape 1992; Rognes 1997).

2.3 Molecular Identification of Larvae

2.3.1 Introduction

The previous section established the value of allozyme electrophoresis as a means of identifying adult carrion-breeding blowflies. Since adults and immatures share the same genotype, molecular techniques have the potential to be used as a reliable means of identifying immature as well as adult blowflies. This is important because immature blowflies are morphologically even more difficult to identify from their morphology than are the adults. The main reason for this is that their external morphology is relatively featureless, with few structures exhibiting the variation that might set species apart. Thus, blowfly species are much more similar as immatures than as adults and closely-related forms may be almost or quite identical.

Although difficulties in identifying larvae can be overcome by rearing them through to adults, this procedure has several practical drawbacks. Specimens require time to complete their development and thus delay identification. Other problems include contamination of cultures by wild blowflies and destruction of irreplaceable specimens by parasitoid Hymenoptera or by the predacious larvae

of certain *Chrysomya* species. As a consequence of the latter two factors there may be no adult specimens for identification.

Here the allozyme electrophoretic profiles of adults and third instar larvae are compared for four carrion-breeding *Calliphora* species - *C. stygia*, *C. dubia*, *C. hilli hilli* and *C. vicina*. The first three of these were chosen as representatives of the *stygia-*, *augur-* and *hilli*-groups which, despite being morphologically distinct as adults, have larvae that are morphologically very similar. The larvae of *hilli*-group species cannot be separated at all on morphological grounds and species identification is only possible with difficulty within the *augur-* and *stygia*-groups (see Section 3.3). *C. vicina* was included here because its larvae are commonly encountered in carrion in urban areas of southern Australia. On morphological grounds it would be regarded as lying within yet another, separate, group. The aim of the present work is to demonstrate that larvae of any of the species in these groups can be identified biochemically by comparison with the profiles of adults. In addition, *C. vicina* is compared with the three species from native Australian species-groups to determine its phylogenetic position.

2.3.2 Materials and Methods

Collection of Specimens

Adults and larvae were obtained from laboratory cultures established by rearing eggs or first-instar larvae oviposited by females caught in Adelaide. Cultures were maintained in cages at 25 ± 1°C under a 12h:12h light:dark regime and were provided with water, sugar and lamb's liver as a medium for oviposition. Larvae were allowed to pupate in wheat chaff beneath the rearing dishes. Puparia were then transferred to fresh cages for the emergence of adults.

Individual adults and third instar larvae were placed in Eppendorf tubes, labelled according to their identity, frozen alive in liquid nitrogen and stored at -80°C to await allozyme analysis.

Allozyme Analyses

The methods used for analyses are as given in Section 2.2.2 and Table 2.2, although in this section the work was done in one rather than in two stages.

Five additional enzyme systems, comprising six loci, were used in the electrophoretic screening of the adults and larvae examined here. Details of the separate methods employed for these systems are given in Table 2.9.

Statistical Analyses

For the purpose of ascertaining the phylogenetic relationship of *C. vicina* with the other species the genetic distances between taxa were calculated as percent fixed differences (%FD). These were then displayed on a phenetic dendrogram constructed using UPGMA (Sneath and Sokal 1973).

2.3.3 Results

Of 42 allozyme markers examined, eight were found to be invariant (*Ak*, *Argk*, *Fum*, *Mdh-1*, *Pgam*, *Pk-1*, *Pk-2* and *Tpi-1*). The profiles of the remaining 34 loci are virtually identical for adults and larvae of the same species (Table 2.10). Table 2.11 summarises the expression of each locus, irrespective of whether it was invariant. It shows that 39 of the 42 allozyme markers examined were expressed and scoreable in both adults and larvae. *Gp* was not expressed in adults, reflecting the fact that this enzyme was not used in the analysis presented in Section 2.2. *Gldh* and *Hk-2* were expressed in adults but not in larvae. However, neither of these loci is important diagnostically for species determination in any of the species-groups (see Section 2.2.3). Therefore larvae

of any of the six native species in the three species-groups should be reliably identifiable by electrophoretic comparison with an adult of the same species.

Larvae of *Calliphora vicina* also present an allozyme profile that is distinctive and easily recognised (Table 2.10). Moreover, when its allele frequencies are compared with those of the species representing the three native species-groups (Fig. 2.6) it is seen to be a quite separate, genetically distinct lineage. *C. stygia* is more distinct than *C. vicina* (74%FD versus 57%FD) but *C. vicina* is more distinct than either *C. dubia* or *C. hilli hilli* (40%FD).

2.3.4 Discussion

The results demonstrate that diagnostic allozyme markers determined from the analysis of adult blowflies are of equal value in the identification of their immatures. Over 90% of the markers expressed in the adult flies in this study, including those shown to be diagnostic, were also expressed in their larvae. This outcome mirrors the success of other workers in using allozyme electrophoresis to identify larvae of e.g. mosquitoes (Corsaro and Munstermann 1983), fruit flies (Dadour *et al.* 1992), pyralid moths (Kioko *et al.* 1995) and chrysomelid beetles (Piedrahita *et al.* 1985).

Molecular identification of northern hemisphere genera of blowfly larvae has recently been accomplished using mitochondrial DNA (Sperling *et al.* 1994). However there have been no reports of the use of allozymes.

Allozyme electrophoresis has some advantages over DNA analysis. Many specimens can be surveyed simultaneously and very quickly, enabling a rapid characterisation of the gene pool of a given taxon. It is also very inexpensive and gives highly reliable results. A disadvantage is that it is strictly comparative; specimens cannot be identified without simultaneous analysis of a control. In contrast, DNA markers are expensive to develop, often hard to

isolate in closely-related species and sometimes found to be lacking in specimens from populations that have not previously been analysed (Hillis *et al.* 1996). However, because DNA analysis does not require specimens that have been frozen, it may still be valuable for blowflies preserved in ethanol.

Misidentification of larvae may have serious consequences. In forensic investigations it could lead to error in the estimation of time since death and in ecological work it could render data meaningless or useless. The technique outlined here provides a ready means of species determination for morphologically difficult larvae.

The genetic affinities revealed for *C. vicina* support its classification by Kurahashi (1989) in subgenus *Calliphora* and by Hardy (1937) in the *C. erythrocephala* [*vicina*]-group, apart from the species of the *C. stygia*-group (*Neocalliphora*) and the *C. augur*- and *hilli*-groups (*Paracalliphora*). *C. vicina* differs genetically from these other subgenera to about the same degree as *Onesia tibialis* (Fig. 2.2) (57%FD and 52%FD, respectively). Such distinctness is not surprising given that *C. vicina* evolved in Europe and was only recently introduced into Australia.

2.4 Conclusions

2.4.1 Molecular Systematics

The results (1) confirm the species status of all forms currently described as species; (2) support a return to the ranking of *C. hilli fallax* as a full species, *C. fallax*; (3) support the recognition of *C.* sp. nov. as a distinct species, and (4) indicate that genetically distinct Kangaroo Island and adjacent mainland subpopulations appear to exist in at least three species. The allozyme data also strongly support the morphologically-based classification of eight of the forms into three separate species-groups and the placing of *C. maritima* in a fourth

(monotypic) group. However, based on these data, the comparative genetic affinities of the parasitic blowfly *Onesia tibialis* suggest that *Calliphora* in its current form may be paraphyletic. Allozyme electrophoresis is also strongly endorsed as a valuable technique for the identification of adult carrion-breeding blowflies.

2.4.2 Molecular Identification of Larvae

Allozyme electrophoresis shows that larvae and adults of a blowfly species are virtually identical genetically. This technique is therefore also valuable as a means of identifying blowfly larvae.

Genetically, *C. vicina* is sufficiently distinct from native *Calliphora* species to warrant its classification in a separate subgenus.

Chapter 3

Morphological Taxonomy and Identification of Third Instar Larvae and Identification of Adults of *Calliphora* Species

3.1 Introduction

This section continues from Chapter 2 to seek means of identifying carrion-breeding *Calliphora* blowflies.

Section 2.3 showed the great potential value of allozyme electrophoresis for identifying immatures. Nevertheless, a morphological approach is also needed. Neither the facilities and materials nor the funds required for molecular analysis are always available. Even when molecular identification is possible it is valuable to have a further means of assessment to cross-check the identification. Identification aside, morphological analysis of immatures is also necessary to gain insights into phylogenetic relationships.

Third instar larvae are dealt with here to the exclusion of the other immature stages since they are the most likely to be encountered in carcasses. Eggs are not produced by all species and, in any case, they usually hatch within a few hours. Well over half of the larval life of a blowfly is spent as a third instar. Therefore reliable identification is a much higher priority for third instar larvae. Blowfly puparia show many of the diagnostic features of the third instar larva and can be identified using their characters (Erzinçlioglu 1985).

Fewer species are treated here than in the previous chapter. Difficulties in obtaining gravid females limited me to species that could be caught in South Australia. Diagnostic descriptions are given for *C. stygia* (Fabricius), *C. albifrontalis* Malloch, *C. dubia* (Macquart), *C. augur* (Fabricius), *C. hilli hilli* Patton, *C.* sp. nov., *C. maritima* Norris and *C. vicina* Robineau-Desvoidy. Some of these species have been fully or partially described previously by other workers. Their results are referred to and discussed below.

A key is also provided for the identification of these eight *Calliphora* species, as well as for their separation from third instar larvae of other genera of carrion-breeding blowflies occurring in this state.

Although the morphological taxonomy of the adults is not addressed in this thesis, their morphological identification is important forensically. A key has therefore also been provided in this chapter for the identification of the adults of the above eight species of *Calliphora*, as well as for the other seven species from southern Australia known or suspected to breed in carrion. This key, like that to the larvae, also separates specimens of *Calliphora* from other genera of carrion-breeding Calliphoridae.

3.2 Materials and Methods

Specimens were obtained by rearing eggs or first instar larvae laid by females that had been wild-caught and, for certain species, bred in colonies established in the laboratory.

Cultures were maintained in cages at 25 ± 1°C under a 12h:12h light:dark regime and were constantly provided with water and sugar. Lamb's liver was used as a medium for oviposition and for the rearing of specimens. Larvae were allowed to pupate in wheat chaff beneath rearing dishes. Puparia were transferred to fresh cages to await the emergence of adults.

Larvae were killed by immersing in near-boiling water, and fixed for 24 hours in acetic alcohol (3 parts 90% ethanol : 1 part glacial acetic acid). They were subsequently preserved in 80% ethanol. A dissecting microscope (mag. x7-x80) was used for the examination of whole larvae, and a compound microscope (mag. x40-x400) for the examination of dissected parts. Such parts were mounted on slides in Swan's Berlese mountant (Smith 1989).

Scanning electron microscopy was investigated as a possible tool for the recognition of diagnostic characters, but was not pursued when it was found that all taxonomically useful features could be seen and studied adequately using light microscopy.

For examination of the cephalopharyngeal skeleton the first three segments were removed and cleared prior to mounting. Clearing was done by macerating the specimen for 30-60 minutes in warm 10% KOH, soaking for 15 minutes in glacial acetic acid to neutralise residual KOH and placing in an equal quantity of Berlese preservative for 24 hours (Smith 1986). The skeleton was mounted on its side so that the lateral profile of exactly one half of the structure was visible.

Line drawings alone were used to illustrate cephalopharyngeal skeletons and distribution of spines because the three-dimensional arrangement of these features cannot be shown satisfactorily in photographs. Drawings were made using a camera lucida. Photographs were used to illustrate the spines because sclerotisation is important but cannot be adequately shown in a drawing. These photographs all show spines in the dorsal region of the anterior spinal band on the first abdominal segment. Photographs were also used to illustrate the microtubercles because of their great abundance in some species.

Measurements were made using an eyepiece graticule.

3.3 Morphology of Third Instar Larvae

3.3.1 General

A blowfly larva comprises a head (Fig. 3.9, H), three thoracic segments (e.g. Fig. 3.9, TS1-3) and eight abdominal segments (e.g. Fig. 3.9, AS1-8). The head bears five pairs of papillae, only two of which are easily seen (Erzinçlioglu 1985). The mouth is ventral in position. On either side of the first thoracic segment lie the *anterior spiracles*. These represent the sclerotised anterior ends of the lateral tracheal trunks that run the length of the larva (Keilin 1944). The first thoracic and each subsequent segment bear *cuticular spines* which assist in locomotion. The spines are mostly arranged in rows grouped into bands. On the posterior surface of the eighth abdominal (last) segment lie seven peripheral pairs of tubercles and the more centrally placed posterior openings of the tracheal trunks - the *posterior spiracles*. The anus (Fig. 3.17, A) is placed ventrally within a longitudinal cleft, surrounded by a pair of lips. These comprise the *perianal pad* (Teskey 1981). Flanking them are a pair of *anal lobes* (Fig. 3.17, AL).

3.3.2 Size

Size in nature varies according to age, resource availability, competition and temperature. However, the minimum and maximum sizes achievable for a species within a specific instar are determined genetically and are thus invariant. The lengths and widths of the smallest and largest third instar specimens were therefore measured. Maximum recorded sizes only have been given for *C. hilli hilli*, *C. maritima* and *C.* sp. nov. because of a lack of early third instar individuals.

The size ranges show that fully-grown larvae of *C. stygia* and *C. albifrontalis* are optimally longer and wider than larvae of the other *Calliphora* considered

here. Furthermore, the smallest third instar larvae of *C. stygia* and *C. albifrontalis* are larger than the smallest third instars of other species.

Because of the factors mentioned above, size is only of limited value in identification. A further limitation is that the length and width of a larva is affected by the medium in which it is killed (Tantawi and Greenberg 1993).

3.3.3 Anterior Spiracles

Each spiracle comprises a number of lobar papillae projecting from a stalk. The spiracular openings lie at the ends of each of these papillae (Teskey 1981). The number of papillae varies both intra- and inter-specifically. The ranges of variation are too great for papilla number to be a reliable means of separating species, although the differences between species are marked and may help in identification. Species of the *stygia*-group always have more than nine papillae per spiracle and may have up to 14. In contrast, the other species may have as few as seven (always at least eight) but never more than 12.

3.3.4 Posterior Spiracles

These are much larger than the anterior spiracles. They are placed side by side and each consists of three slit-like spiracular openings on a circular spiracular plate, surrounded by a heavily sclerotised ring, the *peritreme* (Teskey 1981).

In previous descriptive work the shape of the openings and the shape and degree of sclerotisation of the peritreme have been considered taxonomically significant (e.g. Fuller 1932; Hall 1948; Prins 1982; Liu and Greenberg 1989). Having examined many specimens, I agree with Erzinçlioglu (1985) that in *Calliphora* these characters are too variable to be useful taxonomically.

Holloway (1985, 1991) said she had come to the same conclusion, but nonetheless illustrated these structures for New Zealand blowflies.

Although the structure of the posterior spiracles is too variable to be useful, their greatest diameter can be used to separate species in the *stygia*-group from almost all others. In larvae of this group the spiracles have a greatest diameter ≥ 0.30 mm, while in the remaining species, with the exception of *C. maritima*, the spiracles have a greatest diameter ≤ 0.30 mm. The mean value for this variable was also greater in the *stygia*-group species than in the others - ≥ 0.32 mm vs. ≤ 0.30 mm.

Another character, *the spiracle distance factor* (the distance between the spiracles divided by the maximum diameter of one spiracle) was first used by van Emden (1965) and later by Erzinçlioglu (1985). However, observations made during the present study show that the distance between the spiracles is affected by the degree of cuticular shrinkage and hardening as a result of preservation. This character is therefore not reliable except in specimens that are freshly killed. It was not calculated in this study since all specimens had been preserved for several months or more.

3.3.5 Cephalopharyngeal Skeleton

This structure, extensively investigated by Erzinçlioglu (1985), lies within the head and first thoracic segment and is heavily sclerotised. It consists of a pair of *mouth-hooks* (Fig. 3.1a, MH) , now considered to be maxillary in origin (Griffiths 1994) and various sclerites for muscle attachment, the most prominent of which is the *pharyngeal sclerite*. Like the posterior spiracles, the shape and degree of pigmentation of the cephalopharyngeal skeleton have frequently been used taxonomically. Erzinçlioglu (1985) stressed its great variability. However, after close investigation of the species treated here, it is concluded that while variability makes the cephalopharyngeal skeleton unreliable as a

means of distinguishing all species, *C. vicina* and the species of the *stygia*-group show consistent features. These features are 1) the length of the tooth of the mouth-hook (Fig. 3.1b, LT) (measured from the tip of the tooth to where it joins the base of the mouth-hook) relative to the depth of the base of the mouth-hook (measured dorsoventrally) (Fig. 3.1b, DB); 2) the degree to which the oral sclerite (Fig. 3.1a, OS) is crossed by the *tooth* of the mouth hook (Fig. 3.1a, T); and 3) the shape of the *posterodorsal process* of the *ventral cornu* of the pharyngeal sclerite (Fig. 3.1a, PP,VC). In the *stygia*-group the length of the tooth of the mouth-hook is appreciably greater than the depth of the base of the mouth-hook, whereas in all other species these values are about the same. In *C. vicina* the oral sclerite is only partly crossed by the tooth of the mouth-hook, but it is completely crossed by it in the other species. Finally, the posterodorsal process is much more sharply curved in the *stygia*-group and *C. vicina* than in the other taxa examined.

3.3.6 Spinulation

The shape of the spines and their arrangement on the cuticle is by far the most valuable larval character. There are bands of spines along the anterior and posterior margins of each of TS1-AS8. The anterior spines point posteriorly, while the posterior spines are usually much smaller and point anteriorly. The bands may completely encircle a segment or be restricted to certain parts of its circumference. The anterior bands tend to be less complete on more posterior segments. Conversely, the posterior bands become less complete on the more anterior segments. The arrangement of the bands is virtually identical in all species on TS1-AS3, but differs significantly on AS4-8. In general, *C. vicina* and the *stygia*-group are much more spinose dorsally on AS4-8 than are the other species.

AS1-7 sometimes bear laterally-placed *pleural spines* (Fig. 3.9, PS) (in the fusiform area of Greenberg and Szyska (1984) or on the anterolateral swelling

of Prins (1982)). Erzinçlioglu (1984) and Prins (1982) describe the pleural spines as anterolateral, but close examination shows that they are placed posteriorly on each segment. Pleural spines on all of AS1-7 is diagnostic of the *stygia* and *maritima* species-groups and *C. vicina*, but in all other species these spines, if present, are found only on AS7.

Special attention has been paid to the posterior spinal band surrounding the anus on AS8. The arrangement of these spines is useful for separating the various species-groups. They are in a crude semi-circle in all species other than *C. vicina*. Only *C. stygia* and *C. albifrontalis* have spines between the anus and the anal lobes. Further, these spines are arranged in rows in *C. vicina* and in the *augur* and *maritima* groups, but not in the *stygia* and *hilli* groups.

Finally, the shape and degree of pigmentation of the spines is very useful taxonomically. Each spine comprises a basal region surmounted by a tooth that may be rounded or pointed (Erzinçlioglu 1985). Species of the *stygia*-group are notable for their large rounded spines. Other species have smaller, pointed spines. Spines in some species, such as *C. vicina*, are double-pointed, with a pair of teeth. The sister species in the *stygia*- and *augur*-groups can only be reliably separated using the morphology of the spines.

3.3.7 Microtubercles

These minute rounded elevations of the cuticle (Fig. 3.26, M) occur in the posterolateral regions of the abdominal segments, extending further dorsally the more posterior the segment. On AS6 and 7 they may also occur anterioraly and on AS8 always both dorsally and laterally. The microtubercles are larger and thus more prominent in *C. stygia*, *C. albifrontalis* and *C. vicina* than in the other species. They are particularly abundant in *C. vicina*. As an aid to identification, it is best to examine the microtubercles on the dorsal surface of AS8.

Liu and Greenberg (1989) recorded these structures in several species of *Calliphora* (including *C. vicina*) and *Lucilia* in North America.

3.3.8 Tubercles on AS8

These tubercles are larger and better developed in *C. stygia* and *C. albifrontalis* than in the other species. Otherwise, of the seven pairs of tubercles on AS8, only the three dorsalmost pairs (Fig. 3.15, T1-T3) are useful taxonomically. The distances between these tubercles separate the *stygia*-group species and usually also *C. vicina* from the other taxa. In *C. stygia*, *C. albifrontalis* and usually *C. vicina* the middle tubercle (T2) is closer to the ventralmost tubercle (T3) than to the dorsalmost tubercle (T1), whereas these tubercles are always equidistant in the other species. Erzinçlioglu (1985) also found this character useful.

3.3.9 Intraspecific Variation in Morphology

As noted above, there is considerable intraspecific variation in morphology. This is particularly true of the spines and this should be considered when examining the figures given here. These only represent typical specimens and should be used in conjunction with the text. Moreover, these comments only apply to specimens from South Australia. Several species are also found in other parts of southern Australia and further variation in their larval morphology probably occurs within their geographical ranges. This variability may mean that the key to third instar *Calliphora* larvae will misidentify specimens from other states.

3.4 Species Descriptions of Third Instar Larvae

3.4.1 *Calliphora stygia* (Fabricius, 1782)

Musca stygia Fabricius, 1782: 438.
Calliphora villosa Robineau-Desvoidy, 1830: 437.
Pollenia rufipes Macquart, 1835: 271.
Musca laemica White *in* White and Doubleday, 1843: 291 [1845: 263].

Size

Length = 10.0-22.0 mm; width = 1.8-3.8 mm.

Cephalopharyngeal Skeleton (Figs 3.1a and 3.1b)

Tooth of mouth hook appreciably longer than depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process sharply curved.

Anterior Spiracles

With 9-14 papillae ($\bar{x}$ = 11.3 ± 1.1, n = 50).

Posterior Spiracles

Greatest diameter = 0.30-0.43 mm ($\bar{x}$ = 0.34 mm ± 0.04 mm, n = 25).

Distribution and Structure of Spines

Anterior bands completely encircling TS1-AS5; incomplete dorsally on AS6; present laterally and ventrally on AS7 and 8 (Fig. 3.9). Posterior bands present ventrally on AS2-5 (although only as scattered spines on AS2); dorsally and ventrally on AS6 and 7 (dorsal band on AS7 continuous with pleural spines) (Fig. 3.9). Posterior spines on AS8 present between anus and anal lobes, with

concentration of spines directly dorsal to anus (Fig. 3.16); spines dorsal to anus arranged randomly in rough semicircle (Fig. 3.17). Pleural spines present on AS1-7 (Fig. 3.9). Spines large with rounded teeth; distributed relatively sparsely and evenly in rows (Fig. 3.29).

Microtubercles

Prominent, especially dorsally on AS8 (Fig. 3.26), although somewhat more patchily distributed than in *C. vicina*.

Tubercles on AS8

Large; width of base of T1, T2 and T3 greater than distance between them. T2 closer to T3 than to T1.

Notes

Fuller (1932) concentrated almost exclusively on the structure of the cephalopharyngeal skeleton and posterior spiracles in her description of this species. However, these structures are shown here to be of little use as a means of differentiating *C. stygia* from its relatives. The details given by Zumpt (1965) closely follow Fuller's and are therefore equally inadequate. The comments of Erzinçlioglu (1984) and Holloway (1985, 1991) on this species are consistent with the results of the present study, although they omitted some details. The spines were accurately described and illustrated by Miller (1939) (as *C. laemica* (White)), but like Fuller, he placed too much emphasis on variable features of the posterior spiracles and cephalopharyngeal skeleton. Erzinçlioglu (1987) published a key which separates the third instar larvae of this species from those of *C. augur*.

Calliphora stygia was included in O'Flynn and Moorhouse's (1980) keys to the identification of the eggs and early instar larvae of carrion-breeding Calliphoridae in Queensland.

3.4.2 *Calliphora albifrontalis* Malloch, 1932

Musca australis Boisduval, 1835: 669. [Preoccupied Gmelin, 1790.]
Calliphora albifrontalis Malloch, 1932: 67.
Calliphora maryfullerae Hardy, 1947: 56 (as *maryfulleri*; nov. n. for *australis* Boisduval).

Size

Length = 10.2-20.4 mm; width = 1.8-3.5 mm.

Cephalopharyngeal Skeleton (Fig. 3.2)

Tooth of mouth hook appreciably longer than depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process sharply curved.

Anterior Spiracles

With 10-14 lobes ($\bar{x}$ = 12.0 ± 1.0, n = 50).

Posterior Spiracles

Greatest diameter = 0.30-0.35 mm ($\bar{x}$ = 0.32 mm ± 0.01 mm, n = 25).

Distribution and Structure of Spines

Mainly as in *C. stygia* (Figs 3.9, 3.16 and 3.17). The only reliable difference detected between *albifrontalis* and *stygia* is in the structure of the spines. In *C. albifrontalis* the unpigmented base of each spine is prominently raised above the cuticle below the pigmented portion of the spine. In *C. stygia* the base is not raised to such an extent, so that virtually the entire spine is pigmented. The overall result of this is that spines in *C. albifrontalis* are noticeably longer than in *C. stygia* (Fig. 3.30).

Microtubercles

As in *C. stygia*.

Tubercles on AS8

As in *C. stygia*.

Notes

None of the immature stages of this species have been described previously. Mackerras and Fuller (1937) referred to the larvae of *C. stygia* and *C. albifrontalis* (as *C. australis*) as being "very much alike", but did not specify the nature of any differences.

3.4.3 *Calliphora dubia* (Macquart, 1855)

Musca placida Walker, 1853: 343. [Preoccupied Müller, 1764.]
Rhynchomyia dubia Macquart, 1855: 129(109).
Calliphora nociva Hardy, 1932: 556.

Size

Length = 8.6-19.6 mm; width = 1.6-3.0 mm.

Cephalopharyngeal Skeleton (Fig. 3.3)

Tooth of mouth hook approximately same length as depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process roundly arched.

Anterior Spiracles

With 8-11 lobes ($\bar{x}$ = 8.9 ± 0.7, n = 50).

Posterior Spiracles

Greatest diameter = 0.18-0.28 mm ($\bar{x}$ = 0.24 mm ± 0.03 mm, n = 25).

Distribution and Structure of Spines

Anterior bands completely encircling TS1-AS4 (although faint dorsally on AS4); incomplete dorsally on AS5; present ventrally, lateroventrally and sometimes faintly laterodorsally on AS6, ventrally and lateroventrally on AS7 and ventrally on AS8 (Fig. 3.10). Posterior bands present ventrally on AS2-5, ventrally and sometimes faintly laterodorsally on AS6; absent lateroventrally and usually also in pleural region of AS7, otherwise complete dorsally and ventrally (Fig. 3.10). Posterior spines on segment AS8 absent between anus and anal lobes (Fig. 3.18); spines dorsal to anus tiny and arranged in distinct rows in crude semicircle (Fig. 3.19). Pleural spines lacking. Spines small with tiny pointed teeth, rarely double-pointed, arranged in sets of two or three in rows (Fig. 3.31).

Microtubercles

Extremely tiny and inconspicuous (Fig. 3.27).

Tubercles on AS8

Small. Width of base of T1, T2 and T3 less than distance between them. T1, T2 and T3 equidistant from each other.

Notes

C. dubia was previously described by Morris (1991) (as *C. nociva* Hardy). Her description of the third instar larva is generally consistent with the above, although her value for maximum length is markedly less (15.7 mm vs. 19.7 mm).

3.4.4 *Calliphora augur* (Fabricius, 1775)

Musca augur Fabricius, 1775: 777.
Calliphora oceaniae Robineau-Desvoidy, 1830: 438.
Ochromyia lateralis Macquart, 1843: 291(134).
Calliphora rufiventris Macquart, 1847a: 82 [1847b: 98].
Musca dorsalis Walker, 1849: 907. [Preoccupied Fabricius, 1775.]
Ochromyia nigricornis Macquart, 1851: 218(245).

Size

Length = 7.6-16.0 mm; width = 1.4-3.0 mm.

Cephalopharyngeal Skeleton (Fig. 3.4)

Tooth of mouth hook approximately same length as depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process roundly arched.

Anterior Spiracles

With 8-12 lobes ($\bar{x}$ = 10.2 ± 1.0, n = 50).

Posterior Spiracles

Greatest diameter = 0.23 mm-0.30 mm ($\bar{x}$ = 0.26 mm ± 0.76 mm, n = 25).

Distribution and Structure of Spines

Distribution of spines (Figs 3.11, 3.18 and 3.19) as in *C. dubia*, except that posterior spines always present in pleural area on AS7, faintly dorsally and laterally (including pleural area) on AS6 and sometimes faintly laterodorsally on AS5 (Fig. 3.11). Spines with weaker pigmentation and more often double-pointed than in *C. dubia*, and teeth larger (Fig. 3.32).

Microtubercles

As in *C. dubia.*

Tubercles on AS8

As in *C. dubia.*

Notes

O'Flynn (1976) recorded considerably fewer anterior and posterior spinal bands, particularly on the abdominal segments, than I observed. Otherwise, her description generally tallies with mine. Erzinçlioglu's (1984) description differs from mine in regard to the condition of the anterior spinal bands on segments AS5 and 6 and the posterior band on AS6. He found that these bands were complete dorsally, whereas I found them to be incomplete. This might be geographic variation since Erzinçlioglu's specimens came from Queensland and New South Wales. As with *C. stygia*, Zumpt (1965) and Fuller (1932) emphasised the cephalopharyngeal skeleton and posterior spiracles. Again, in the present study they are found to be inadequate for identification. As referred to above, Erzinçlioglu's (1987) key separates the third instar larvae of this species from those of *C. stygia.*

Mackerras and Fuller (1937) referred to the larvae of *C. augur* and *C. dubia* (as *C. nociva*) as being "difficult to distinguish from each other", but did not say whether it was nonetheless possible.

Descriptions of the first and second instars were given by Erzinçlioglu (1984), O'Flynn (1976), and O'Flynn and Moorhouse (1980).

3.4.5 *Calliphora hilli hilli* Patton, 1925

Calliphora hilli Patton, 1925: 400.
Calliphora milleri Hardy, 1937: 22.

Size

Maximum length = 16.0 mm; maximum width = 2.6 mm.

Cephalopharyngeal Skeleton (Fig. 3.5)

Tooth of mouth hook approximately same length as depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process roundly arched.

Anterior Spiracles

With 7-12 lobes ($\bar{x}$ = 10.1 ± 0.8, n = 50).

Posterior Spiracles

Greatest diameter = 0.23 mm-0.30 mm ($\bar{x}$ = 0.27 mm ± 0.71 mm, n = 25).

Distribution and Structure of Spines

Anterior bands completely encircling TS1-AS3; incomplete dorsally on AS4; present ventrally, lateroventrally and sometimes faintly laterodorsally on AS5, ventrally on AS6-8 (Fig. 3.12). Posterior bands present ventrally on segments AS2-5 (although only as scattered spines on AS2 and 3), ventrally and sometimes faintly laterodorsally on AS6; encircling AS7, except for a break ventral to pleural area (Fig. 3.12). Posterior spines on segment AS8 absent between anus and anal lobes (Fig. 3.20); spines dorsal to anus arranged randomly in crude semicircle (Fig. 3.21). Pleural spines lacking. Spines small with pointed teeth, arranged evenly in rows (Fig. 3.30).

Microtubercles

As in *C. dubia.*

Tubercles on AS8

As in *C. dubia.*

Notes

Descriptive details of the third instar were given by Fuller (1932) (as *C. fallax* Hardy). The comments above concerning Fuller's descriptions of *C. stygia* and *C. augur* also apply to this species. Zumpt (1965) stated that 'the figures she [Fuller] gives do not coincide...with specimens named *C. hilli* which I received...'. However, he did not provide a redescription. Miller's (1939) description of *C. hilli hilli* (as *C. rufipes* (Macquart)), like his description of *C. stygia* (see above), provides inaccurate details concerning the posterior spiracles and cephalopharyngeal skeleton but he describes the structure of spines correctly. Descriptive details were also given by Holloway (1985, 1991), whose findings accord with my own.

3.4.6 *Calliphora* sp. nov.

Size

Maximum length = 16.0 mm; maximum width = 3.2 mm.

Cephalopharyngeal Skeleton (Fig. 3.6)

Tooth of mouth hook approximately same length as depth of base of mouth-hook; tooth completely crossing oral sclerite. Ventral cornu with posterodorsal process roundly arched.

Anterior Spiracles

With 8-12 lobes ($\bar{x}$ = 9.8 ± 0.9, n = 30).

Posterior Spiracles

Greatest diameter = 0.25-0.28 mm ($\bar{x}$ = 0.26 mm ± 0.01 mm, n = 20).

Distribution and Structure of Spines (Figs 3.12, 3.20, 3.21 and 3.34)

As in *C. hilli hilli*.

Microtubercles

As in *C. dubia*.

Tubercles on AS8

As in *C. dubia*.

Notes

Since this species has not been described formally, the larvae have not been described previously.

3.4.7 *Calliphora maritima* Norris, 1994

Calliphora maritima Norris, 1994: 1344.

Size

Maximum length = 19.0 mm; maximum width = 3.2 mm.

Cephalopharyngeal Skeleton (Fig. 3.7)

Tooth of mouth-hook approximately same length as depth of base of mouth hook; tooth completely crossing oral sclerite. Ventral process with posterodorsal process roundly arched.

Anterior Spiracles

With 7-10 lobes ($\bar{x}$ = 8.8 ± 0.7, n = 50).

Posterior Spiracles

Greatest diameter = 0.28 mm-0.33 mm ($\bar{x}$ = 0.30 mm ± 0.65 mm, n = 25).

Distribution and Structure of Spines

Anterior bands completely encircling TS1-AS4, incomplete dorsally on AS5, present ventrally, lateroventrally and faintly laterodorsally on AS6, ventrally and lateroventrally on AS7 and 8 (Fig. 3.13). Posterior bands present ventrally on AS2-5 (although only as scattered spines on AS2 and 3); ventrally and laterodorsally on AS6; encircling AS7, except for a break ventral to pleural area (Fig. 3.13). Posterior spines on segment AS8 absent between anus and anal lobes (Fig. 3.22); spines dorsal to anus arranged in sparse rows in crude semicircle (Fig. 3.23). Pleural spines on AS1-7, although only sparse on AS4-6 (Fig. 3.13). Spines small with prominent pointed teeth, arranged somewhat unevenly in rows (Fig. 3.35).

Microtubercles

As in *C. dubia.*

Tubercles on AS8

As in *C. dubia.*

Notes

The immature stages of this species have not been described previously.

3.4.8 *Calliphora vicina* Robineau-Desvoidy, 1830

Musca carnivora Fabricius, 1794: 313.
Musca erythrocephala Meigen, 1826: 62. [Preoccupied De Geer, 1776.]
Calliphora vicina Robineau-Desvoidy, 1830: 435.
Calliphora littoralis Robineau-Desvoidy, 1830: 435.
Calliphora spitzbergensis Robineau-Desvoidy, 1830: 435.
Calliphora monspeliaca Robineau-Desvoidy, 1830: 436.
Calliphora musca Robineau-Desvoidy, 1830: 436.
Calliphora nana Robineau-Desvoidy, 1830: 436.
Calliphora scutellata Macquart, 1834: 161.
Musca thuscia Walker, 1849: 897.
Calliphora rufifacies Macquart, 1851: 216 [243].
Musca aucta Walker, 1853: 334.
Calliphora insidiosa Robineau-Desvoidy, 1863: 695.
Calliphora turanica Rohdendorf, 1926: 90.

Size

Length = 7.2-17.0 mm; width = 1.2-2.6 mm.

Cephalopharyngeal Skeleton (Fig. 3.8)

Tooth of mouth hook appreciably longer than depth of base of mouth hook; tooth only partly crossing oral sclerite. Ventral cornu with posterodorsal process sharply bent.

Anterior Spiracles

With 7-11 lobes ($\bar{x}$ = 8.3 ± 1.0, n = 30).

Posterior Spiracles

Greatest diameter = 0.20-0.28 mm ($\bar{x}$ = 0.24 mm ± 0.03 mm, n = 25).

Distribution and Structure of Spines

Anterior bands completely encircling TS1-AS5 (although faint and sometimes incomplete dorsally on AS5); present ventrally, lateroventrally and faintly laterodorsally on AS6; ventrally and lateroventrally on AS7 and 8 (Fig. 3.14). Posterior bands present ventrally on AS2-4 (although sometimes faintly laterodorsally and even dorsally on AS4); ventrally, faintly laterodorsally (sometimes dorsally and sometimes also faintly lateroventrally (segment may be completely encircled)) on AS5; completely encircling AS6 and 7 (Fig. 3.14). Posterior spines on AS8 absent between anus and anal lobes (Fig. 3.24); spines dorsal to anus tiny and arranged in distinct rows without a semicircular pattern (Fig. 3.25). Pleural bands present faintly (Fig. 3.14). Spines small and weakly pigmented with tiny pointed teeth, sometimes double-pointed, arranged unevenly in broken rows (Fig. 3.36).

Microtubercles

Prominent and particularly numerous, especially dorsally on AS7 and dorsally and laterally on AS8 (Fig. 3.28).

Tubercles on AS8

Small. Width of base of T1, T2 and T3 smaller than distance between them. T2 usually closer to T3 than to T1, although in some specimens the three tubercles are equidistant.

Notes

Miller (1939) described this species (as *C. erythrocephala*), but gave only details of the spiracles of the third instar larva. Hall's (1948), Schumann's (1953/1954) and Liu and Greenberg's (1989) descriptions of the distribution of spines in the third instar tally with mine, except that they describe the posterior spinal band of AS5 as complete circumferentially (Liu and Greenberg also say that the band on AS4 is sometimes complete), but AS5 was usually incomplete in my specimens and AS4 always so. Since these authors were studying northern hemisphere larvae the differences are not surprising. Conversely, Holloway's (1985, 1991) descriptions of specimens from New Zealand are totally consistent with mine. So too, for the most part, is Erzinçlioglu's (1985) description of British specimens, although his value for maximum length is considerably below that recorded here (13.75 mm vs. 17.0 mm).

3.5 Discussion

3.5.1 Identification

Although third instar larvae of most species examined here can now be identified morphologically, the sister species in each species-group are very similar. Consequently their separation is not easy and identification would be unreliable without experience. Future work on these sister species may show that their developmental biology is virtually identical, especially since they are so close phylogenetically. From an ecological and forensic perspective the separation of such species may not be a priority. However, as shown in Chapter 2, sister taxa may be reliably separated using molecular analysis.

3.5.2 Phylogenetic Relationships

The morphological features identified here reinforce both the composition of the species-groups recognised in Chapter 2 and the subgenera recognised by Kurahashi (1971, 1989). They also support the distinctness of *C. vicina*, the sole representative of a third subgenus, *Calliphora s. str.* (Kurahashi 1989), which was included in my molecular study of larvae (Section 2.3).

The species of the *stygia*-group are least divergent morphologically. This is shown by the structure of the spiracles, mouth-hooks, dorsal papillae on AS8 and spines, and by the distribution of the bands of spines. *C. vicina* is the next closest taxon, sharing some features with the *stygia*-group but standing alone in the position of the oral sclerite and the distribution of its spines. Within *Paracalliphora*, *C. maritima* stands out in possessing pleural bands on AS1-6. The species of the *augur*-group appear to be the next most closely related to *C. maritima* since they only differ from it in the morphology and distribution of the spines. Finally, the species of the *hilli*-group appear to be the most divergent based on third instar larval morphology. Although they are similar to *augur*-group species, the form and arrangement of the spines in *C. hilli hilli* and *C.* sp. nov. is quite different and the anterior band of spines on AS4 is incomplete dorsally. This tentative arrangement of the species is similar to that arrived at biochemically in Chapter 2 by analysing the adults. It differs in that *C. maritima* is morphologically closer to the *stygia*-group, rather than the *augur*-group as suggested by the molecular study. However, these species cannot be fully evaluated without also considering the structure of the adults.

3.6 Identification of Adults

Although a comprehensive study of the morphology of the adults of the carrion-breeding *Calliphora* from southern Australia would further clarify their phylogenetic affinities, it has not been undertaken here because a full systematic

analysis is beyond the scope of this thesis. In any case, the adults of all species, other than *C.* sp. nov., have already been described (although mostly only the males).

Nevertheless, the broad external morphological differences between adults are valuable forensically because they permit the reliable identification of each species without molecular analysis. Prior to my molecular studies it was not known if this was possible because some taxa could have comprised complexes of cryptic species. These differences were studied, as a precursor to the molecular work, so that the various forms could be recognised (the exception to this is *C. ochracea*, which was studied later). The separating features have been used to devise a key, given below, which enables the identification of both sexes of the adults of all 11 species of carrion-breeding *Calliphora* known from southern Australia, along with four species that are suspected to be carrion-breeders. No key to adults which is as comprehensive has been published previously.

Like the larvae, adults display a certain degree of intraspecific variability that may be geographically related. However, the characters used in the key to adults take account of such variability because adults are easier to collect than larvae, and specimens could therefore be examined from a broad range of localities within the geographic distribution of each species.

3.7 Keys

3.7.1 Key to Third Instar Larvae of Carrion-breeding Genera of Calliphoridae and to Known Third Instar Larvae of Carrion-breeding Species of *Calliphora* in South Australia

1. Posterior spiracular peritreme incomplete .....*Chrysomya*
   Posterior spiracular peritreme complete 2
2. Oral sclerite unpigmented .....*Lucilia*
   Oral sclerite pigmented (*Calliphora*) 3
3. Microtubules conspicuous dorsally on AS8 (e.g. Fig. 3.28) 4
   Microtubules inconspicuous dorsally on AS8 (e.g. Fig. 3.27) 6
4. Spines small with pointed teeth (Fig. 3.36) .....
   .....*Calliphora vicina* Robineau-Desvoidy
   Spines large with rounded teeth (e.g. Fig. 3.29) 5
5. Spines with raised base (Fig. 3.30) .....*Calliphora albifrontalis* Malloch
   Spines with base not raised (Fig. 3.29) .....*Calliphora stygia* (Fabricius)
6. Pleural spines present on AS1-7 (Fig. 3.13) .....
   .....*Calliphora maritima* Norris
   Pleural spines absent on AS1-7 (e.g. Fig. 3.12) 7
7. Spines arranged evenly in rows (e.g. Fig. 3.33); anterior spine band on AS4 incomplete dorsally; anterior spines only sometimes present laterodorsally on AS5 (Fig. 3.12) .....
   .....*Calliphora hilli hilli* Patton and *Calliphora* sp. nov.
   Spines arranged in sets of two or three in rows (e.g. Fig. 3.31); anterior spine band on AS4 complete dorsally; anterior spines always present laterodorsally on AS5 (Fig. 3.10) 8
8. Spines darker, with teeth smaller and only rarely in pairs (Fig. 3.31) .....
   .....*Calliphora dubia* (Macquart)
   Spines paler, with teeth larger and more often in pairs (Fig. 3.32) .....
   .....*Calliphora augur* (Fabricius)

3.7.2 Key to Adults of Carrion-breeding Genera of Calliphoridae and of Species of *Calliphora* known or suspected to breed in carrion in Southern Australia

(The known carrion-breeding species are asterisked.)

1. Base of stem-vein setulose dorsally .....*Chrysomya*
   Base of stem-vein bare dorsally 2
2. Lower calypter bare dorsally; body metallic green .....*Lucilia*
   Lower calypter haired dorsally; body metallic blue to blue-black or yellow to brown (*Calliphora*) 3
3. Three pairs of presutural acrostichal setae 4
   Two pairs of presutural acrostichal setae 6
4. Postocular region with gold dusting; coxae yellow-orange .....
   .....*Calliphora fulvicoxa* Hardy
   Postocular region with silver dusting; coxae darkened 5
5. Femora uniformly orange on outer surface; fronto-orbital plates and parafacials with gold dusting; ♂: frons minimum width less than $\frac{1}{2}$ the width of anterior ocellus; ommatidia on anterior upper $\frac{2}{3}$ of eyes considerably enlarged to about 2x width of others .....
   .....*Calliphora stygia* (Fabricius)*
   Fore femora darkened on proximal $\frac{1}{4}$ of upper-most $\frac{1}{2}$ of outer surface; fronto-orbital plates and parafacials with silver dusting; ♂: frons minimum width about width of anterior ocellus; ommatidia on anterior upper $\frac{2}{3}$ of eyes only slightly larger than others .....
   .....*Calliphora albifrontalis* Malloch*
6. Legs black 7
   Legs yellow, orange or brown 8
7. Abdomen with blue sheen .....*Calliphora vicina* Robineau-Desvoidy*
   Abdomen mottled golden .....*Calliphora canimicans bezzii* Hardy

8. Abdomen with metallic blue or green median vitta 9
   Abdomen lacking metallic median vitta 10
9. Abdomen with greenish-blue sheen; dusting on fourth visible tergite yellowish .....*Calliphora augur* (Fabricius)*
   Abdomen with rich blue or purplish sheen; dusting on fourth visible tergite vivid white .....*Calliphora dubia* (Macquart)*
10. Abdomen entirely uniform dark orange; eyes with dense erect yellow hairs 11
    Abdomen entirely mottled olive-green or golden; eyes glabrous 12
11. Mesonotum with yellowish dusting .....*Calliphora ochracea* Schiner*
    Mesonotum with whitish dusting .....*Calliphora nigrithorax* Hardy
12. Fore femora darkened on proximal $\frac{1}{2}$ of inner surface; dorsum of abdomen with olive-green sheen .....*Calliphora maritima* Norris*
    Fore femora orange on proximal $\frac{1}{2}$ of inner surface; dorsum of abdomen rather lustreless golden 13
13. Parafacials with a small brilliant white spot; ♂: ommatidia on anterior upper $\frac{2}{3}$ of eyes considerably enlarged to about 2x width of others .....
    .....*Calliphora gilesi* Norris
    Parafacials lacking a small brilliant white spot; ♂: ommatidia on anterior upper $\frac{2}{3}$ of eyes only slightly larger than others 14
14. Parafacials with silver dusting .....*Calliphora varifrons* Malloch*
    Parafacials with gold dusting 15
15. ♂: frons minimum width about width of anterior ocellus; ♀: frons width at level of lunule < 35% of head width .....
    .....*Calliphora fallax* Hardy*
    ♂: frons minimum width at least about 2x width of anterior ocellus; ♀: frons width at level of lunule > 35% of head width 16

16. Postocular region with gold dusting; ♂: frons minimum width about 2x width of anterior ocellus .....*Calliphora hilli hilli* Patton*

Postocular region with silver dusting; ♂: frons minimum width about 6x width of anterior ocellus .....*Calliphora* sp. nov.*

Note

The following four species are possibly carrion-breeders: *C. nigrithorax* Hardy, *C. fulvicoxa* Hardy, *C. gilesi* Norris and *C. canimicans bezzii* Hardy. All have been collected at liver-baited traps, but have not been observed to breed in carcasses in the field. Some may yet be shown to be, like *Onesia tibialis*, parasites of earthworms, visiting carrion to feed rather than to reproduce. It is important that these species should be included in the key since workers studying carrion may well encounter them. Their immature stages have not been described. If there is any doubt about the identity of larvae the specimens should be reared through to the adult stage, or a molecular approach to their identification should be adopted.

Calliphora nigrithorax is the sister species of *C. ochracea* and thus is probably also a carrion-breeder. It is known from South Australia, Victoria, the Australian Capital Territory, New South Wales and Tasmania. These species, along with *C. quadrimaculata* (Swederus) from New Zealand, are probably the most primitive living *Calliphora* (Dear 1985).

Calliphora fulvicoxa has been recorded from all Australian states and territories except the Northern Territory. It has been shown to oviposit on carrion in the laboratory (Hardy 1937). It was placed in *Paracalliphora* by Kurahashi (1971).

Calliphora gilesi is known from south-western Western Australia and in South Australia from Minnipa, Flinders Island and Kangaroo Island. It is very similar

morphologically to *C. hilli hilli*, but distinguished as shown in the key (characters of Norris 1994).

Calliphora canimicans bezzii is known only from central coastal Victoria. It has been reared from liver (K. R. Norris, Division of Entomology, CSIRO, pers. comm.). Kurahashi (1971) placed it in the subgenus *Australocalliphora*.

3.8 Conclusions

3.8.1 Morphological Taxonomy and Identification of Third Instar Larvae

Study of the morphology of third instar larvae of eight species of *Calliphora* has provided characters that make it possible to identify all species other than *C. hilli hilli* and *C.* sp. nov. These species are inseparable. However, the sister species within the *stygia*- and *augur*-groups are also very difficult to separate, confirming the continued need for molecular identification in certain cases. Overall, substantial morphological distinctness exists only at the species-group level. Morphological differences at this level largely support the species-group and subgeneric arrangements proposed on the basis of the biochemical study in Chapter 2, as well as the separate status of *C. vicina*.

3.8.2 Identification of Adults

Examination of the external morphology of both sexes of the adults of the 11 known species of carrion-breeding *Calliphora* in southern Australia reveals features that enable them all to be separated unambiguously.

Chapter 4

Thermogenesis in Blowfly Larvae

4.1 Introduction

When animals aggregate closely they are less dependent on external sources of energy for heat. This is because every member of the aggregation experiences a reduction in the surface area that is exposed to the surrounding air and thus a reduction in the heat that is lost to it (Schmidt-Nielsen 1997). Such energy-saving behaviour is seen in some vertebrates, e.g. 'huddling' in emperor penguins in the Antarctic (Ancel *et al.* 1997). Among the invertebrates, a number of reports concern aggregation in insects.

In general, insects have low body temperatures. Their small body mass results in rapid convective cooling that prevents an appreciable increase in body temperature from endothermy or solar radiation (Heinrich 1993). However, by aggregating they reduce heat loss. This is particularly important to immatures, in which the resultant increased temperature raises their metabolic rate, thus producing faster growth.

Accounts of adult insects that have evolved aggregation as a behavioural strategy are rare. One example is the honey bee, *Apis mellifera* Linnaeus. Honey bees regulate the temperature inside the hive during winter by forming clusters. Gates (1914) measured a temperature of 31°C in such a cluster of bees, when the temperature outside the hive was only -28°C - a remarkable difference of 59°C! Aggregation is put to an altogether different purpose by the Japanese honey bee, *Apis cerana japonica* Radoszkowski. Bees of this subspecies defend themselves from the predatory hornets, *Vespa simillima xanthoptera* Cameron

and *V. mandarinia japonica* Radoszkowski, by 'balling' around individual hornets until the temperature rises to ~47°C, which kills the hornet, but not the bees (Ono *et al.*, 1987; 1995).

In several species of Lepidoptera the development time of the larvae is decreased as a consequence of their aggregation. For example, the eastern tent caterpillar, *Malacosoma americanum* (Fabricius), spins silken tents into which the animals crowd when not feeding. The tents allow solar radiation to produce heating. They also reduce convective loss of metabolically-produced heat, thus retarding cooling (Knapp and Casey 1986). As a result, temperatures in these tents may be up to 43°C above air temperature (Joos *et al.* 1988).

Another example is that of the wax moth, *Galleria mellonella* Linnaeus, whose larvae infest bee hives. They feed on the wax of the old brood combs and other materials while being insulated from convective heat loss (Heinrich 1993). Buchmann and Spangler (1992) recorded a maximum temperature of 41.3°C in a hive empty of bees but infested with these larvae. The simultaneous air temperature outside it was only 28°C.

Even in insects that do not specifically aggregate there may be a significant effect. For example, Pimental (1958) showed an increment in temperature in cultures of the flour beetles, *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst), proportional to the number of adult insects present. Further, when 300 larvae were placed together they raised the temperature of the surrounding medium by a greater amount than the same number of adults (0.6°C above control vs. 0.3°C). Spradbery (1992) found that when puparia of the screw-worm fly, *Chrysomya bezziana* Villeneuve, were stored in bulk, just before eclosion the temperature elevation at an ambient temperature of 25°C was as much as 14°C. As in *Tribolium*, the magnitude of the temperature elevation was proportional to the number of individuals present.

Despite the spectacular nature of some of these observations, most of the reports of heat generation by insect aggregations in the literature refer to blowfly larvae in carrion. The first report was by Girard (1869, cited in Heinrich 1993) who noted that a box full of maggots of *Lucilia caesar* (Linnaeus) generated a temperature excess of 32°C. Other examples, discussed later in this chapter, establish that aggregations of blowfly larvae often elevate the temperature in carcasses above that of the surrounding air to a substantial degree. However, despite the importance of larval thermogenesis to our understanding of the ecology and physiology of blowflies and especially to forensic entomology, most reports are anecdotal and form parts of broader studies of the ecology of carcass decomposition.

Of particular interest is the effect of the temperature of the ambient air on the thermal environment of the larvae in the carcass. Factors that might influence the temperatures generated by blowfly larvae are their taxonomic identity, their number, their stage of development, the mass and stage of decomposition of the carcass in which they are feeding, and whether the carcass is sunlit or shaded. Blowfly larvae are also commonly parasitised by minute Hymenoptera (Erzinçlioglu 1996) and this would almost certainly affect their metabolism and therefore also thermogenesis.

The work presented here is more detailed than in any previous blowfly study and was designed to investigate several of these factors systematically. Experiments were carried out on larval thermogenesis during cool and warm parts of the year, and in small and large carcasses. A major weakness of previous work has been the lack of a control (an uninfested carcass or carcasses), which would enable the possible role of bacteria in heat generation to be estimated. In the present work, all carcasses infested with larvae were compared simultaneously with carcasses from which blowflies were excluded. Measuring temperature in a part of an infested carcass apart from the feeding larvae would be inconclusive

because the temperature there might be influenced by larval heat conducted through the body tissues.

My studies are presented below as three main sets of experiments:- 1) Thermogenesis in larvae in small carcasses; 2) thermogenesis in larvae in large carcasses and 3) effect of solar radiation on thermogenesis. In the treatment of each set of experiments discussion is confined to issues of direct relevance to that set; discussion of the general trends observed in the experiments as a whole and their applied significance is found in Section 4.7. The experiments in each set are presented in the order in which they were carried out; later experiments often dealt with issues or questions raised by earlier experiments.

4.2 Thermogenesis in Larvae in Small Carcasses

4.2.1 Introduction

Small carcasses are a more limited food resource than large carcasses, and would be expected to contain smaller aggregations of blowfly larvae. They would also provide less insulation for developing larvae than large carcasses, and this might mean that aggregations of equivalent size would produce lower temperatures in a small carcass than in a large one. Nevertheless, there might still be enough heat conserved within a small carcass to accelerate the development of larvae beyond the rate that might be expected from air temperatures.

The aim of the experiments described here was to determine the extent to which heat is generated in small aggregations in small carcasses at different times of year. Blowflies differ in their thermal tolerances (Norris 1965) and therefore some species are only active in particular seasons. Thus, the season in which the experiment was done determined which species were available to be studied.

There were two experiments, one in late June and early July, 1996 (winter) and the other in January, 1997 (summer). The summer experiment also helped examine (using a shaded control) the effect of solar radiation on thermogenesis (see Section 4.4).

4.2.2 Experiment 1: Thermogenesis in *Calliphora* Larvae in a Piglet Carcass in Winter

Materials and Methods

In the winter experiment I used two carcasses of baby pigs, *Sus scrofa* Linnaeus, each initially weighing approximately 2 kg. These had died overnight of natural causes and their carcasses were obtained the following morning. They were kept refrigerated at 4°C for a day until being placed outdoors on the morning of 24 June. Each carcass was placed on its side on a bed of wheat chaff in a rectangular plastic container. The chaff was intended as a pupation medium for larvae once they had ceased feeding and left the carcass. The experimental carcass was infested with larvae by being exposed to blowflies for five days, until 29 June. Its container was then covered with very fine nylon fabric to prevent further oviposition. This ensured that the number of larvae in the carcass was not excessive. The fabric was sufficiently fine to prevent the entry of eggs and first instar larvae but not to impede the passage of air.

The second carcass was a control from which blowflies were excluded using nylon fabric throughout the experiment. Both carcasses were placed in stout wire cages to exclude vertebrate scavengers. They were also sheltered and constantly heavily shaded beneath a corrugated iron roof to exclude the confounding variables of rain and solar radiation. These variables have both been shown to affect larval mortality (Waterhouse (1947) and Experiment 5, respectively). Temperatures were recorded from midday on 27 June, the time when first instar larvae were first detected on the exposed piglet. The

experiment was carried out in the grounds of the North Terrace campus of the University of Adelaide. The study site (Fig. 4.1) consisted of flat bare earth beneath large overhanging deciduous trees (leafless at this time of year), bordered by high fences to the east and north. Although the fence, and to a certain extent also the trees, shaded the site for much of each day, it was sunlit for a part of each afternoon.

Temperatures in the carcasses were recorded every 15 minutes using copper-constantan (type T) thermocouples attached to an automated data logger (1200 series Squirrel meter/logger, Grant Instruments Ltd). Recorded values were accurate ± 0.5°C. Thermocouples were inserted into the carcasses by threading them through thin glass tubes. For each carcass one thermocouple was inserted into the mouth and a second was positioned in the rectum. In the experimental carcass three others were spaced equidistantly within the centre of the torso by inserting them through small incisions made on the ventral surface. In the control carcass only two additional thermocouples were used. A final thermocouple, for recording the temperature of the ambient air, was fixed above the ground in a shaded spot adjacent to the carcasses.

Although the thermocouples were in fixed positions at the start of the experiment, those in the infested carcass were repositioned as necessary to ensure that at least one measured the temperature of the larval aggregation. Supplementary temperature measurements (accurate ± 0.1°C) were made at regular intervals during each day of the experiment using a hand-held digital thermometer. This was done to determine if thermocouples needed be moved to locations of higher temperature and to provide a check on the effectiveness of the fixed thermocouples for detecting temperature maxima. Observations were continued until 23 July, by which time most larvae had left the infested carcass and the temperatures generated by the remaining larvae no longer exceeded daytime ambient temperatures.

In analysing the results, the maximum temperatures recorded in each carcass and in the surrounding air during each 15 minute interval was determined. Henceforth, for the sake of economy, these temperatures shall be referred to as T_i (maximum temperatures in the infested carcass), T_c (maximum temperatures in the control carcass) and T_a (maximum temperatures of the ambient air). Elevation of T_i above T_c for the same 15 minute period was assumed to be produced by the larvae; it was ensured as far as possible that at least one thermocouple was always located among feeding larvae. Although there were often significantly different temperatures recorded by thermocouples in different parts of the infested carcass, temperatures other than the maxima have not been given. Such lower temperatures could be misleading because it was not possible to determine the extent to which they would have been produced by larval, as opposed to bacterial heating. In the control carcass, there were no significant differences between the temperatures recorded by the thermocouples in different parts of the body.

Values for T_i, T_c and simultaneous T_a were plotted against time. A second graph shows the deviation of T_i from T_c (T_i-T_c) and deviations of T_i and T_c from T_a (T_i-T_a and T_c-T_a). Summary data for these six variables were also tabulated.

Results

Blowfly eggs were observed on the exposed carcass by the end of the first day of exposure, in and under the exposed ear, in the mouth and between the two front legs. However, as mentioned above, first instar larvae were not evident until three days later, on 27 June. For future reference, day 1 of the experiment is regarded as starting at midday on 27 June.

The data reveal that the temperature profile of the infested carcass differed significantly from that of the control carcass as well from that of the air. Figs 4.2 and 4.3 show that for the first six days of larval activity T_i closely followed T_c, to within about 1°C. T_i and T_c were lower than daytime T_a but higher than

night-time T_a. For the first three days, while larvae were still in the first instar, T_i and T_c were virtually indistinguishable. However, from day four until day six, when most larvae were in the second instar, T_i was slightly higher than T_c, although mostly still by less than 1°C. On day seven, when most larvae had reached the third instar, T_i began to rise substantially above control temperatures and on day eight rose above daytime T_a. Thereafter T_i remained above T_a until day 20, when most larvae left the carcass. Throughout the experiment, daytime maximum and night-time minimum T_i occurred about three hours later than maximum and minimum T_a.

From day seven until day 19 T_i fluctuated within a limited range of 6.3°C around a mean of 14.0°C (Table 4.1). The range during this period was less than seen in the whole experiment (8.3°C), although T_a also varied less between days seven and 19 (10.8°C vs. 15.7°C). The mean T_i during this period was markedly greater than the mean T_a (10.1°C), with an average elevation above ambient of 3.9°C. However, the average elevation of T_i above T_a across the whole experiment was only 2.6°C. The maximum elevation of T_i above T_a was 7.8°C. This occurred at midnight on day 17. Like the elevation of T_i above T_a, the average elevation of T_i above T_c was also greater between days 7 and 19 than during the whole experiment (3.1°C vs. 2.1°C). The maximum elevation of T_i above T_c was 5.4°C, occurring at 10.15 a.m. on day 14.

From day 20 the deviation of T_i from T_a steadily decreased (Figs 4.2 and 4.3). T_i also progressively approached T_c. From day 20 until the end of the experiment only a few larvae remained in the carcass.

Throughout the experiment, T_a was much closer to T_c than to T_i; the former two sets of temperatures fluctuated around approximately the same mean (11.3°C vs. 11.7°C). T_c deviated from T_a by an average of only 0.5°C. However, the range and timing of the fluctuation of T_c was closer to the pattern exhibited by T_i (9.1°C vs. 8.3°C).

The infested carcass in this experiment was occupied by larvae of *C. stygia* and *C. vicina*. It was not possible to determine the relative contribution of each to the dynamics of the larval aggregation because, as shown in Chapter 3, they are cannot be identified without the aid of a microscope. Infestation began in the head of the carcass and proceeded posteriorly. By day 11 the larval mass had mostly left the head and had become established in the vicinity of the thorax. By day 15 the main aggregation was feeding in the abdomen, before largely dispersing on day 19. From day 20 onward, small numbers of dispersed larvae remained feeding beneath the thorax. Unfortunately, it was not possible to determine the number of larvae that had been in the carcass. Rather than all of the post-feeding larvae moving into the chaff beneath the carcass and pupating there, many unexpectedly escaped from the container altogether. This occurred because condensation during the night wetted the sides of the container enough to give the larvae purchase. They were then further able to escape from the protective nylon material because its edges were only folded beneath the container. Future experiments of this sort during winter should involve better security of the material covering the container.

4.2.3 Experiment 2: Thermogenesis in *Calliphora* Larvae in a Piglet Carcass in Summer

Materials and Methods

In the summer experiment I also used a pair of carcasses each of an initial weight of about 2 kg. Again, maggots were allowed to infest one carcass while the other acted as an uninfested control. The methodology was as in the winter experiment, except for the way in which the experimental carcass was infested with larvae. In the summer experiment the carcass was not exposed to wild flies because a pilot experiment showed that a much heavier infestation than in the winter would have resulted after only a very brief exposure. Instead, the carcass was exposed to captive flies for a period that limited the resulting larval

infestation to a size comparable with that of the winter experiment. This was achieved by exposing the carcass to 40 captive gravid females of *Calliphora dubia* for 24 hours. *Calliphora dubia* was used because it is thermophilic, being the species found most abundantly in carcasses during summer. The carcasses were placed outdoors on the morning of 16 January, and temperatures were recorded from midday. The experiment was concluded on 20 January when the larvae had dispersed and left the carcass. The site for the experiment was on the flat roof of the Fisher Building on the North Terrace campus of the University of Adelaide (Fig. 4.4). The site was different to that of Experiment 1 because the experiment was done in conjunction with Experiment 5, which required a totally exposed location. Although this site was totally unshaded, the carcasses were nevertheless maintained in constant heavy shade using a metal canopy.

Results

Once the experimental carcass in this experiment had been exposed to the captive *C. dubia*, and prior to its placement outdoors, it was examined for the presence of larvae. Numerous first instars were seen in the mouth. It was expected that around 2000 larvae might be present, since 40 mature females of *C. dubia* were used and the sister-species of *C. dubia*, *C. augur*, has been shown to produce, on average, 50 larvae per female (Mackerras 1933).

The temperature data for this experiment have been set out similarly to the winter study (Figs 4.5 and 4.6 and Table 4.2). Maximum carcass temperature values were used as before. As in the previous experiment, elevation of T_i above T_c was assumed to be produced by the larvae, and again, temperatures did not vary appreciably within the control carcass. However, the duration of the summer experiment was much shorter than the winter study. This was due to the far higher air temperatures (average of 24.4°C vs. 11.3°C) and the consequently accelerated development of the larvae. Despite this, the same general trends are evident in the relationships between T_i and T_c and T_a.

Figs 4.5 and 4.6 show that T_i was close to, although mostly slightly higher than T_a for about the first six hours of the experiment. Thereafter, T_i exceeded ambient for the next three days, despite still following the same general fluctuations as T_a. Larvae reached the third instar after about 18 hours, and this corresponds with the substantial divergence of T_i from T_c at this time. Nevertheless, T_i was still higher than T_c for the first 18 hours of the experiment, while the larvae were mostly in the second instar. Over the course of the experiment T_i averaged 30.6°C, an average of 6.3°C above T_a. The maximum elevation above T_a was 12.5°C. This occurred at 1.15 a.m. on day 3 of the experiment. The maximum deviation of T_i from T_c was 9.3°C, occurring at 9.15 a.m. on day 3. On day four T_i dropped briefly below T_a for the first time. This corresponded with the departure of the larvae from the head of the pig and resultant dispersal of the larvae. However, T_i remained above T_a even after this had occurred.

T_c fluctuated around a mean of 25.7°C, and was therefore closer to T_a than T_i. T_c deviated by an average of only 1.3°C from T_a but by 5.0°C from T_i. However, the ranges of T_i and T_c were much closer to each other (22.2°C and 22.3°C, respectively) than to the range of T_a (27.8°C). Daytime maxima and night-time minima in T_c were generally about 5°C lower or higher, respectively, than the equivalent T_a values.

The *C. dubia* larvae commenced their development in the head of the piglet and remained there and in the thorax until they moved into the chaff beneath the carcass at the end of day three. Once the larvae had pupated, they were counted and found to number 1058. This was considerably fewer than expected. Nevertheless, this degree of infestation appeared similar to that in the winter experiment.

4.2.4 Discussion

The results demonstrate that aggregations of blowfly larvae in small carcasses produce heat. This heat production is more accurately determined by comparing temperatures in infested carcasses with temperatures in uninfested carcasses of similar mass rather than by omitting the control and comparing them with air temperatures, as has been done by previous workers.

Although larval aggregations of a similar size were established in the carcass in each season, the larvae in the summer experiment elevated their collective temperature further above both T_a and T_c than did the larvae in the winter experiment. There are several possible explanations for this. First, the larval aggregation in the summer experiment may have been sufficiently larger to cause the difference in heat production. Although every effort was made to equalise the degree of infestation of the carcasses, this could only be done subjectively, since it was impossible to determine the number of larvae in the winter experiment. Also, the larvae in the winter experiment were not all equal in age. This was because the carcass was exposed to blowflies for several days so that eggs were deposited on the carcass at different times. As a result, it is unlikely that, once all the eggs had hatched, the larvae present would have produced as much collective heat as they would if they had all been of the same age.

A second explanation may have been the difference in the species of blowfly used. Larvae of *C. dubia* may produce more heat than those of *C. stygia* and *C. vicina* because they require warmer conditions for development.

A further possibility is that the amount of heat larvae produce is related to the ambient temperature, and thus their metabolic rate. Larvae may be unable to elevate the temperature of their surroundings substantially unless the ambient air temperature is high enough to adequately raise their metabolism.

A number of workers have observed small carcasses containing small aggregations of larvae and some of these studies have referred to changes in temperature in the carcasses over time. Work has been carried out on the carcasses of dogs (Reed 1958), cats (Early and Goff 1986) and, as in my own research, piglets (Payne 1965; Tullis and Goff 1987; Wallman 1990). However, none of these studies specifically investigated the temperatures generated by the larvae present in the carcasses. The general approach has been to record temperatures at one location in a carcass using a thermometer that is fixed in position. This does not take account of the inevitable movement of aggregations of larvae as the carcass decomposes. The information provided by these authors on temperatures in carcasses is therefore not a reliable guide to the dynamics of larval thermogenesis.

More recently, two studies have focused on the temperatures present among relatively small aggregations of the larvae themselves, not just within a carcass as a whole. Greenberg (1991) recorded maxima of 13°C above ambient for larvae in a shaded rabbit carcass in spring and 18°C above ambient for larvae in beef packed inside a human skull. His observations support my own observation that larvae produce their highest temperatures in the third instar. However, his findings are otherwise difficult to compare with my results because he used no control and did not define the prevailing ambient temperatures.

Turner and Howard (1992) also used no controls in their work on rabbit carcasses. They recorded temperatures in a larval aggregation within a shaded rabbit carcass in summer in excess of 40°C over 10 hours. The maximum elevation above ambient was approximately 25°C. The high elevation of larval temperature above ambient in this experiment is probably partially due to the use of rabbit carcasses and the added insulation provided by their hair: it seems likely that less heat would be lost from a thickly furred rabbit than from an almost hairless piglet.

I have only found one study that compared temperatures in an infested carcass with temperatures in an uninfested carcass. This study, by Wobeser and Galmut (1984), was also done on small carcasses - those of ducks. These workers measured maximum temperatures in infested carcasses of between 40°C and 47°C. These maxima were 14°C to 32°C higher than the simultaneous air temperatures. By contrast, duck carcasses shielded from blowflies exhibited temperatures only slightly above ambient, although several degrees higher than seen in my experiments. One reason for this is that all carcasses in Wobeser and Galmut's experiments were exposed to solar radiation. This may cause substantial elevation of temperatures in small carcasses even when no larvae are present (see Section 4.4). Also, the plumage of birds provides effective insulation, like the hair of the rabbit mentioned above. Heat is therefore likely to be also relatively well conserved in a bird carcass.

4.3 Thermogenesis in Larvae in Large Carcasses

4.3.1 Introduction

Larvae are likely to reach greater numbers in large than in small carcasses because of the greater mass of food available to them. Larger aggregations and hence higher and more sustained temperatures might therefore be expected among larvae in large carcasses. Another reason for higher temperatures might be the potentially greater insulation provided by large carcasses. For both reasons larvae in large carcasses seem likely to develop at a faster rate than larvae of the same species in a small carcass.

The aim of the experiments described in this section was to examine in detail the temperatures produced in large larval aggregations in large carcasses at seasonally divergent times of year. The experimental method was essentially as in Section 4.2 and the experiments were carried out in the same location as the winter study on small carcasses.

Again there were two experiments - the first carried out in late March to early April, 1997 (autumn) and the second between late July and late November, 1997 (winter and spring).

4.3.2 Experiment 3: Thermogenesis in *Calliphora*, *Lucilia* and *Chrysomya* Larvae in a Pig Carcass in Autumn

Materials and Methods

Two large pigs were used, initially weighing approximately 42 and 48 kg. The smaller was used as a control from which flies were excluded. Exclusion was again achieved by covering the carcass securely with fine nylon fabric. Flies were given unlimited access to the other carcass to allow the development of as large a larval aggregation as possible. Both pigs were laid on their sides on the ground and were well sheltered from sun and rain beneath a large roof of corrugated sheets of metal and plastic. Shade was also provided by the fences partly bordering the study site (see Section 4.2.2), and the remaining foliage of the overhanging deciduous trees. In each carcass six thermocouples were variously positioned in the head and rectum and at four other equally-spaced positions in the torso.

The pigs were obtained on 19 March and placed outside late on the same day. Temperatures were recorded from midday on 20 March.

Results

The results are shown in Figs 4.7 and 4.8 and Table 4.3. Temperature data are given from midday on 21 March, designated as the start of the experiment (day 1) (a day later than when first recorded) because blowfly immature stages were not observed until the afternoon of that day. These were first instar larvae of

Calliphora dubia in a small wound on the side of the pig's head. On the afternoon of day 3 the first blowfly eggs appeared, scattered over the head.

For the first four days of larval activity T_i generally varied from T_c by less than 2°C (see Figs 4.7 and 4.8), rising slightly above T_c during the day and dipping slightly below it at night. However, just before the start of day 5 there was a sharp increase in T_i to above both T_c and T_a. This rise in T_i was not related to the larvae of *C. dubia*, which by this stage had developed well into their third instar. Rather, it followed the hatching of innumerable eggs of *Chrysomya rufifacies* and was measured within of an aggregation of many first instar larvae of this species in and beneath the mouth of the carcass. From day 5 until the termination of the experiment T_i remained above T_c and T_a. As in Experiments 1 and 2, daytime maximum and night-time minimum T_i occurred roughly three hours behind the maximum and minimum T_a.

Day 5 to day 17 was the period of greatest larval activity. T_i averaged 30.1°C during this time as compared with a mean T_a of (17.3°C) (Table 4.3). The mean elevation of T_i above T_a was 12.8°C. Throughout the whole experiment it was 10.2°C. The maximum elevation of T_i above T_a was 21.2°C, recorded at 3.15 a.m. on day 10. From day 5 to day 13 the daily fluctuation of T_i was quite wide (range = 15.1°C), in association with T_a (range = 19.2°C). After day 13 this fluctuation lessened markedly, apparently influenced by a reduction in the range of T_a. The mean elevation of T_i above T_c during days 5-17 was about 2°C lower (11.0°C) than the mean difference between T_i and T_a. This corresponds with an elevation of mean T_c above mean T_a of about 2°C (19.1°C vs. 17.3°C). Mean T_i-T_c for the whole experiment was 8.4°C. The mean elevations of T_i above both T_a and T_c for days 5-17 were therefore each higher by 2.6°C than the equivalent values for the whole experiment. The maximum elevation of T_i above T_c was 19.2°C, occurring at 3.00 p.m. on day nine.

The last three days of the experiment, days 18-20, was the period when most larvae had left the carcass and it was populated by only a few remnant individuals. Consequently, there was a marked reduction in the elevation of T_i above both T_a and T_c of about 3°C.

T_c deviated above T_a by only 6.0°C during the experiment. As mentioned above, the greatest fluctuations were in T_i and T_a. Although the range of T_i and T_a was similar from day 5 onwards, as was expected, mean T_a (16.8°C) remained closer overall to mean T_c (18.6°C) than to mean T_i (27.0°C).

In this experiment the exposed pig carcass was infested with larvae of *C. stygia*, *C. dubia*, *Lucilia sericata*, *Ch. rufifacies*, and the muscid, *Hydrotaea rostrata*. Although not seen, larvae of the small *Chrysomya varipes* (Macquart) were possibly also present because adults were observed frequenting the carcass. However, *Ch. rufifacies* was by far the most dominant species and it is therefore probable that the bulk of the heat generated in the carcass resulted from the metabolism of this species. *Hydrotaea rostrata* is a tertiary fly, as mentioned in Chapter 1, and its larvae did not appear until day 18, by which time the numbers of larvae of the other species had dropped considerably.

Infestation began in and around the head; a huge aggregation of *Ch. rufifacies* (many thousands), already referred to, became established there just prior to day 5. Late on day 7 a further large aggregation of *Ch. rufifacies* larvae appeared between the rear legs and between the ground and the posteroventral area of the torso. On day 8 the anus and rectum became infested with a third aggregation. After this larvae encroached further into the anterior and posterior ends of the body, although not into the centre of the thorax. Larvae, particularly those of *H. rostrata*, also fed beneath the carcass among the exudates there.

4.3.3 Experiment 4: Thermogenesis in *Calliphora* and *Hydrotaea* Larvae in a Pig Carcass in Winter and Spring

Materials and Methods

In this experiment the methods used were as in the autumn study. To the nearest kilogram the pigs initially weighed 37 and 45 kg. The smaller carcass was the control and the larger one was exposed. The study site was not as well shaded as in Experiment 3 because the overhanging trees had lost their foliage, however the carcasses were just as well protected from solar radiation and rain.

The carcasses were obtained on 29 July and placed outside late on the same day. Temperatures were recorded from midday on 31 July.

Results

Temperature data have been given from midday on 6 August (day 1), since first instar larvae were not present until the afternoon of that day. The results are given in Figs 4.9-4.14 and in Tables 4.4a-c.

This experiment ran for much longer than the autumn experiment (108 days vs. 20 days). There are two main reasons for this. First, from the start of the experiment until the departure of the majority of blowfly larvae, mean T_a was lower (12.5°C; days 1-37) than in the autumn experiment (16.8°C; days 1-20). This resulted in a less intense larval infestation because both the numbers and diversity of adult blowflies attracted to it were reduced. The carcass therefore took longer to decompose. The second reason is that in this experiment it was decided to investigate the role in carcass heating of the tertiary muscid, *Hydrotaea rostrata*, totally exclusive of heat produced by blowfly larvae. To do this it was necessary to collect temperature data beyond the period of activity of all blowfly larvae.

Although the temperatures have only been given from 6 August, observations prior to that time were nevertheless made of the oviposition by flies on the exposed carcass. The carcass was exposed on 29 July, but blowfly eggs were not detected on it until 1 August. These were deposited in the mouth. Between 1 August and 6 August, further eggs were deposited by flies each day, all of them concentrated in the mouth and at the interface between the carcass and the ground.

During the first 6 days, when larvae were present in the carcass for the first time, there was minimal mean elevation of T_i above either T_a (0.4°C) or T_c (0.6°C) (Figs 4.9 and 4.12 and Table 4.4a). Larvae were confined to the mouth, and did not reach the third instar during this period. Further eggs were deposited on top of the head, in the crevices formed by the overlap of the fore and hind legs, and around the anus.

Between days 7 and 23 the mean elevation of T_i above T_a and T_c increased by about 4°C (4.5°C and 4.3°C, respectively) (Figs 4.9 and 4.12 and Table 4.4a). This increase corresponded with the larvae in the mouth of the pig reaching the third instar. During this period two further small aggregations appeared between the fore legs (day 10) and between the hind legs (day 15). Having reached the third instar, the larvae infesting the carcass were identified as an approximately equally mixed aggregations of *Calliphora stygia* and *C. vicina*.

The greatest elevation of T_i occurred between days 24 and 37, when the aggregation between the fore legs had developed considerably and invaded the chest region (Figs 4.9 and 4.10 and 4.12 and 4.13). During this period the mean T_i was 25.6°C, 12.1°C above mean T_a and 12.2°C above mean T_c (Table 4.4a). By comparison, during the whole period while blowfly larvae were present (days 1-53), the mean T_i was only 19.3°C and the mean T_i-T_a only 8.6°C and mean T_i-T_c only 6.5°C (Table 4.4b). As in the autumn experiment, the daily fluctuation of T_i (overall range = 9.4°C) was much less during this period of greatest larval

infestation than at other times in the experiment. To a certain extent this reduction appears to be linked to a lower range of T_a during this time (14.3°C), but this does not seem to account entirely for the reduced fluctuation. The maximum elevation of T_i above T_a was 16.9°C, at 5.45 a.m. on day 29 and the maximum elevation of T_i above T_c was 15.6°C, at 2.30 p.m. on day 32.

On day 38 larvae of *H. rostrata* appeared for the first time, in the mush beneath the carcass. However, the carcass also continued to be populated with larvae of *C. stygia* and *C. vicina* until day 53. During this time the elevation of T_i above T_a and T_c continued, but the means were about half those seen between days 24 and 37 (mean T_i-T_a = 6.2°C and mean T_i-T_c = 6.0°C) (Figs 4.10 and 4.13 and Table 4.4a).

From day 54 until the end of the experiment on day 108 (a total of 55 days), *H. rostrata* larvae populated the carcass exclusively. The mean T_i-T_a and mean T_i-T_c during this period in the area of infestation beneath the carcass were 5.0°C and 4.6°C, respectively (Figs 4.10 and 4.11 and 4.13 and 4.14 and Table 4.4c). Although these values are lower than the equivalent means for days 1-53 when blowfly larvae were present (8.6°C and 6.5°C), they are not markedly lower.

4.3.4 Discussion

These experiments show that in large carcasses much heat is produced by blowfly and muscid larvae. As demonstrated in the previous section, this is more accurately assessed by reference to temperatures in a control carcass than to the temperature of the ambient air, although this distinction was more obvious in the autumn experiment. Importantly, heat production is able to be sustained beyond the departure of blowfly larvae by the larvae of the muscid, *Hydrotaea rostrata*.

Interestingly, during the period of greatest larval infestation, the mean difference between T_i and T_a was very similar in both experiments (12.8°C in autumn vs. 12.1°C in winter and spring). The difference between T_i and T_c was also small (11.0°C vs. 12.2°C). However, maximum values for T_i-T_a and T_i-T_c were markedly higher in the autumn experiment. One explanation for this might be that a larger aggregation was present in that experiment. Certainly very great aggregations of *Ch. rufifacies* larvae occurred, however they were often not well insulated from heat loss because they were alongside the carcass, rather than within it. Another possibility is therefore that larvae of *Ch. rufifacies* produce more heat than *Calliphora* and *Lucilia* larvae.

The difference between mean T_i-T_a and mean T_i-T_c was slightly less in the winter-spring experiment than in the autumn experiment. This is linked with the fact that mean T_c was less elevated above mean T_a in the winter-spring experiment. A hypothesis that would explain why T_c was more elevated above T_a in the autumn experiment (by about 2°C overall) is that bacterial activity in carcasses does not reach a level where noticeable heat is produced until the ambient temperature reaches a certain threshold, which was crossed in the autumn experiment.

Another interesting result of the autumn experiment was that a substantial elevation of T_i above T_a and T_c occurred even when the larvae in the largest aggregation were only in the first instar. This suggests that larval thermogenesis might be a product of the actual mass of larvae present, rather than the stage of development *per se*. Maximum heat is doubtless generated by third instars, as discussed earlier, because each larva is then at its largest mass. However, an equivalent mass of earlier instars (a much greater number of larvae) might provide an equivalent amount of heat.

There were several notable features of the winter-spring experiment. To begin with, there was a long delay of six days before larvae were recorded on the

exposed carcass. A further six days elapsed (12 in total) before T_i was sustained above T_a and T_c. By contrast, this stage was reached after only 4 days in the autumn experiment. Another interesting feature of this experiment was that despite the extremely prolonged presence of blowfly larvae in the carcass (almost two months), the heat produced remained above both T_a and T_c throughout.

As discussed in Section 4.2.4, most studies on carcasses have not specifically recorded temperatures among developing larvae. This applies equally to experiments on large and small carcasses, examples of the latter having already been given. In large carcasses, Waterhouse (1947) found the average daily temperature of dead sheep in winter to be about 22°C higher than the average daily air temperature, although at times it was as high as about 35°C. Williams (1987) measured a maximum carcass core temperature of ~30°C above ambient temperature in a dead sheep in summer, while Anderson and VanLaerhoven (1996) recorded a core value of ~20°C above ambient, also in summer, in a pig carcass. However, in none of these studies was it stated whether these temperature elevations were actually produced within larval aggregations.

Nevertheless, some recent studies have measured larval temperatures in large carcasses, although rather imprecisely. Two such studies show results close to those of the present autumn experiment.

Cianci and Sheldon (1990) experimented on shaded 20 kg pig carcasses in summer and autumn and recorded a maximum elevation of larval temperature above ambient of approximately 20°C. Such a maximum elevation is very close to that recorded in my own autumn study (21.2°C). Cianci and Sheldon also noted that this maximum elevation coincided with the appearance of third instar larvae. I also found this to be the case in my experiments on large carcasses (and also in Experiments 1 and 2). However, my large carcasses were over twice as heavy as Cianci and Sheldon's.

Morris (1993) studied larval aggregations in unshaded dead sheep, pigs and goats in spring, summer and autumn. Unfortunately, most of her data on temperatures in these carcasses are of little use in revealing changes in larval thermogenesis over time because each thermometer was fixed in place for the entirety of the experiment. However, one manually-recorded reading that she gives from a summer experiment on an unspecified carcass showed a maximum elevation of larval temperature above ambient of 20.5°C. Again, this tallies with my own results from the autumn experiment.

The experiments of Catts (1992) were done on pig carcasses in Washington during summer and autumn, but he did not state their initial weight nor whether they were shaded from the sun. He found that, regardless of season, maggot temperatures ranged as much as 35-45°C above daily ambient minima and about 20°C above daily ambient maxima. However, these figures are probably not representative of the true difference between larval and ambient temperatures because larval and air temperatures were not measured simultaneously, as in my work. If Catts had done this, the observed elevation of T_i above T_a might have been much smaller.

All of the studies referred to so far were on larvae in carcasses in relatively warm weather. However, two northern hemisphere workers reported observations carried out in winter on unshaded carcasses. Of these, Deonier (1940) recorded a maximum elevation of larval temperature above ambient in a goat carcass of more than 27.8°C. More commonly, he measured temperatures among larvae of between about 38 and 43°C when ambient temperatures were between about 15 and 20°C (an average elevation of about 22°C). Heinrich (1993) reported a temperature above ambient of about 20°C among larvae in a deer carcass. Although these values are somewhat higher than mine for larval thermogenesis in winter (max. T_i-T_a = 16.9°C), the difference could be explained by the thermal insulation provided by the fur coats of the animals studied by Deonier and Heinrich, or by the effect of extra heat coming from

incident solar radiation. They were also dealing with other fly species, whose physiological attributes may have differed from those of the species in my study.

4.4 Effect of Solar Radiation on Thermogenesis in Larvae

4.4.1 Introduction

Most solar energy reaches the Earth as short-wave radiation. Some is absorbed by the earth or objects upon it and re-radiated as long-wave radiation. Objects in the sun gain heat by short-wave radiation and get hot mostly because the incoming short-wave radiation exceeds the outgoing long-wave radiation to the sky. Such heating is greater on a clear day than on an overcast day because the radiation from the sun is reduced by passing through clouds or objects such as foliage (Unwin and Corbet 1991). Therefore a carcass exposed to the sun should be warmer than one in the shade (see earlier in this chapter). However, so far this effect has not been addressed in any of my experiments, i.e. all carcasses have been shaded, precisely in order to minimise solar heating as a confounding variable. But what effect does such heating have on blowfly larvae developing in a carcass? This has not previously been investigated in detail.

The experiment described here (Experiment 5) was designed to elucidate the effect of solar radiation on larval thermogenesis, as well as larval heating in small carcasses during summer (see Experiment 2; Section 4.2.3). The results led to questions about the survival of blowfly larvae in carcasses in summer. These were investigated with a further experiment on thermal death points of larvae (Experiment 6).

4.4.2 Experiment 5: Effect of Solar Radiation on Thermogenesis in *Calliphora* Larvae in a Piglet Carcass

Materials and Methods

These are mainly as for Experiment 2, as is the study site, although some details are repeated here for the sake of clarity.

The experiment was carried out in January, 1997. Four piglet carcasses were used, each initially weighing ca. 2 kg. Two were constantly shaded from the sun by a tin roof while the others remained totally exposed to the sun throughout. One shaded and one unshaded carcass were controls from which blowflies were excluded. As in previous experiments this was achieved using fine nylon fabric. The other carcasses were each infested by larvae from 40 gravid female *C. dubia* which had been allowed access to them for 24 hours. They were also covered with fabric during the experiment to avoid further random oviposition. The use of the nylon fabric affected the temperatures in the carcasses exposed to the sun by shading them slightly. Preliminary measurements showed a minor difference between the temperature above and below a piece of fabric upon which sunlight was allowed to fall of less than 1.0°C. In any case, both the carcasses shaded by a roof and those that were not beneath a roof were similarly shrouded.

All carcasses were placed outdoors on the morning of 16 January and logging of temperatures commenced at midday the same day.

Results

The results are displayed in Figs 4.15-4.17 and Table 4.5. The results for the shaded carcasses have already been given in Section 4.2.3. Nevertheless, some are also referred to here for comparison with the data for the sun-exposed carcasses.

For about the first day of the experiment there was only a slight elevation of sun-exposed T_i above T_c, but an elevation of T_i above T_a of up to 19.5°C, closely matching T_c-T_a. From day 2 onwards T_i in the sun remained below T_c, although still very close to it (mean overall T_i-T_c = -1.0°C). Early on day 2 there were greater increases in sun-exposed T_i and T_c than had occurred on day 1, up to maxima above 50°C. Similar increases occurred each day, during daylight hours, for the remainder of the experiment. At night, by contrast, T_i and T_c for the sun-exposed carcasses actually dropped below ambient (min. T_i-T_a = -2.5°C and min. T_c-T_a = -2.3°C). The ranges of sun-exposed T_i and T_c (45.3°C and 46.0°C, respectively) were much greater than the range of T_a (28.4°C). However, in the shade the ranges of T_i and T_c were about half as great as in the sun (22.2°C and 22.3°C, respectively) and much closer to T_a. In fact, the range of T_a in the shade (27.8°C) was about the same as in the sun, and T_a in the sun and shade differed little (mean difference = 2.1°C). T_i differed between the sun and shade considerably less than did T_c (4.9°C vs. 10.8°C) because of the heat generated within the infested carcass in the shade; mean T_i-T_c in the shade was 5.0°C, 6.0°C above the mean difference in the sun. The maximum T_i-T_a in the sun was 24.3°C at 4.15 p.m. on day 2 and the maximum T_c-T_a in the sun was 28.5°C at 3.30 p.m. on day 2. These elevations were, respectively, 16.9°C and 16.7°C higher than the simultaneous values for T_i-T_a and T_c-T_a in the shade. (The maximum value in the shade for T_i-T_a was only 12.5°C and for T_c-T_a only 7.4°C.)

After day 1 of the experiment there was no evidence of larval activity in the sun-exposed carcass that had initially been infested with larvae. Maximum temperatures in that carcass remained above 40°C for about six hours on day 1 and such intense heat presumably killed the larvae. There was certainly no clear separation between T_i and T_c as measured for the shaded piglets or in the other shaded carcasses analysed in this chapter.

The results pose important questions regarding the ability of larvae of different species to survive in carcasses that are exposed to solar radiation and what their thermal death points might be. The next experiment in this chapter helps address this issue.

4.4.3 Experiment 6: Thermal Death Points of *Calliphora* and *Chrysomya* Larvae

Materials and Methods

Ten larvae were each placed in the bottom of a glass test tube of diameter 2.5 cm with two drops of water per tube to keep them moist for the duration of the experiment. The water was important, because carcasses are moist and when organisms are surrounded by moisture heat is conducted to their bodies a lot faster than in dry air. The reason for this is that the thermal conductance of water is about 25 times that of air (Schmidt-Nielsen 1997). The larvae were restrained at the bottoms of the tubes with plugs of cotton wool placed directly above them. Approximately the bottom one third of each tube was immersed in a water bath heated to 25°C, the constant temperature at which the larvae had been developing. A copper-constantan (type-T) thermocouple was placed at the bottom of each tube to measure the temperature surrounding the larva. The temperature of the water bath was increased at a rate such that the temperature surrounding the larvae rose by about 0.2°C per minute. Such gradual heating was intended to approximate what might occur in a carcass exposed to solar radiation. Activity of the larvae was monitored closely. When larvae became motionless an intense cold light was shone directly on them. Since larvae are negatively phototactic they continued to move slightly in response to this stimulus until death. The death point of a larva was therefore taken to be the point at which the cold light failed to elicit any movement.

A further 10 larvae were then exposed to a second experimental regime where the temperature was increased from 25°C at a rate of about 0.6°C per minute, about three times as fast as previously.

The heating regimes were applied to third instar larvae of *Calliphora dubia* (the species used in Experiment 5), as well as to *C. vicina* and *Chrysomya rufifacies*. The latter two species were chosen to represent those featuring prominently in the large carcass experiments in winter and spring (Experiment 4) and autumn (Experiment 3) respectively.

Results

In general, larvae crawled about in the bottoms of the tubes with increasing rapidity as the temperature increased. However, once the temperature reached a point about 2.0°C below the fatal temperature they stopped crawling and lay on their lateral or dorsal surfaces. Their only movements were then slight twitches, which within about 1.0°C of the death point could only be elicited by shining the cold light upon them.

All replicate larvae died at the same temperature. Table 4.6 shows that the thermal death point of each species depended on the rate at which the temperature had been increased above 25°C. Death points were lower when the rate of heating was lower and consequently when the number of degree-minutes of heating was higher. Death points also differed between species. *Chrysomya rufifacies* tolerated higher temperatures than the other species, particularly when heating was rapid. When the two sets of results are considered together, the two *Calliphora* species had the most similar thermal death points.

4.4.4 Discussion

Experiment 5

The unexpectedly high temperatures during Experiment 5 most likely killed the larvae in the unshaded infested carcass. Nevertheless, the results demonstrate again the value of comparing temperatures in infested carcasses with those in uninfested carcasses. Sun-exposed T_i was much closer to T_c than to T_a, particularly at night, than would have been expected if larvae had been present. In fact, a control would be even more important for temperature measurements in carcasses exposed to solar radiation than in shaded ones because T_i of a sun-exposed carcass is so much higher than T_a. If the temperatures in Experiment 5 had been lower and the larvae had survived, the control carcass would have been a much better indicator than the ambient air of the minimum temperatures which the larvae could have experienced.

In future work it would be useful to expand this experiment by examining large carcasses in similarly high summer temperatures. Solar radiation experiments are difficult to carry out in winter because of the need to control for the confounding effects of rain and clouds. Large and small carcasses exposed to solar radiation should gain heat at the same rate per unit area and thus might be expected to ultimately attain the same temperature. However, in reality, large carcasses should be warmer because larger objects lose less heat to the environment by radiation and convection (Monteith and Unsworth 1990). Also, large carcasses have a smaller surface area relative to their volume and thus would not heat up as quickly by absorption of heat from solar radiation, nor would they lose heat as quickly as small carcasses once the heat source was removed, i.e. at nightfall. It might therefore be concluded that large carcasses pose even more inhospitable barriers to larval colonisation than small ones, especially during periods of high ambient temperatures, such as in Experiment 5.

Despite this, the possibility should be considered that large carcasses might not always be exposed to sunlight for long enough to build up fatally high temperatures - some carcasses might be too large and thus heat up too slowly during sunlight hours for such temperatures to develop. Others might indeed develop such high temperatures but retain some cooler regions within them where development could be accomplished successfully. Thus, larvae in large sun-exposed carcasses might be able to survive high ambient temperatures that would prove fatal to larvae in small carcasses.

An important consideration is that some blowfly species favour shaded or sunlit locations at certain times of year because of the thermal tolerances of the adults (Green 1951; Norris 1966). This might influence the numbers and identity of adults that visit particular carcasses. For example, the temperatures of the surfaces of sun-exposed carcasses in summer might at times prevent some species from ovipositing, but not others. There is also evidence to show that adults may prefer sun or shade independently of temperature, particularly in winter (Nuorteva 1965; Hanski and Nuorteva 1975). Consequently, differences would probably exist in nature between the numbers of larvae in shaded and unshaded maggot-infested carcasses, depending on species and time of year, which would in turn influence the temperatures generated in them.

I am aware of only one study that simultaneously compared temperatures in shaded and sun-exposed carcasses. This was the experiment of Shean *et al.* (1993) who took measurements only once a day. However, they found that the maximum elevation above ambient was greater by as much as 9°C in an exposed carcass and the maximum temperature of the larval aggregation was as much as 12°C higher. No controls (carcasses without larvae) were used in this study, so no clear account could be taken of the contributions of the two sources of heat, solar radiation and larval thermogenesis.

Experiment 6

This experiment was done in response to the unexpected outcome of Experiment 5 and helps clarify the response of blowfly larvae to high temperatures. As shown in Experiment 5, in an animal carcass exposed to the sun the temperature varies widely during the course of a day. Potentially deadly temperatures may only exist for parts of the day or, as suggested above, only in some parts of a carcass. It is therefore important to know how larvae respond to gradual heating over time, as would be experienced in a sun-exposed carcass.

The results show that even when heat is increased quite quickly, larvae of all the species studied endure temperatures well over 40°C before succumbing. Despite *C. dubia* being found to survive up to around 45°C, it would not have survived in the sun-exposed infested carcass on day 1 of Experiment 5 because T_i rose far above that temperature. The death points of *C. vicina* and *Ch. rufifacies* suggest that these species could have survived temperatures well in excess of those measured in Experiments 3 and 4 if the carcasses in those experiments had been unshaded.

It might have been expected that gradual heating of the environment of a larva would allow acclimation, so that the larva would tolerate higher temperatures than if it had been exposed to faster heating or an instantaneous jump in temperature. In fact, the results suggest that the opposite is true; gradual heating appears to reduce tolerance to higher temperatures such that lower temperatures in fact become those that are fatal. The same phenomenon was seen by Feder et al. (1997) in *Drosophila* larvae. An explanation for this might be that thermal damage to a larva is cumulative over time: that at a slow rate of heating it experiences a higher number of degree-minutes of heat than when heated quickly. If this hypothesis is correct, such cumulative heat damage must occur only above a certain temperature threshold. This is possibly the temperature or narrow range of temperatures at which larvae die without completing their development. Although such a threshold appears not to have

been determined for *C. dubia*, Willliams and Richardson (1984) reported it to be between 30°C and 35°C in *C. vicina*, a range further supported by Ratcliffe (1935). This is considerably below the thermal death points for this species as determined here. Other species studied by Williams and Richardson were *C. hilli hilli* and *C. stygia* (35-40°C) and *Lucilia cuprina* (40-45°C). Wigglesworth (1967) reported that larval development ceased at 39°C in *C. stygia* and at 45°C in the northern hemisphere genus *Phormia*. Waterhouse (1947) presented evidence to show that *Ch. rufifacies* is able to develop successfully at 40°C.

Some workers have recorded very high temperatures within larval aggregations and such findings confirm that larvae are at least occasionally exposed to such temperatures. For example, Deonier (1940) recorded temperatures of over 48.9°C among larvae in a goat carcass, Waterhouse (1947) recorded larval temperatures up to 46°C in sheep carcasses and Catts (1992) measured temperatures in larval aggregations in pig carcasses as high as 50°C. Morris (1993) recorded a maximum of 47.5°C among larvae in an unidentified carcass, and Turner and Howard (1992) noted that larval temperatures in a dead rabbit exceeded 40°C for up to 10 hours. Unfortunately, in none of these studies were the larvae identified, thus greatly reducing their value.

4.5 Behaviour of Larvae in Carcasses

During the present work it was observed that larvae tend to feed in a more compact aggregation and deeper within the carcass when the ambient temperatures are low, such as at night.

Such adjustment by larvae of their position relative to others and relative to the surface either increases or decreases the amount of heat loss. Readings made with a manual thermometer showed that the temperatures among the larvae in the aggregation were related to the distance between individuals. Tightly aggregated feeding larvae align themselves side by side with their posterior ends

and spiracles directed outwards. Temperatures seemed always to be highest when larvae were arranged in this way. When they moved away from one another and became less tightly aggregated the temperature decreased.

When larval densities were low, as during advanced stages of decomposition, larvae appeared to position themselves in parts of the carcass that enabled them to conserve heat. They were therefore often found beneath areas of solid bone, such as the pelvis, femur and skull, rather than beneath areas of dried skin and tissue. These bony coverings presumably reduce heat loss.

Differences were noted between the behaviour of larvae of different taxa. In Experiment 3, *Calliphora* and *Lucilia* larvae appeared to aggregate apart from the larvae of *Chrysomya rufifacies*. This was probably because of the predatory habits of the latter (Fuller 1934). As already mentioned, *Ch. rufifacies* larvae often fed in exudates alongside the carcass. In all of the experiments, larvae of *Calliphora* and *Lucilia* were rarely exposed in this way, but were within or beneath the carcass. Larvae of the latter genera did not appear to aggregate apart from one other.

In Experiments 3 and 4 many *Calliphora* and *Lucilia* larvae, particularly young ones, moved about individually in the surface tissues of the carcasses, in spaces beneath the epidermis of the skin. These larvae generally started their migrations from either the anus or mouth and proceeded gradually towards the middle of the torso and over onto the back. There was no appreciable difference in temperature between the air above and below this raised epidermis. Such behaviour was also noted by Braack (1984) in larvae of *Chrysomya marginalis* (Wiedemann) on antelope carcasses. However, in the present work it was only observed in *Calliphora* and *Lucilia* larvae. Perhaps the prominent processes of *Ch. rufifacies* larvae prevent them from behaving in this way. Braack (1984) termed the behaviour 'epidermal streaming'. He suggested that it was used by larvae to explore the surface of a carcass in search of an entrance, and that by

moving about beneath the epidermis larvae are protected from predators and are better able to avoid tumbling off the side of the carcass.

In my experiments larvae of the muscid, *Hydrotaea rostrata*, did not form tight aggregations in the way that calliphorids did. Nor were they usually found within the carcass. Instead they were scattered or loosely clumped in the mush beneath it, subject to how many of them were present.

4.6 Sources of Error

Although multiple thermocouples in the carcasses recorded temperatures every 15 minutes, it is unlikely that actual maxima were recorded as often as that. This is particularly so in the infested carcasses where maximum temperatures were within moving aggregations of larvae, even though every effort was made to ensure that at least one thermocouple was placed in every aggregation. As a result, the data should only be assumed to closely approximate the maximum thermal conditions within or beneath carcasses. Even so, they are far more accurate and detailed than any previous temperature measurements in carcasses.

It would have been ideal if the temperatures recorded by the thermocouples could have been checked every few minutes and the thermocouples repositioned as necessary, but this was logistically impossible. Another option would have been to use many more thermocouples, but this was also not possible because of the limited equipment available to me.

It was mentioned in the introduction to this chapter that parasitism of blowfly larvae by parasitoid wasps probably effects larval thermogenesis. Although such parasitism was not detected in the present field experiments, it doubtless occurred nonetheless where developing larvae were unprotected by nylon fabric and may have influenced the results. Future work should investigate the effect of these wasps on larval metabolism.

4.7 Discussion

4.7.1 General

The experiments in this chapter show that both blowfly and muscid larvae produce heat. In blowflies the heat appears to be generated from the time of the first instar, the earliest stage of development, although it increases with the growth of the larvae and the consequent increase in their mass: larval metabolism, and thus heat production, increases with increasing mass during the development of feeding larvae (Hanski 1976; Putman 1977; Williams and Richardson 1984).

Maximum temperatures in infested carcasses follow trends in ambient temperatures, but temperatures in uninfested carcasses of similar mass are a better standard for comparison with the temperatures in infested carcasses because of the insulation they provide from extremes in T_a.

Developing fly larvae can apparently thermoregulate by altering their position within the carcass and their position relative to other larvae. Larval blowflies need to develop as quickly as possible in order to maximise the use of available resources, in often keen competition with other larvae, and to minimise predation (Ullyett 1950). To do this one would expect them to optimise their thermal environment. Williams and Richardson (1984) determined the temperatures of optimal growth to be 30°C in *Calliphora vicina* and *C. hilli*, 35°C in *C. stygia*, and 40°C in *Lucilia cuprina*. It should be expected that the behaviour of larvae of these species would cause them to concentrate in thermal environments as close to these temperatures as possible, and that other species would behave similarly. Byrd and Butler (1997) showed that aggregations of *Chrysomya rufifacies* formed on a linear temperature gradient at a mean temperature of 35°C, but that the temperature preferred by feeding larvae lowered to about 31°C prior to the post-feeding stage. In the present experiments, larvae appeared to move to optimise conservation of heat and the

temperature of their surroundings, but the highest temperatures within carcasses containing feeding larvae were always among the larvae themselves.

In Experiments 1, 3 and 4, larval aggregations comprised mixtures of species. These mixed aggregations contained mainly *Calliphora* larvae, but *Lucilia sericata* also occurred in Experiment 3. There would presumably have been competition between these species, which may have affected their growth and thus also their metabolism and heat output. Several laboratory studies have investigated interspecific competition between blowfly larvae (e.g. Ullyet 1950; Goodbrod and Goff 1990; Wells and Greenberg 1992), often focusing upon the effect of predatory species, such as *Chrysomya rufifacies*. Interspecific competition not involving predatory species appears to produce a similar result to intraspecific competition, giving rise to many under-sized adults, and with increasing intensity, fewer of them (Fuller 1934). Future laboratory studies should investigate thermogenesis in species that aggregate with each other in nature to determine how their thermogenesis is influenced by competition.

Blowfly larvae may die if they are unable to avoid temperatures that are above optimal, as in Experiments 5 and 6. Although this was not observed in the present experiments, larvae would be expected to move to other parts of a carcass, or to areas alongside it, if such places were cooler. There is anecdotal evidence to support this (e.g. Waterhouse 1947).

An example of another insect whose individuals adjust their position relative to one another to regulate temperature is the honey bee (Wilson 1971). In winter, bees cluster together in the hive more tightly as the outside temperature falls. When ambient temperatures are sufficiently high they do not aggregate at all.

4.7.2 Bacterial Thermogenesis

The use of control carcasses, free of fly larvae, was an important feature of the experiments described in this chapter. Their aim was to provide a standard for comparison with infested carcasses and to assist in determining the possible role of bacteria in carcass heating. It is well known that bacteria produce heat from their metabolic activities in decomposing organic matter, e.g. compost heaps (Lamanna *et al.* 1973; Doetsch and Cook 1974). However, the extent to which the bacterial species found in animal remains produce heat that has been mistakenly attributed to larval metabolism or solar radiation appears not be known. The experiments described here show negligible heating of uninfested carcasses in the cool months of the year, but a slight elevation of T_c over T_a in the summer and autumn. Although the carcasses were heavily shaded, this elevation might nonetheless have been a consequence of reflected solar radiation. Another explanation for it could lie with the temperature requirements of the species of bacterium that is credited with the greatest role in the decomposition of animals, *Clostridium perfringens* (Veillon and Zuber) (Corry 1978). This bacterium has an optimum growth temperature of about 45°C (Buchanan and Gibbons 1974), but will not grow below about 15°C (Corry 1978). The small elevation of T_c over T_a in the summer and autumn experiments could have been because mean T_a was generally above 15°C, permitting growth of *C. perfringens*, whereas in the winter and winter-spring experiments mean T_a was generally below 15°C, inhibiting growth of the bacterium. Unfortunately, this hypothesis cannot be substantiated because, as implied above, no studies appear to have been done on the specific thermogenic abilities of *C. perfringens* and other bacteria found in decomposing carcasses.

Even so, it is possible, and even likely, that bacteria do contribute a certain amount of heat to that which is measured in infested carcasses, at least during warm months. In fact, larvae may hasten the establishment of bacterial populations in carcasses, or even be a requirement for this to occur, because

without the heat from the larvae the temperature in the carcasses would be too low to enable the growth of some bacteria.

I know of only two studies in which temperatures were recorded in large carcasses which were free of fly larvae. Rodriguez and Bass (1985) measured temperatures in four human bodies buried at various depths. By comparison with the temperature of the surrounding soil, all bodies produced heat. However, when the bodies were later exhumed, the two that had been buried more shallowly were found to be infested with blowfly larvae. These larvae probably contributed heat, thus influencing the results, although this was not considered by the authors. The two bodies that had been buried more deeply exhibited no insect activity when exhumed. They nevertheless showed a mean temperature elevation above soil temperature of 3.4°C and 5.0°C. If larvae really were absent, such heat production could only have been caused by microbial activity during decomposition.

Deonier (1940) made limited temperature observations in sheep and goat carcasses in winter, prior to infestation with blowfly larvae. Carcasses in the shade maintained temperatures appreciably lower than ambient, whereas the temperatures of those in the sun rose above ambient. However, Deonier did not specify the actual temperature differences that were observed.

4.7.3 Thermogenesis in Larvae in Small vs. Large Carcasses

In the experiments in this chapter greater elevations above both ambient and control temperatures were produced in large infested carcasses than in small infested carcasses. This was probably due to a combined effect of the larger aggregations in the larger carcasses, coupled with the greater insulation that such carcasses provide. More heat would probably be conserved in an aggregation of a particular mass if the aggregation were in a large rather than

small carcass. This could only be substantiated by establishing equal numbers of larvae in two carcasses of different sizes.

Only one study seems to have specifically compared temperatures in carcasses of different sizes, but these were exposed to natural infestation:- Hewadikaram and Goff (1991) compared temperatures in two pig carcasses, of masses 8.4 and 15.1 kg, and recorded consistently higher temperatures (by about 8°C) in the larger carcass during the period of most intense maggot activity. However, this corresponded with a larger number of *Chrysomya* larvae in the larger carcass. In addition, the maximum recorded temperature elevation above ambient was about the same for each.

The temperatures observed by Deonier (1940) in the blowfly-infested carcasses of rabbits, lambs, cats and dogs during winter were not substantially above ambient, although the opposite was true of sheep and goat carcasses (as referred to above).

4.7.4 Forensic Implications

From a forensic perspective, an understanding of larval thermogenesis is important because rate of development is crucial to the estimation of the age of larvae and thus the time since death of the body in which they are found. However, this depends on the assumption that temperatures measured within aggregations actually influence the rate of development of individual larvae. There is clear evidence, at least in some *Chrysomya* species, that a significant difference exists between the rates of development of larvae aggregated at different densities. Goodbrod and Goff (1990) measured times of development and temperatures in larval densities in *Ch. megacephala* and *Ch. rufifacies* ranging between one and 40 larvae per gram of liver. Statistically significant differences were found in total duration of larval development between most densities and particularly between low and high ones. As larval density

increased there was an associated increase in the difference between the temperature among the larvae and the ambient air temperature. Of course, the influence of thermogenesis in larval aggregations on rate of development may differ in other species. Further studies should therefore to be done to clarify the relationship between rate of development and temperature in aggregations of different sizes in a range of species.

There is no question that larvae develop faster at high temperatures than at low temperatures. However, in an aggregation one is measuring the temperature produced collectively by a number of larvae. Perhaps, over time, individual larvae do not experience the temperatures measured in the entire aggregation because of their movements within it. If this were so their rates of development would not be influenced to the extent indicated by the temperature of the aggregation. The temperatures in the immediate surroundings of a single larva would be impossible to follow with existing technology.

If one assumes, as is probably likely, that the temperatures measured in larval aggregations in the experiments in this chapter are close to those influencing individual larvae, it would have been very erroneous to base an estimate of the time of their initial infestation on ambient air temperatures. Indeed, if the prevailing air temperatures had been the sole guide to the rate of development of the larvae, and the pigs in these experiments had been actual human victims, the time elapsed since their death would have been seriously overestimated.

An estimate of time since death of the carcasses in the winter experiments would also have been badly *under*estimated without the benefit of knowing the substantial delay prior to the first oviposition by blowflies. Issues related to the estimation of such delayed arrival of adult blowflies at a carcass are explored in Chapter 5.

4.8 Conclusions

4.8.1 General

Blowfly larvae in carcasses generate heat from the earliest stage of development. Their heat production is more accurately determined by comparing temperatures in infested carcasses with temperatures in uninfested carcasses of similar mass, rather than with ambient air temperatures. Nevertheless, the amount of heat produced is influenced by the temperature of the ambient air, as well as by the size of the larval aggregation and the size of the carcass in which the larvae are feeding.

Solar radiation may also have a profound influence on larvae, especially in small carcasses, although details of its effect on thermogenesis remain obscure. The behaviour of blowfly larvae appears to optimise the temperature of their surroundings and thus their rate of development.

4.8.2 Thermogenesis in Larvae in Small Carcasses

Blowfly larvae in small carcasses produce heat. In the summer experiment T_i - T_a was as high as 12.5°C and T_i-T_c as high as 9.3°C. The heat produced was less in winter: T_i-T_a reached only 7.8°C and T_i-T_c only 5.4°C.

4.8.3 Thermogenesis in Larvae in Large Carcasses

Blowfly larvae in large carcasses produce substantially more heat than the larvae in small carcasses. In the autumn experiment T_i-T_a was as high as 21.2°C and T_i-T_c as high as 19.2°C. These variables were lower in winter-spring, T_i-T_a reaching only 16.9°C and T_i-T_c only 15.6°C.

Significant heat was also generated in carcasses infested exclusively with larvae of the muscid, *Hydrotaea rostrata*.

4.8.4 Effect of Solar Radiation on Thermogenesis in Larvae

Much higher temperatures were produced in small sun-exposed carcasses than in small carcasses in the shade, even though air temperatures did not differ any where near as greatly. Maximum T_i-T_a in the sun was about twice that in the shade, and maximum T_i-T_c in the sun about three times higher than in the shade. Such temperatures (maxima of over 60°C) killed the larvae in the initially infested sun-exposed carcass.

Investigation of the thermal death points of the larvae of three species of blowfly under regimes of constantly increasing heat suggest that more gradual heating is more damaging to larvae. Larvae of *Chrysomya rufifacies* are probably able to tolerate higher temperatures than *Calliphora* larvae.

Chapter 5

Influence of Ambient Temperature on Activity of Adult Blowflies

5.1 Introduction

This chapter examines the relationship between blowflies and the temperature of their surroundings. Whereas the last chapter dealt with larvae, this deals with the adults.

In Chapter 1 it was indicated that an important consideration in forensic entomology is that time of death may not equal the time of initial oviposition by blowflies. There are several possible reasons for this, one of which is that flies are physically incapable of ovipositing on the body or of seeking it out because the air temperature is too low or too high. Clarification of the relationship between temperature and adult activity would therefore be useful for improving the accuracy of estimates of the time elapsing before infestation.

Any study of the relationship between ambient temperature and activity must examine the role of *acclimatisation*, a compensatory response to one or more altered environmental conditions. Acclimatisation is an example of 'phenotypic plasticity', the effect of the environment on an organism's phenotype (Huey and Berrigan 1996). Acclimatisation applies to organisms in their natural environment. The same process may be experimentally induced in the laboratory by varying a single environmental condition such as temperature. The laboratory effect is called *acclimation*, to distinguish it from acclimatisation, which occurs in the field (Randall *et al.* 1997).

Because their metabolic rates are mostly dependent upon the temperature of the environment, this phenomenon has been extensively studied in ectothermic animals. It is particularly important in helping animals to counter the effects of seasonal changes in climate. An example of this is the carabid, *Pterostichus brevicornis* (Kirby), from Alaska. In summer these beetles usually die when temperatures fall to -6.6°C. However, in winter the same individuals survive freezing at temperatures below -35°C (Miller 1969).

A complication is that a number of other environmental variables, such as oxygen, nutrition and photoperiod, also influence the relationship between temperature and acclimatisation. For example, in goldfish, photoperiod controls thermal tolerance at different times of year, even when the water temperature remains constant (Hoar 1956).

In blowflies, behavioural compensation in response to variations in ambient temperature has scarcely been studied. However, Nicholson (1934) found that captive *Lucilia cuprina* exhibited quite different flight activity when temperatures were rising as compared with when they were constant. This was presumably because the rate of adjustment is varied in such situations: the faster the rise in temperature the greater the difference between the temperature to which an animal is adjusted and that to which it is exposed (Digby 1958a). In adult *Calliphora vicina*, Tribe and Bowler (1968) showed that at certain temperatures the rate of oxygen consumption was greater in cold-acclimated flies than in warm-acclimated flies.

In nature, it should be expected that the metabolism of a blowfly would be partly a product of its thermal history: that the thermal environment in which it develops, or which it has experienced as an adult since emerging from the puparium, might influence its subsequent level of activity in response to temperature. Acclimatisation occurring during development would be more notable than if it occurred during adulthood, because the effects would need to

persist through the profound morphological and physiological changes of endopterygote metamorphosis. Such persistent acclimatisation, at whatever stage in life it occurs, might particularly affect the ability of the fly to seek out a carcass and oviposit on it. This area of blowfly biology is therefore potentially important in ecology and forensics.

The experiments presented in this chapter investigated thermal acclimation in blowflies. The first three experiments investigated the possible effect of ambient temperature at different stages in life on subsequent adult activity. The fourth experiment explored the possible role of carrion odour in enhancing adult activity.

5.2 General Material and Methods

5.2.1 Source of Individuals Used in Experiments

Flies were obtained from laboratory cultures derived from wild-caught females. These cultures had been maintained at a constant temperature of 25 ± 0.5°C and 12h:12h light:dark photoperiod for several generations before use in the experiments. Densities of developing larvae were kept low to avoid the temperature among them rising more than 1.0°C above ambient.

A pilot study had indicated that there were no noticeable consistent differences between the activity of males and females. Therefore, except in Experiment 4, flies were randomly selected for use.

5.2.1 Measurement of Activity

Five transparent plastic cages were used, each containing five flies. Each cage contained supplies of granulated sugar in a petri dish and water in a container

provided with a cotton wick. The cages were placed in a constant temperature cabinet (accurate ± 0.5°C) with a fluorescent tube providing the same light:dark regime as during the flies' development. Because the tube was at the top of the cabinet the flies in some cages received more intense light than others. However, this did not appear to affect levels of activity. The flies were given at least 24 hours to acclimate to the temperature in the cabinet. Their behaviour in each cage was then observed through the glass door of the cabinet and recorded on each of 15 separate occasions. Preliminary observations had determined that 15 observations were sufficient to obtain a representative measure of activity. In total, therefore, 75 separate observations were made. Behaviour was quantified on a scale of 1-5 using the method of Nicholson (1934):

1. *coma*, or comatose motionlessness: the fly was either on its back with its legs curled up or clinging to the side of the cage or the sugar or water container in a lopsided manner;
2. *rest*: the fly was on its feet in a normal resting posture;
3. *'movement'* of any sort, such as cleaning and feeding, not involving locomotion;
4. *crawling*;
5. *flight*.

5.2.2 Data Analysis

A mean activity value for each cage, on the scale of 1-5 as detailed above, with associated variance, was arrived at by first multiplying the number of observations of a particular activity state by a ranked value of that behaviour (i.e. coma = 1, rest = 2, etc.) for each separate observation. These weightings were applied to each activity in an attempt to quantify the amount of energy, and thus intensity of activity, expended in each activity state. The above products were then added together for a particular observation and the result divided by the number of flies on which the observation was based (because of mortality

there were sometimes fewer than five flies in a cage). Mean and variance activity values for each cage were then calculated for the 15 observations.

Weighted means and variances of the activity scores for all five cages were then calculated and standard errors further calculated from the weighted variances. The means and standard errors were then plotted. *Weighted* means and variances were used because of the mortality referred to above. Where this occurred there were unequal numbers of flies in the five cages.

5.3 Experiment 1: Effect of Ambient Temperature During Maternal Egg Development on Subsequent Adult Activity

5.3.1 Aim

The aim was to determine if the ambient temperature experienced by females during development of their eggs affects the behaviour of their adult progeny. Previous studies have shown a parental influence on offspring determined by the environmental conditions experienced by the parents, particularly the mother. For example, Saunders (1987) demonstrated that the photoperiod experienced by females of *Calliphora vicina* affected the incidence and duration of diapause in their larvae. Zamudio *et al.* (1995) showed that in *Drosophila melanogaster* Meigen, males whose parents had been reared at 25°C had higher mating success than those whose parents had been reared at 18°C.

It was assumed in this experiment that the temperature experienced by the eggs was very close to that at which the mother was kept. There appears to be no published data on abdominal temperatures in blowflies. However, the highest internal temperature in adult flies is found in the thorax, where the flight muscles are located (Heinrich 1993). Yurkiewicz and Smyth (1966a) measured thoracic temperatures in flying *Lucilia sericata* of up to 1.2°C above ambient.

In resting individuals it was only 0-0.2°C above ambient. It therefore seems reasonable to assume that abdominal temperature in blowflies would not be appreciably greater than ambient.

5.3.2 Methods

Calliphora vicina and *Calliphora dubia* were chosen for this experiment because they are winter-adapted and summer-adapted respectively, and therefore might be expected to differ in their responses to temperature. In southern Australia, *C. vicina* undergoes diapause during summer. Although *C. dubia* may be active all year round, it is particularly abundant in summer. Both species are easily cultured.

The experimental flies were cultured under four different combinations of temperature conditions, referred to hereafter as treatments (abbreviations used for each treatment are given in brackets):

1. (15°C→15°C). Parent flies were placed in a constant temperature cabinet at 15°C, directly after eclosion, and permitted to breed. Their offspring were reared at the same temperature until eclosion. After an interval of two days the flies were run through the experimental procedure. The two day delay was inserted because activity in blowflies has been shown to be reduced during the 24-48 hours after emergence (e.g. Kitching and Roberts 1975).
2. (25°C→15°C). As for 1, except that the parent flies were bred at 25°C.
3. (15°C→25°C). Parent flies were placed in a constant temperature cabinet at 15°C, directly after eclosion, and permitted to breed. Their offspring were reared at 25°C until eclosion, when after the two-day interval they were run through the experimental procedure.
4. (25°C→25°C). As for 3, except that the parent flies were bred at 25°C.

Using experimental flies from treatments 1-4, observations were begun at 5°C and continued in five degree increments up to 35°C. Activity was therefore assessed at seven different temperatures. It was not possible to examine activity at lower or higher temperatures than these because of the limitations of the constant temperature cabinet. Although 24 hours generally elapsed between measurements at different temperatures, on some occasions up to 48 hours separated them. Measurements were made at about the same time each day (mid-morning) to minimise skewing of the data by any endogenous activity rhythms.

Since this experiment was concerned with the temperature of egg development within the mother flies (the temperature at which the mother flies were kept), comparisons were made between treatment 1 and treatment 2, and between treatment 3 and treatment 4.

The results are presented as graphs of activity against ambient temperature in which the data for two treatments, given as the weighted means and standard errors mentioned earlier, are compared. It was not possible to apply any statistical analysis to the sets of data to determine significance because of inherent limitations of the data. One of these was that the activity of each fly at each temperature could not be recorded independently of the activity of any other fly. This was unavoidable, given that no automated means of measuring the activity of single flies was available to me. Instead, activity was recorded as shown by sets of flies. A consequence of this is that the data do not have the level of independence desirable for statistical tests. Also, because activity does not vary with increasing temperature in the same way in each treatment, the data do not lend themselves to being fitted to a mathematical model of the sort that would ideally enable comparisons to be made.

In addition to measuring activity as described in Section 5.2.1, supplementary data were recorded on the number of flights made by all flies at each

temperature during a five minute period. It was decided to make these supplementary observations because preliminary work had shown that of the five behavioural attributes recorded, flight was least well represented. This was because the duration of each flight was usually only a few seconds, whereas other activities lasted much longer. It was particularly important to be clear about the relationship between temperature and flight because of the importance of this activity to flies seeking carcasses. Flight data are plotted as number of flights per fly per minute against temperature.

5.3.3 Results

General Activity

In treatments 1 and 2 (post-oviposition temperature 15°C) there were no marked differences in activity for either species at each of the seven temperatures (Fig. 5.1).

In treatments 3 and 4 (post-oviposition temperature 25°C) there were more substantial differences in activity at each temperature, especially in *C. vicina* (Fig. 5.2). In this species, flies in the 25°C treatment were, except at 30°C, always more active than flies in the 15°C treatment. The lower activity at 30°C is an anomalous reversal of the overall trend. Similar anomalies occurred at 15°C and 30°C in *C. dubia*: its activity in the two treatments was almost identical at most temperatures.

Flight Activity

The results for flight activity (Figs 5.6 and 5.7, except treatments 25°C→25°C→15°C and 25°C→25°C→25°C, which only concern Experiment 3) mostly parallel the results for general activity. Thus there was little difference between the number of flights per minute of *C. dubia* individuals in the

15°C→15°C and 25°C→15°C treatments (treatments 1 and 2). However, in *C. vicina* there were marked differences between these treatments in the flight activity at 15°C and to a lesser degree 35°C. At 15°C the number of flights was substantially greater in the 15°C→15°C treatment. At 35°C there was also a marked difference in flight number, but in the reverse direction.

Especial differences between the two species were observed for the 15°C→25°C and 25°C→25°C treatments at the temperatures for which major differences were observed in the general activity data. These temperatures were between 15°C and 30°C in *C. vicina* (more flights per fly in the 25°C→25°C treatment) and at 30°C in *C. dubia* (more flights per fly in the 15°C→25°C treatment). In addition, the number of flights per minute of *C. dubia* individuals in the 25°C→25°C treatment was notably greater at 35°C.

5.3.4 Discussion

The results suggest that in *C. dubia* and *C. vicina* there may be some effect on adult activity from maternal temperature acclimation. However, the data also imply that the temperature of post-oviposition development may be important in revealing temperature effects that occurred earlier in ontogeny.

In *C. vicina*, individuals whose post-oviposition developmental temperature was 25°C, and whose mother was kept at 25°C, produced higher adult activity at almost all temperatures than those whose mother was kept at 15°C. The same overall trends may apply to *C. dubia*, but a comparison is impeded by the fact that *C. vicina* is winter-adapted, and hence tends to be more active at intermediate temperatures, while *C. dubia* is summer-adapted.

If the above interpretation of the results is correct, how might it be explained? One explanation would be in terms of an 'anticipatory response' to future

environmental conditions on the part of the female parent fly (Huey and Berrigan 1996). A maternal response to a cue of low or high temperatures might be to prepare the progeny physiologically for optimum fitness given a continuation of such temperatures. Thus, the offspring of females kept at a higher temperature might be acclimated to deal better with and thus be more active at, higher temperatures. However, this hypothesis is not borne out by the data for *C. vicina* at and below 15°C (Fig. 5.2), where the activity of progeny was still higher when mothers were kept at 25°C. For some reason the fitness of progeny appears to decrease at lower temperatures. This may be because the physiological changes occurring during acclimation at low temperatures are aimed more at survival once more extreme conditions develop (Hoffmann 1995).

5.4 Experiment 2: Effect of Ambient Temperature During Post-Oviposition Development on Subsequent Adult Activity

5.4.1 Aim

This experiment is related to the previous one because the results of the same experimental treatments were shared by both experiments, although compared in different ways. Here, the aim was to examine the effect on activity of the temperature of the surroundings after oviposition.

It has been shown that the adults of some insects are acclimated by exposure to different ambient temperatures during the larval stage. For example, Zamudio *et al.* (1995) showed that male *Drosophila melanogaster* had greater mating success when reared and acclimated at 25°C than at 18°C, regardless of the temperature at which they were tested. This was probably because the metabolic efficiency of this species is greater at 25°C. Berrigan (1997), also

working with *D. melanogaster*, demonstrated higher metabolic rates at the same temperature in cold-acclimated flies than in warm-acclimated flies.

Because *C. dubia* is ovoviviparous and *C. vicina* is oviparous, this experiment looked at the influence of temperature only on the larval and pupal stages of development in the former species, whereas in the latter species the influence also applied to at least most of egg development following fertilisation. The experiment could not discriminate between influences that might apply at different stages during immature development.

5.4.2 Methods

As stated above, some data were used in both Experiment 1 and Experiment 2 by altering the comparisons that were made between the four treatments described in Section 5.3.1. Here, data from treatment 1 are compared with those from treatment 3, and data from treatment 2 are compared with data from treatment 4. In all other respects the methods are as for Experiment 1.

5.4.3 Results

General Activity

Fig. 5.3 shows the activity rates of *C. dubia* and *C. vicina* adults when the temperature of maternal egg development was 15°C. In the summer-adapted *C. dubia* there was much higher activity at temperatures of 25°C and above in the individuals in the 25°C treatment than in those in the 15°C treatment. Below 20°C, activity of the flies in the 25°C treatment was lower, although not markedly so. In the winter-adapted *C. vicina*, flies in the 15°C treatment showed greater activity in the centre of the temperature range and lower activity at the lower and upper ends of the range.

When egg development in the mother was at 25°C (Fig. 5.4), the differences between the 15°C and 25°C post-oviposition treatments in *C. dubia* were similar to those observed when egg development in the mother was at 15°C (Fig. 5.3), although the differences were greater around the centre of the temperature range. By comparison, in *C. vicina*, flies in the 25°C post-oviposition treatment were generally more active than those in the 15°C treatment at temperatures below 20°C, and less active at and above 20°C (Fig. 5.4).

Flight Activity

Fig. 5.6 shows that individuals of *C. dubia* in the 15°C→25°C treatment made considerably more flights per minute at 25°C and 30°C than those in the 15°C→15°C treatment. This result is similar to that observed in the general activity data. By contrast, individuals of *C. vicina* in the 15°C→25°C treatment made noticeably fewer flights per minute at 25°C and 30°C, and also far fewer at 15°C and 20°C (Fig. 5.7). Such differences were not as evident in the general activity data for this species.

The number of flights per minute of *C. dubia* in the 25°C→25°C treatment was generally greater than its number of flights per minute in the 25°C→15°C treatment, particularly above 20°C (Fig. 5.6). Equally marked differences were evident between the equivalent treatments in the flight activity of *C. vicina*, this time mostly at 15°C, 20°C and 25°C (Fig. 5.7).

5.4.4 Discussion

In contrast with the previous experiment, here ambient temperature appears to have affected adult activity in all of the treatments for both species. In *C. dubia*, the general trend when maternal egg development temperature is both 15°C and 25°C is that post-oviposition development at 15°C produces higher activity at low temperatures, and lower activity at high temperatures. In *C.*

vicina, the effect tends to be reversed. In the two treatments with a post-oviposition development temperature of 15°C there is a tendency towards lower activity at low temperatures and higher activity at high temperatures.

A possible explanation for the difference between the two species is that the winter-adapted *C. vicina* is genotypically better suited to lower temperatures than the summer-adapted *C. dubia*. Thus, acclimation to a high temperature causes lower activity at high temperatures in *C. vicina* than in *C. dubia*, but higher activity at the low temperatures to which the former is better adapted. *C. dubia*, on the other hand, shows the reverse because it is a warm-adapted species. This would minimise stress over the ranges of temperatures to which the two species are exposed in the wild.

5.5 Experiment 3: Effect of Ambient Temperature During the First Two Weeks of Adult Life on Subsequent Adult Activity

5.5.1 Aims

The aim of this experiment was to determine the influence of ambient temperature during the early life of the adult on its subsequent level of activity. It might be expected that prolonged exposure to a certain temperature could have a sustained phenotypic influence upon adults, even if subsequent environmental temperatures vary, just as it appears to effect the phenotypes of flies when exposure occurs earlier in their development. For example, flies that have been kept at a low temperature might be expected to be more active at low temperatures than flies that have been kept at a higher temperature.

Acclimatisation is well known to occur in adult insects, including blowflies, and may in fact be quite rapid. The Queensland fruit fly, *Dacus tryoni* (Froggatt),

was found to be capable of acclimatisation to lower temperatures, as shown by changes in its thresholds for torpor and flight. This may occur as quickly as 1°C per minute (Meats 1974). Thus, acclimatisation in some flies may be effectively immediate.

It is an obvious adaptive advantage for adult insects to be able to acclimatise rapidly to their surroundings.

The purpose of this experiment is to reveal, as an indication of their ability to acclimatise, the extent to which thermal acclimation at divergent temperatures occurs in adult blowflies, and whether differences are discernible between species.

5.5.2 Methods

The methodology was as in Experiment 1, although different treatments were used. In this experiment comparison was made between the following:

1. (25°C→25°C→15°C). Flies whose parents and immatures had been kept at a constant temperature of 25°C and which were kept at 15°C for 14 days after eclosion. After this time they were run through the experimental procedure.

2. (25°C→25°C→25°C). As above, except that the flies were kept at 25°C for 14 days after eclosion.

5.5.3 Results

General Activity

Fig. 5.5 reveals that individuals of *C. dubia* kept at 15°C were more active at most temperatures than those kept at 25°C, particularly between 15°C and 30°C. By contrast, there was no clear difference between the activity of *C. vicina* from the two treatments. However, the activity of those from the 15°C treatment was also slightly higher between 15°C and 30°C.

Flight Activity

The data for flight activity (Figs 5.6 and 5.7) show that in both species more flights occurred in the flies that had been kept at 15°C, reflecting the results for general activity. Paradoxically, differences were particularly marked in *C. vicina*, the species which showed the smallest difference in general activity.

It is interesting that the flight activity of both species in the two treatments was substantially below that seen when the flies experienced an equivalent thermal history until eclosion i.e. 25°C→25°C. However, these flies were at least a week younger because they were used in the treatment sooner after eclosion. The biggest difference between the flight activity of these younger flies and the older ones used in this experiment was between the 25°C→25°C treatment and the 25°C→25°C→25°C treatment. The flies in the latter flew far less often. Indeed, they were the least active of all six treatment groups, whereas the former were the most active.

5.5.4 Discussion

In *C. dubia*, individuals kept at 15°C during the first two weeks of adult life appeared to produce higher activity overall than those kept at 25°C. A similar

effect may have occurred in *C. vicina*, although the data did not support it as strongly.

It might have been expected that acclimation of adult flies to 15°C and 25°C (Fig. 5.5) would give activity results similar to those in Experiment 2 (Figs 5.3 and 5.4). However, although the results are similar at some temperatures, they differ substantially at low temperatures for *C. vicina* and at high temperatures for *C. dubia*. This outcome may again be explained by genotypic characteristics of the two species. Because overall both species are more active at 25°C than at 15°C, it could be that they were physiologically more fatigued after two weeks at the former temperature than at the latter. Hence the lower activity at 25°C and higher activity at 15°C. This may parallel the observations of Yurkiewicz and Smyth (1966b) and Yurkiewicz (1968) that as environmental temperature increased, metabolism in *Lucilia sericata* also increased, but that flight range decreased.

Activity in the 25°C-acclimated flies, at both low temperatures in *C. vicina* and high temperatures in *C. dubia*, is lower than in Experiment 2 because these are the temperatures to which they are better adapted. However, the overall difference between activity at 25°C and at 15°C is greater in *C. dubia*. This could be because, being relatively warm-adapted, this species is even more active when acclimated to 25°C than is *C. vicina* when acclimated to 15°C.

The outcome for *C. vicina* closely resembles Tribe and Bowler's results (1968) for this species. Although they found adult oxygen consumption at 20°C and 30°C to be higher in flies acclimated to the former temperature, they did not find it any higher at 10°C.

The results of the flight data (Figs 5.6 and 5.7) support the above hypothesis but also suggest that flight activity diminishes with age. This is because flight activity was so much lower in the 25°C→25°C→15°C and 25°C→25°C→25°C

treatments than in the 25°C→25°C treatment (Experiments 1 and 2) in which the flies were younger.

5.6 Experiment 4: Effect of Liver Odour on Activity

5.6.1 Aims

As mentioned above, changes in behaviour at certain temperatures are likely to be influenced by environmental variables other than temperature itself. In carrion-breeding blowflies one such variable is odour. The odour arising from a carcass might affect the behaviour of females, in conjunction with, or independent of, ambient temperature.

The purpose of this experiment was to examine the influence of carrion odour on adult activity. It was hypothesised that flies - at least the female flies - in Experiments 1-3 may have shown different activity if exposed to the odour of carrion. Such an odour would be expected to act as a stimulus to females requiring a protein meal for ovarian development or to gravid females seeking a site for oviposition.

5.6.2 Methods

This experiment utilised only one species, *C. vicina*, and one ambient temperature, 20°C, which was the temperature at which it was found to be most active. Flies were reared, and their mothers kept at 25°C. Activity was measured for three sets of flies: 1) three-day-old males; 2) three-day-old females deprived of protein; 3) 14-day-old females that had not been deprived of protein and were gravid. As a control, flies were first observed 15 times in the constant temperature cabinet when it was free of any necrotic odour. An open container of putrid liver was then left for five minutes inside the cabinet. To ensure that

the odour diffused effectively, the container was placed in front of the fan responsible for air circulation within the cabinet. Activity was then recorded a second time. Following this, the container was removed and the cabinet aired for five minutes to remove residual odour. A second set of control activity measurements were then made.

The protocol used for measuring activity was as in previous experiments.

5.6.3 Results

General Activity

Fig. 5.8 shows, on the basis of the standard errors around the means, that females displayed a marked positive response to liver odour, but this response was effectively absent in three-day-old male flies. The level of activity of males and females of the same age under odour-free conditions did not appear to differ appreciably. Three-day-old females deprived of protein showed a noticeable increase in activity in response to liver odour. Fourteen days after eclosion, gravid females also showed a clear response. The gravid females displayed slightly higher mean levels of activity than the protein-deprived females. The level of activity shown by males and females (*C. vicina*) was within the range exhibited by *C. vicina* at 20°C in the previous experiments.

Flight Activity

The data for flight activity in the three sets of flies are shown in Fig. 5.9. These show an actual reduction in activity of male flies in response to liver odour. In the females, flight activity increased in response to the odour, however a subsequent reduction in activity on return to the odour-free environment was only evident in the protein-deprived females. In the gravid individuals flight activity anomalously increased further during this third measurement.

5.6.4 Discussion

The results suggest that females alone increase their activity levels in response to liver odour. This was probably seen in both of the sets of females examined because the younger ones were responding to a stimulus that indicated the presence of a protein source for egg maturation, while the older females were stimulated because the odour suggested the presence of a medium for oviposition.

Because these data show an effect at only one temperature, they would need to be repeated at a full range of temperatures to reveal the true impact of liver odour on the activity of females. Although I have already said that preliminary studies for this chapter disclosed no difference between the sexes in their activity at different temperatures, the present results suggest that the overall activity of female blowflies in nature would often be greater, at least at optimal temperatures.

Other studies also have revealed a carrion odour response in female blowflies, although again at only one temperature. Wall and Warnes (1994), working with *Lucilia sericata*, observed an increase in the number and velocity of flights in three-day-old protein-deprived females and nine-day old gravid females exposed to liver odour. Similar increases in spontaneous activity of this species were also seen by Ashworth and Wall (1995) in seven-day old protein-deprived females and in gravid females.

5.7 General Observations

5.7.1 Development Time

In each experimental treatment described in Experiments 1-3, I noted how long flies took to develop to eclosion. Although the numbers of flies emerging each

day were not recorded, it is still useful to compare the range of development times for each treatment. Table 5.1 shows no major difference between the ranges of development times for either *C. dubia* or C *vicina* when the temperature of maternal egg development varied but the temperature of development outside the mother was constant at 25°C. When the post-oviposition temperature was 15°C, development in *C. dubia* was substantially slower if the maternal egg development temperature was 15°C than if it was 25°C. However, there appeared to be no clear difference between these treatments in *C. vicina*. This result indicates that in *C. dubia* there may be a maternal effect of low temperature that retards subsequent development time. Although such an effect was not apparent in the present data at a post-oviposition developmental temperature of 25°C, it may also have become apparent there if the mean time of development had been established, rather than just the range.

Ranges of development times in both species were much lower when post-oviposition development was at 25°C rather than 15°C.

There were no major differences between these species in the development time ranges of the treatments, except for 15°C→15°C, where development was markedly slower in *C. dubia* (already referred to above) than in *C. vicina*.

Except for the *C. dubia* 15°C→15°C treatment, these development times are consistent with the findings of Greenberg (1991) for *C. vicina* (19.5 days at 25°C) and of Morris (1993) for *C. dubia* (35.8 days at 15°C; 16.6 days at 25°C).

5.7.2 Adult Behaviour

(The comments in this section refer to Experiments 1-3 only.)

General Activity

Flies of both species were more active at higher than lower temperatures until a certain temperature was reached at which this trend was reversed. This temperature varied according to species. *Calliphora dubia* was usually most active at 25°C, whereas *C. vicina* was most active at 20°C. Both species were therefore generally less active at temperatures above and below these values. The difference between the species is presumably related to the fact that *C. dubia* is summer-adapted while *C. vicina* is winter-adapted. Similar general patterns of activity were recorded in several different blowfly species by Nicholson (1934).

C. vicina was markedly more active than *C. dubia* at 15°C and 20°C in all treatments. At lower temperatures (5°C and 10°C) *C. vicina* was less active than *C. dubia* when the temperature of post-oviposition development was 15°C. Conversely, in these two treatments, at 25°C and above *C. vicina* was consistently more active than *C. dubia*.

Specific Activity States Other Than Flight

Differences in the fraction of flies exhibiting the various behaviours between individual treatments are not examined here because the variability between cages was too great to do so meaningfully. The flights observed during the general activity observations were too infrequent and their numbers too variable between cages to withstand scrutiny, thus endorsing the decision to gather extra data on this behaviour (see below).

Both species exhibited coma only at 5°C. Table 5.2 shows the weighted mean occurrence of this behaviour in each treatment. The absence of standard errors

for most means indicates that individual flies remained comatose throughout the period of observation, rather than exhibiting the behaviour intermittently, as with the other activity states. Although *C. dubia* seems to display this behaviour at 5°C rather more than *C. vicina*, there appear to be no other obvious trends relating to this activity state in the different treatments.

Figs 5.10-5.15 show the weighted means and standard errors for the fractions of flies per observation exhibiting the three commonest activity states: rest, 'movement' and crawling. These are shown for all thermal histories, plotted against temperature.

For both species, rest in most treatments occurred less often at temperatures in the middle of the temperature range, between 15°C and 25°C, than at lower or higher temperatures (Figs 5.10 and 5.11). The least rest occurred around 20-25°C in *C. dubia*, but at a lower range of 15-20°C in *C. vicina*. The fraction of flies exhibiting rest was markedly greater between 15°C and 25°C in *C. dubia* than in *C. vicina*, although generally slightly smaller in *C. dubia* at 5°C and 10°C. The level of rest was similar in both species at 30°C and 35°C.

'Movement' was absent in almost all treatments in *C. vicina* at 5°C (Fig. 5.13), although surprisingly this was not so in *C. dubia* (Fig. 5.12). *C. dubia* was also more active at 10°C. At other temperatures 'movement' was generally more prevalent in *C. vicina*. In both species it occurred most between 15°C and 25°C.

Crawling behaviour was entirely lacking at 5°C in *C. vicina* (Fig. 5.15), but was observed to some extent in *C. dubia* (Fig. 5.14). Both species crawled most between 15°C and 30°C, subject to treatment, although differences between the fractions crawling at these temperatures were generally not great. However, crawling in *C. dubia* occurred more at 25°C and 30°C than at other temperatures, whereas in *C. vicina*, it occurred more at 20-30°C.

The mainly higher activity at higher temperatures in *C. dubia* and at lower temperatures in *C. vicina* is again presumably related to the summer versus winter adaptation of these species.

The general trends in rest, 'movement' and crawling activity in relation to temperature were very similar to the activity curves obtained for these same behaviours by Nicholson (1934) for *Lucilia cuprina.*

Flight Activity

The general relationship between ambient temperature and flight activity was similar to the relationship between ambient temperature and overall activity and thus also reflected the species' seasonal adaptation. However, the optimum temperatures for flight activity were higher. *Calliphora dubia* tended to fly most at 30°C - although not in all treatments - while *C. vicina* flew most at lower temperatures (Figs 5.6 and 5.7).

For each treatment, *Calliphora vicina* produced more flights per minute than *C. dubia* at almost every temperature. The difference between species was particularly great between 15°C and 30°C.

Only *C. dubia* was observed to fly at 5°C. Three flights were taken by a single individual in one treatment during the five-minute observation period. In view of the summer adaptation of this species, such activity was surprising

Nicholson (1934) derived flight activity plots for four blowfly species across a similar range of temperatures. The plot for the one *Calliphora* species, *C. stygia*, was very similar to my result for *C. dubia*, although numbers of flights per minute in *Lucilia* and *Chrysomya* species were much greater at 25°C +. The threshold temperature for flight in *C. stygia* was 10°C.

Norris (1966) trapped blowflies at different times of year and obtained *C. stygia* at temperatures as low as 5°C and *C. augur*, the sister species of *C. dubia*, down to 8°C. It seems from my results and those of other authors that flight in *Calliphora* species cannot occur below about 5-10°C. Deonier (1940) said that the minimum temperature for activity of *Calliphora* in winter was 1.7°C, although the usual minimum lay between about 4.5°C and 10°C. However, he did not define 'activity' so may have been referring to movement other than flight.

Temperature-specific Behaviour

Two behaviours in both species were observed particularly at the lowest (5°C) and highest (35°C) temperatures. At 5°C flies huddled together in groups numbering between two and five on the walls of their cage or on the side of the water container. This behaviour was presumably a means for conserving heat, paralleling larval aggregations in carcasses.

At 35°C, and also less commonly at 30°C, both species were seen to extrude a droplet of liquid from the tip of the mouthparts and hold it there for several seconds before reingesting it. This behaviour was also recorded by Nicholson (1934) in *Calliphora*, *Lucilia* and *Chrysomya* species, and by Thomas (1991) in *Cochliomyia*. Heinrich (1976, 1979) demonstrated the value of this behaviour for bumblebees and honeybees for thermoregulation. If the behaviour plays a similar role in blowflies it could explain its occurrence at 30°C and 35°C but not at lower temperatures. If the flies are stressed by these temperatures the droplet could be a means of losing excess heat by evaporation. At 35°C a high proportion of flies were observed on the cotton wick protruding from their water container. The flies may have been there in order to lose heat. Since there would have been evaporation from the wicks their surfaces would have been cooler.

5.8 Sources of Error

In work of this kind it is difficult to be sure that the methods used to quantify behaviour are the most appropriate for testing the particular questions asked. Activity in flies has been assessed by a variety of methods other than the one I used. The most similar method was that of Thomas (1991), who produced a time-activity budget for *Cochliomyia hominivorax* (Coquerel). Flight alone has been examined as an indicator of activity in blowflies (Wall 1993) and tstetse flies (Wall 1988), using a computer-operated actograph, in which a flight is recorded when a fly alights on a circular target. Flight has also been analysed in blowflies using a video recorder (Wall and Warnes 1994). Spontaneous activity has been assessed in blowflies using a tilting actograph, where movement is recorded when a fly moves from one side of a balanced chamber (Green 1964; Ashworth and Wall 1995). Of course, activity levels can also be determined indirectly by measuring metabolic rate, as indicated by oxygen consumption (e.g. Berrigan 1997).

The results of the present study may have been different if one of the above methods had been used. However, the available resources were limited. Given the possible limitations of the methodology for measuring activity, it is also possible that clearer trends in the data would have been apparent if the data had been based upon more flies or more replicates.

One aspect of the methodology that may have influenced the results, at least in Experiments 1-3, was that the flies in each cage were moved sequentially from one temperature to another, rather than different sets of flies being tested at each temperature. In consequence when flies were tested at higher temperatures they were older and had been exposed to a greater range of temperatures than flies tested at lower temperatures. I could only have avoided this by producing flies of identical ages for observations at each temperature. However, this would have been logistically impossible as I had to rear all flies myself.

Finally, activity levels may have been greater if the flies had been deprived of food. Studies on *Phormia regina* (Meigen) have demonstrated a marked reduction in flight activity when food-deprived adults are fed with sucrose solutions (Barton Browne and Evans 1960; Green 1964).

5.9 Discussion

The experiments described in this chapter show that ambient temperature effects activity levels in adult blowflies. On the one hand, flies behaved differently at different temperatures no matter what their thermal history. However, when the thermal history was altered, further differences were seen in such temperature responses. These further differences were assumed to be the result of acclimation to the changed temperature conditions. The acclimation effect varied according to the stage in life at which the environmental temperature was altered and was able to be explained in terms of adaptation. In addition, other environmental factors, such as carrion odour, are likely to further complicate the relationship between adult activity and temperature.

Although it is tempting to explain the results in terms of the adaptive traits of the two species, other possible explanations must be considered. Ideally, the adaptive explanations advanced by me would need to be tested further, preferably using field experiments, which would necessarily involve fluctuating rather than constant temperatures. This approach has been firmly advocated by Huey and Berrigan (1996). However, if the behavioural responses to temperature observed here apply in nature, they imply that blowflies are strongly affected by the internal and external environmental temperatures to which they have been exposed throughout.

This is important in relation to the work presented in the previous chapter. I showed there that carcass temperature may influence rate of development of blowfly larvae, independent of ambient temperature. However, from the results

of this chapter, carcass temperature may also profoundly affect the level of activity of the adult flies which the larvae eventually become.

Another point is that the adaptation of blowflies to temperature might be expected to vary according to the populations to which they belong, just as intraspecific morphology and molecular characteristics in blowflies may vary geographically. Thus, care should be taken not to generalise about the effects of temperature on the activity of flies of the same species from different localities.

The results are important forensically because any factor that influences the behaviour of blowflies, particularly blowfly females, may affect estimates of time since death using these insects. In such estimates it is very important to determine when the body became infested. The present experiments did not specifically investigate the effect of temperature on oviposition. However, the results suggest that temperature may at least be important indirectly. A delay in the arrival of a female blowfly could be caused by long past environmental temperatures in the history of the blowfly, causing a reduction in activity, in turn reducing its effectiveness in seeking carrion and hence delaying oviposition.

There may be additional acclimation effects of temperature that influence willingness to oviposit. Further work should be done to clarify this. Studies on female *Lucilia sericata* have shown that they will not lay eggs below about 14°C (Macleod 1947), and that this species lays more eggs at 30-40°C than at lower temperatures (Cragg 1956). It would be interesting to see if such reproductive thresholds are influenced by thermal history.

If further studies reveal more clearly the role of environmental temperature in controlling adult activity, it might be possible to predict with more confidence the likely delays in oviposition using meteorological temperature data. However, the potential for this would be limited if the major influence of

temperature is found to be at the larval feeding stage, where environmental temperatures may differ considerably from ambient temperatures.

At present, the best that can be said is that infestation by *Calliphora* is unlikely to occur below about 5-10°C, and is possibly unlikely at higher temperatures as well, depending upon the thermal history of the individuals in the area. Infestation is also unlikely during periods of rain, high wind or at night, because blowflies are generally not active at these times (Green, 1951; Digby 1958a, 1958b).

5.10 Conclusions

5.10.1 Effect of Ambient Temperature During Maternal Egg Development on Subsequent Adult Activity

Adult *C. dubia* and *C. vicina* appear to exhibit different activity levels in response to ambient temperature, dependent upon the temperature to which their mothers were exposed. However, this effect only occurred when post-ovipositional development was at 25°C and was more apparent in *C. vicina* than in *C. dubia*.

5.10.2 Effect of Ambient Temperature During Post-oviposition Development on Subsequent Adult Activity

In *C. dubia* and *C. vicina* the ambient temperature between oviposition and eclosion appeared to influence the subsequent behaviour of adults of these species in response to ambient temperature. Activity levels at low and high temperatures differed according to developmental temperature and species.

5.10.3 Effect of Ambient Temperature During the First Two Weeks of Adult Life on Subsequent Adult Activity

Adult *C. dubia* and *C. vicina* appeared to display greater activity in response to ambient temperature after having been kept for two weeks at 15°C rather than 25°C.

5.10.4 Effect of Liver Odour on Adult Activity

Female three-day-old protein-deprived and 14-day-old gravid *C. vicina* displayed greater activity when exposed to carrion odour. Male flies did not appear to respond in this way.

Chapter 6

General Discussion: A Matter of Life and Death

6.1 Preamble

Great care should be exercised in forensic entomology, as in all areas of science, when drawing conclusions from the available evidence. In published work on this subject, often too little attention is paid to its fallibility. The impression may be received that deriving forensic inferences from insects is relatively straightforward. In reality, this process is rendered difficult by the subtlety of the morphological features distinguishing some species and by the host of factors in an insect's environment that affect its biology. Therefore the difficulties in using insects forensically, particularly in estimating time since death, must not be underestimated. From a social standpoint, a great deal may depend upon the accuracy of such estimations.

This thesis deals with the problems involved in identifying carrion-breeding blowflies, in estimating the length of time that has elapsed since their eggs or larvae were deposited and in estimating any delay in oviposition that may have occurred.

6.2 Identification

Chapters 2 and 3, dealing with the systematics of southern Australian carrion-breeding *Calliphora*, have confirmed that they are difficult to identify, and one suspects that determinations must at times have been erroneous. However, the taxonomic status of

the known forms is now made clear and the means have been provided for accurately identifying both adults and immatures. This is possible using the morphology of the adults of all species and of the third instar larvae of most. Difficulty with morphological identification can now be overcome by the use of allozyme electrophoresis. Doubtless future research will also develop DNA analysis as a valuable adjunct to this molecular technique. Allozyme analysis of *Calliphora* has also posed interesting questions about the evolution of the group, and these too await further investigation.

The taxonomy of Australian blowflies appears to be more complex than that of northern hemisphere blowflies, and moreover there are still undescribed and very likely also uncollected carrion-breeding species in Australia. Therefore it is important to be cautious in naming blowflies found in carrion, particularly in a forensic context.

6.3 Developmental Temperature

Although blowfly systematics requires further work, this is not where the greatest problems lie. The second part of this thesis shows that the biology of blowflies, particularly their thermobiology, is very complex.

When blowfly larvae are discovered in a carcass, the current temperature conditions are known, but their past thermal environment is uncertain. It has traditionally been assumed that the latter can be estimated by examining air temperatures as recorded by nearby meteorological stations. However, the experiments presented here on larval thermogenesis suggest strongly that this is untrue for almost all of the time spent by the larvae in carrion. This is because to a large extent the larvae create their own thermal environment, which differs considerably from that indicated by meteorology. These experiments should be extended to examine the actual time taken for larvae of different species to develop at different times of year in carcasses in which temperature is

monitored closely. If it can be assumed that the temperature in an aggregation is an accurate reflection of the developmental temperature of individual larvae (see Section 4.7.4), these data should then be compared with the results of laboratory studies using various constant and fluctuating temperature regimes. This approach may ultimately produce a database enabling the time of development of our local species to be determined with much greater accuracy than is currently possible. To provide additional rigour, a statistical measure of confidence in the estimated age of larvae, such as that developed by Wells and LaMotte (1995), would also be desirable.

6.4 Delayed Infestation

Even if one can be confident about the identification and rate of development of blowflies in carcasses, there remains the question of when the body first became open to colonisation. This is important because in forensics a time of death is sought, rather than just a time of infestation. It seems from the present work that delays in oviposition on both small and large carcasses that may amount to several days are not uncommon in winter. This has been noted previously by O'Flynn (1976) and Wallman (1990). The interval between time of exposure of a body (assumed time of death) and time of first infestation may also be difficult to predict in warmer months when blowflies are more active. In forensic cases it is often not possible to give an actual time of death, but only to state that death occurred somewhere between the time of the last confirmed sighting of the deceased person and the estimated time of earliest infestation. Because this period may be longer than just the delay in the arrival of blowflies, it would be helpful to know how great the latter delay might have been. Further studies on the effect of the thermal history of blowflies on their activity levels may help to make this more possible in the future. Special attention should also be paid to oviposition behaviour.

6.5 Conclusion

Greenberg (1988) warned that if we give the wrong impression about the inherent difficulties of forensic entomology it could tempt the unqualified to offer opinions outside their field of expertise. Peterson and Murdock (1989) included misrepresentation of expertise as one of a number of ethical dilemmas to be faced by all forensic scientists. Such issues are to be taken seriously because miscarriages of justice have occurred in western societies in recent years as a result of flawed forensic evidence. Several of the most notorious of these have been in Australia (Gerber 1987). Such failures of the judicial system may threaten a person's liberty, or, in some parts of the world, even their life. Erroneous scientific conclusions are unlikely to be detected in the courts because the level of scientific literacy among the legal fraternity and especially among the general public, from which juries are drawn, is poor. As a result, lawyers and judges are ill-equipped to evaluate the rigour of scientific evidence and juries may accept or reject evidence for totally extraneous reasons, such as the amount of confidence shown by the witness (Tipple 1986). Erroneous evidence might be challenged if a second opinion was sought by either defence or prosecution. However, this rarely occurs in forensic entomology in Australia because there are few workers with the necessary experience and all are in different states.

Thus, for diverse scientific as well as social reasons, the use of carrion-breeding blowflies in forensic entomology has serious limitations. Future research may improve the forensic applicability of these extraordinary insects. In the meantime, they can continue to play a role in criminal investigations if applied conservatively.

Bibliography

Adams, M., Baverstock, P. R., Watts, C. H. S., and Reardon, T. (1987). Electrophoretic resolution of species boundaries in Australian Microchiroptera. I. *Eptesicus* (Chiroptera: Verspertilionidae). *Australian Journal of Biological Sciences* **40,** 143-162.

Ancel, A., Visser, H., Handrich, Y., Masman, D., and Le Maho, Y. (1997). Energy saving in huddling penguins. *Nature* **385,** 304-305.

Anderson, G. S., and VanLaerhoven, S. L. (1996). Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences* **41,** 617-625.

Ashworth, J. R., and Wall, R. (1995). Effects of ovarian development and protein deprivation on the activity and locomotor reponses of the blowfly, *Lucilia sericata*, to liver odour. *Physiological Entomology* **20,** 281-285.

Avise, J. C. (1994). 'Molecular Markers, Natural History and Evolution.' (Chapman and Hall: New York.)

Barton Brown, L., and Evans, D. R. (1960). Locomotor activity of the blowfly as a function of feeding and starvation. *Journal of Insect Physiology* **4,** 27-37.

Baumgartner, D. L., and Greenberg, B. (1984). The genus *Chrysomya* (Diptera: Calliphoridae) in the New World. *Journal of Medical Entomology* **21,** 105-113.

Baverstock, P. R. (1988). Applications of molecular genetic techniques in zoology. *Australian Zoological Review* **1,** 1-13.

Belbin, L. (1987). 'PATN: Pattern Analysis Package. Reference Manual.' (C.S.I.R.O. Division of Wildlife and Ecology: Gungahlin, Canberra.)

Berrigan, D. (1997). Acclimation of metabolic rate in response to developmental temperature in *Drosophila melanogaster*. *Journal of Thermal Biology* **22,** 213-218.

Bezzi, M. (1927). Some Calliphoridae (Dipt.) from the South Pacific Islands and Australia. *Bulletin of Entomological Research* **17,** 231-247.

Boisduval, J. B. A. D. (1835). Faune entomologique de l'Océan pacifique, avec l'illustration des insectes nouveaux recuieillis pendant le voyage. Deuxième partie. Coléoptères et autres ordres. In 'Voyage de découvertes de L'Astralobe exécuté parordre du Roi, pendant les années 1826-1827-1828-1829, sous le commandement de M.J. Dumont d'Urville'. (J. Tastu: Paris.)

Braack, L. E. O. (1984). 'Epidermal streaming' and associated phenomena displayed by larvae of *Chrysomyia marginalis* (Wd.) (Diptera: Calliphoridae). *Koedoe* **27,** 9-12.

Buchanan, R. E., and Gibbons, N. G. (1974). 'Bergey's Manual of Determinative Bacteriology.' (The Williams and Wilkins Company: Baltimore.)

Buchmann, S. L., and Spangler, H. G. (1991). Thermoregulation by greater wax moth larvae. *American Bee Journal* **December,** 772-773.

Byrd, J. H., and Butler, J. F. (1997). Effects of temperature on *Chrysomya rufifacies* (Diptera: Calliphoridae) development. *Journal of Medical Entomology* **34,** 353-358.

Catts, E. P. (1992). Problems estimating the postmortem interval in death investigations. *Journal of Agricultural Entomology* **9,** 245-255.

Catts, E. P., and Goff, M. L. (1992). Forensic entomology in criminal investigations. *Annual Review of Entomology* **37,** 253-272.

Cianci, T. J., and Sheldon, J. K. (1990). Endothermic generation by blow fly larvae *Phormia regina* developing in pig carcasses. *Bulletin of the Society of Vector Ecologists* **15,** 33-40.

Corry, J. E. L. (1978). A review. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. *Journal of Applied Bacteriology* **44,** 1-56.

Corsaro, B. G., and Munstermann, L. (1983). Electrophoretic separation of northern Indiana *Culex* species. *Proceedings of the Indiana Academy of Science* **92,** 236.

Cragg, J. B. (1956). The olfactory behaviour of *Lucilia* species (Diptera) under natural conditions. *Annals of Applied Biology* **44,** 467-477.

Crosskey, R. W., and Lane, R. P. (1993). House-flies, blow-flies and their allies (calyptrate Diptera). In 'Medical Insects and Arachnids'. (Eds R. P. Lane and R. W. Crosskey) pp. 403-428. (Chapman & Hall: London.)

Dadour, I. R., Yeates D. K., and Postle, A.C. (1992). Two rapid diagnostic techniques for distinguishing Mediterranean fruit fly from *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Journal of Economic Entomology* **85,** 208-11.

Dear, J. P. (1985). Calliphoridae (Insecta: Diptera). *Fauna of New Zealand* **8,** 1-86.

Deonier, C. C. (1940). Carcass temperatures and their relation to winter blowfly populations and activity in the Southwest. *Journal of Economic Entomology* **33,** 166-170.

Digby, P. S. B. (1958a). Flight activity in the blowfly *Calliphora erythrocephala*, in relation to light and radiant heat, with special reference to adaptation. *Journal of Experimental Biology* **35,** 1-19.

Digby, P. S. B. (1958b). Flight activity in the blowfly, *Calliphora erythrocephala*, in relation to wind speed, with special reference to adaptation. *Journal of Experimental Biology* **35,** 1-19.

Doetsch, R. N., and Cook, T. M. (1974). 'Introduction to Bacteria and their Ecobiology.' (University Park Press: Baltimore.)

Donnellan, S., Adams, M., Hutchinson, M., and Baverstock, P. R. (1993). The identification of cryptic species in the Australian herpetofauna: a high research priority. In 'Herpetology in Australia: a diverse discipline'. (Eds D. Lunney and D. Eyres.) pp. 121-126. (Surrey Beatty & Sons Pty Ltd: Sydney.)

Dujardin, J. P., Le Pont, F., Cruz, M., Leon, R., Tarrieu, F., Gudarian, R., Echeverria, R., and Tibayrenc, M. (1996). Cryptic speciation in *Lutzomyia* (*Nyssomyia*) *trapidoi* (Fairchild & Hertig) (Diptera: Psychodidae) detected by multilocus enzyme electrophoresis. *American Journal of Tropical Medicine and Hygiene* **54,** 42-45.

Early, M., and Goff, M. L. (1986). Arthropod succession patterns in exposed carrion on the island of O'ahn, Hawaiian Islands, U.S.A. *Journal of Medical Entomology* **23,** 520-531.

Emden, F. I. van (1965). Diptera. Vol. 7. Muscidae. Part 1. In 'The Fauna of India and the Adjacent Countries'. (Eds R. B. S. Sewell and M. L. Roonwal) (Government of India: Delhi.)

Erzinçlioglu, Y. Z. (1984). 'Studies on the morphology and taxonomy of the immature stages of Calliphoridae, with analysis of phylogenetic relationships within the family, and between it and other groups in the Cyclorrhapha (Diptera).' Ph.D. Thesis. (University of Durham: Durham.)

Erzinçlioglu, Y. Z. (1985). Immature stages of British *Calliphora* and *Cynomya*, with a re-evaluation of the taxonomic characters of larval Calliphoridae (Diptera). *Journal of Natural History* **19,** 69-96.

Erzinçlioglu, Y. Z. (1987). The larvae of some blowflies of medical and veterinary importance. *Medical and Veterinary Entomology* **1,** 121-125.

Erzinçlioglu, Z. (1996). 'Blowflies.' (The Richmond Publishing Co. Ltd: Slough.)

Fabricius, J. C. (1775). 'Systema entomologiae, sistens insectorum classes, ordines, genera, species adiectis synonymis, locis, descriptionibus, observationibus.' (Kortii: Flensburg & Leipzig.)

Fabricius, J. C. (1782). 'Species insectorum exhibentes eorum differentias specificas, synonyma, auctorum loca natalia, metamorphosin adiectis observationibus, descriptionibus.' Tome II. (C. E. Bohnii: Hamburg & Cologne.)

Fabricius, J. C. (1794). 'Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species, adjectis synonimis, locis observationibus, descriptionibus.' Tome 4. (Hafniae.)

Farris, J. S. (1972). Estimating phylogenetic trees from distance matrices. *American Naturalist* **106,** 645-668.

Feder, M. E., Blair, N., and Figueras, H. (1997). Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Functional Ecology* **11,** 90-100.

Foley, D. H., Meek, S. R., and Bryan, J. H. (1994). The *Anopheles punctulatus* group of mosquitoes in the Solomon Islands and Vanuatu surveyed by allozyme electrophoresis. *Medical and Veterinary Entomology* **8,** 340-350.

Fuller, M. E. (1931). The life history of *Calliphora ochracea* Schiner (Diptera: Calliphoridae). *Proceedings of the Linnean Society of New South Wales* **56,** 172-181.

Fuller, M. E. (1932). The larvae of the Australian sheep blowflies. *Proceedings of the Linnean Society of New South Wales* **57,** 77-91.

Fuller, M. E. (1934). The insect inhabitants of carrion: a study in animal ecology. *Bulletin of the Council for Scientific and Industrial Research* **82,** 1-63.

Gates, B. N. (1914). The temperature of the bee colony. *Bulletin, United States Department of Agriculture* **96,** 1-29.

Gerber, P. (1987). Playing dice with expert evidence: the lessons to emerge from *Regina* v. *Chamberlain. The Medical Journal of Australia* **147,** 243-247.

Girard, M. (1869). Études sur la chaleur libre degagée par les animaux invertebrés et spécialement les insectes. *Annales des Sciences Naturelles. Zoologie et Biologie Animale, ser. 5* **11,** 135-274.

Goff, M. L., Charbonneau, S., and Sullivan, W. (1991). Presence of fecal material in diapers as a potential source of error in estimations of

postmortem interval using arthropod development rates. *Journal of Forensic Sciences* **36**, 1603-6.

Goodbrod, J. R., and Goff, M. L. (1990). Effects of larval population density on rates of development and interactions between two species of *Chrysomya* (Diptera: Calliphoridae) in laboratory culture. *Journal of Medical Entomology* **27,** 338-343.

Green, A. A. (1951). The control of blowflies infesting slaughter-houses. 1. Field observations of the habits of blowflies. *Annals of Applied Biology* **38,** 475-494.

Green, G. W. (1964). The control of spontaneous locomotor activity in *Phormia regina* Meigen. I. Locomotor activity of intact flies. *Journal of Insect Physiology* **10,** 711-726.

Greenberg, B. (1988). Book Review. 'A Manual of Forensic Entomology.' *Journal of the New York Entomological Society* **96,** 489-491.

Greenberg, B. (1990). Nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae). *Journal of Medical Entomology* **27,** 807-810.

Greenberg, B. (1991). Flies as forensic indicators. *Journal of Medical Entomology* **28,** 565-577.

Greenberg, B., and Szyska, M. L. (1984). Immature stages and biology of fifteen species of Peruvian Calliphoridae (Diptera). *Annals of the Entomological Society of America.* 77: 488-517.

Griffin, T. L., and McGaskill, M. (Eds) (1986). 'Atlas of South Australia.' (South Australian Government Printing Division in association with Wakefield Press on behalf of the South Australian Jubilee 150 Board: Adelaide.)

Griffiths, G. C. D. (1994). Relationships among the major subgroups of Brachycera (Diptera): a critical review. *The Canadian Entomologist* **126,** 861-880.

Gurney, W. B., and Woodhill, A. R. (1926). Investigations on sheep blowflies. Part 1. Range of flight and longevity. *New South Wales Department of Agricultural Science Bulletin* **27,** 1-19.

Hall, D. G. (1948). 'The Blowflies of North America.' (The Thomas Say Foundation: Baltimore, Maryland.)

Hall, M. J. R., and Wall, R. (1995). Myiasis of humans and domestic animals. *Advances in Parasitology* **35,** 257-334.

Hanski, I. (1976). Assimilation by *Lucilia illustris* (Diptera) larvae in constant and changing temperatures. *Oikos* **27,** 288-299.

Hanski, I., and Nuorteva, P. (1975). Trap survay of flies and their diel periodicity in the subartic Kevo National Reserve, Northern Finland. *Annales Entomologici Fennici* **41,** 56-64.

Hardy, G. H. (1930). The Queensland species of *Calliphora*, subgenus *Neopollenia*. *Bulletin of Entomological Research* **21,** 441-448.

Hardy, G. H. (1932). Some Australian species of *Calliphora* (subgenera *Neopollenia* and *Proekon*). *Bulletin of Entomological Research* **23,** 549-558.

Hardy, G. H. (1937). Notes on the genus *Calliphora* (Diptera). Classification, synonomy, distribution and phylogeny. *Proceedings of the Linnean Society of New South Wales* **62,** 17-26.

Hardy, G. H. (1940). Notes on the Australian Muscoidea, V. Calliphoridae. *Proceedings of the Royal Society of Queensland* **51,** 133-146.

Hardy, G. H. (1947). Notes on Australian Muscoidea, VI. *Calliphora* in Australia and New Zealand. *Proceedings of the Royal Society of Queensland* **57,** 53-56.

Hawkins, B. A., Goeden, R. D., and Gagne, R. J. (1986). Ecology and taxonomy of the *Asphondylia* spp. (Diptera: Cecidomyiidae) forming galls on *Atriplex* spp. (Chenopodiaceae) in southern California (USA). *Entomography* **4,** 55-108.

Heinrich, B. (1976). Heat exchange in relation to blood flow between the thorax and abdomen in bumblebees. *Journal of Experimental Biology* **64,** 561-585.

Heinrich, B. (1979). Keeping a cool head: honeybee thermoregulation. *Science* **205,** 1269-1271.

Heinrich, B. (1993). 'The hot-blooded insects.' (Harvard University Press: Cambridge, Massachusetts.)

Hewadikaram, K. A., and Goff, M. L. (1991). Effect of carcass size on rate of decomposition and arthropod succession patterns. *The American Journal of Forensic Medicine and Pathology* **12,** 235-240.

Hillis, D. M., Mable, B. K., and Moritz, C. (1996). Applications of molecular systematics. In 'Molecular Systematics'. (Eds D. M. Hillis, C. Moritz, and B. K. Mable.) pp. 515-543. (Sinauer Associates: Sunderland.)

Hoar, W. S. (1956). Photoperiodism and thermal resistance of goldfish. *Nature* **178,** 364-365.

Hoffmann, A. A. (1995). Acclimation: increasing survival at a cost. *Trends in Ecology and Evoloution* **10,** 1-2.

Holbrook, F. R., and Tabachnick, W. J. (1995). *Culicoides variipennis* (Diptera: Ceratopogonidae) complex in California. *Journal of Medical Entomology* **32,** 413-419.

Holloway, B. A. (1985). Immature stages of New Zealand Calliphoridae. In J. P. Dear, 'Calliphoridae (Insecta: Diptera)'. *Fauna of New Zealand* **8,** 12-14 and 80-83.

Holloway, B. A. (1991). Identification of third-instar larvae of flystrike and carrion-asscociated blowflies in New Zealand (Diptera: Calliphoridae). *New Zealand Entomologist* **14,** 24-28.

Huey, R. B., and Berrigan, D. (1996). Testing evolutionary hypotheses of acclimation. In 'Animals and Temperature. Phenotypic and Evolutionary Adaptation'. (Eds I. A. Johnston and A. F. Bennett) pp. 205-237. (Cambridge University Press: Cambridge.)

Joos, B., Casey, T. M., Fitzgerald, T. D., and Buttemer, W. A. (1988). Roles of the tent in behavioral thermoregulation of eastern tent caterpillars. *Ecology* **69,** 2004-2011.

Kashyap, V. K., and Pillay, V. V. (1989). Efficacy of entomological method in estimation of postmortem interval: a comparative analysis. *Forensic Science International* **40,** 245-250.

Kioko, E. N., Overholt, W. A., Omwega, C.O., and Mueke J. M. (1995). Taxonomic significance of isoenzymes in two stem borers (Lepidoptera: Pyralidae) of maize and sorghum in Kenya. *African Entomology* **3,** 167-71.

Kitching, R. L., and Roberts, J. A. (1975). Laboratory observations on the teneral period in sheep blowflies, *Lucilia cuprina* (Diptera; Calliphoridae). *Entomologia Experimentalis et Applicata* **18,** 220-225.

Knapp, R., and Casey, T. M. (1986). Thermal ecology, behavior and growth of gypsy moth and eastern tent caterpillars. *Ecology* **67,** 598-608.

Kurahashi, H. (1970). The tribe Calliphorini from Australian and Oriental regions, I. *Melinda*-group (Diptera: Calliphoridae). *Pacific Insects* **12,** 519-542.

Kurahashi, H. (1971). The tribe Calliphorini from Australian and Oriental regions, II. *Calliphora*-group (Diptera: Calliphoridae). *Pacific Insects* **13,** 141-204.

Kurahashi, H. (1972). The tribe Calliphorini from Australian and Oriental regions, III. A new *Calliphora* from Phoenix Island, with an establishment of a new subgenus (Diptera: Calliphoridae). *Pacific Insects* **14,** 435-438.

Kurahashi, H. (1989). Family Calliphoridae. In 'Catalog of the Diptera of the Australasian and Oceanian Region'. (Ed. N. L. Evenhuis) pp. 702-718. (Bishop Museum Press: Honolulu, Hawaii.)

Lamanna, C., Mallette, M. F., and Zimmerman, L. (1973). 'Basic Bacteriology. Its Biological and Chemical Background.' (The Williams and Wilkins Company: Baltimore.)

Lanser J., Adams M., Doyle R., Sangster N., and Steele T. W. (1990). Genetic relatedness of *Legionella longbeacheae* isolates from human and environmental sources in Australia. *Applied and Environmental Microbiology* **56,** 2784-2790.

Liu, D., and Greenberg, B. (1989). Immature stages of some flies of forensic importance. *Annals of the Entomological Society of America* **82,** 80-93.

Loeschcke, V., Nielsen, B. O., and Andersen, D. (1994). Relationship among *Hydrotaea* species based on allozymes, karyotype and morphology (Diptera: Muscidae). *Hereditas* **121,** 103-111.

Mackerras, M. J. (1933). Observations on the life-histories, nutritional requirements and fecundity of blowflies. *Bulletin of Entomological Research* **24,** 353-362.

Mackerras, M. J., and Fuller, M. E. (1937). A survey of the Australian sheep blowflies. *Journal of the Council for Scientific and Industrial Research* **10,** 261-270.

Macleod, J. (1947). The climatology of blowfly myiasis. I. Weather and oviposition. *Bulletin of Entomological Research* **38,** 285-303.

Macquart, J. (1834). Insectes diptères du nord de la France. Athéricères: creophiles, oestrides, myopaires, conopsaires, scénopiniens, céphalopsides. *Mémoires de la Société (Royale) des Sciences, de l'Agriculture et des Arts de Lille* **1833,** 137-368.

Macquart, J. (1835). 'Histoire naturelle des insectes. Diptères.' Tome deuxième. Ouvrage accompagné de planches. (Roret: Paris.)

Macquart, J. (1843). Diptères exotiques nouveaux ou peu connus. -3.^{e} partie. *Mémoires de la Société (Royale) des Sciences, de l'Agriculture et des Arts de Lille* **1842,** 162-460.

Macquart, J. (1847a). 'Diptères exotiques nouveaux ou peu connus.' 2.^{e} supplément. (Roret: Paris.)

Macquart, J. (1847b). Diptères exotiques nouveaux ou peu connus. 2.^{e} supplément. *Mémoires de la Société (Royale) des Sciences, de l'Agriculture et des Arts de Lille* **1846,** 21-120.

Macquart, J. (1851). Diptères exotiques nouveaux ou peu connus. Suite du 4.^{e} supplément publié dans les mémoires d^{e} 1849. *Mémoires de la Société (Royale) des Sciences, de l'Agriculture et des Arts de Lille* **1850,** 134-294.

Macquart, J. (1855). Diptères exotiques nouveaux ou peu connus. 5.e supplément. *Mémoires de la Société (Royale) des Sciences, de l'Agriculture et des Arts de Lille* **1,** 25-156.

Malloch, J. R. (1927). Notes on Australian Diptera. No. XI. *Proceedings of the Linnean Society of New South Wales* **52,** 299-335.

Malloch, J. R. (1932). Notes on Australian Diptera. XXX. *Proceedings of the Linnean Society of New South Wales* **57,** 64-68.

Manchenko G. P. (1994). 'Handbook of detection of enzymes on electrophoretic gels.' (CRC Press: Boca Raton, Florida.)

Manguin, S. (1990). Population genetics and biochemical systematics of marsh flies in the *Sepedon fuscipennis* group (Diptera: Sciomyzidae). *Biochemical Systematics and Ecology* **18,** 447-452.

Meats, A. (1974). Rapid acclimatization to low temperature in the Queensland fruit fly, *Dacus tryoni*. *Journal of Insect Physiology* **19,** 1903-1911.

Meigen, J. W. (1826). 'Systematische Beschreibung der bekannten europäishen zweiflügeligen Insekten.' Fünfter Theil. (Schulz: Hamm.)

Miller, D. (1939). Blow-flies (Calliphoridae) and their asscociates in New Zealand. *Cawthron Institute Monographs* **2,** 1-68.

Miller, L. K. (1969). Freezing tolerance in an adult insect. *Science* **166,** 105-106.

Monteith, J. L., and Unsworth, M. H. (1990). 'Principles of Environmental Physics.' (Edward Arnold: London.)

Monzu, N. (1977). Coexistence of carrion-breeding Calliphoridae (Diptera) in Western Australia. Ph.D. Thesis, University of Western Australia, Perth.

Morris, B. (1991). Description of the life history stages of *Calliphora nociva* Hardy (Diptera: Calliphoridae). *Journal of the Australian Entomological Society* **30,** 79-82.

Morris, B. (1993). Physiology and taxonomy of blowflies. M.Sc. Thesis, The University of Adelaide, Adelaide.

Nei, M. (1987). 'Molecular Evolutionary Genetics.' (Columbia University Press: New York.)

Nicholson, A. J. (1934). The influence of temperature on the activity of sheep-blowflies. *Bulletin of Entomological Research* **25,** 85-99.

Norris, K. R. (1959). The ecology of sheep blowflies in Australia. *Monographiae Biologicae* **8,** 514-544.

Norris, K. R. (1965). The bionomics of blowflies. *Annual Review of Entomology* **10,** 47-68.

Norris, K. R. (1966). Daily patterns of flight activity of blowflies (Calliphoridae: Diptera) in the Canberra district as indicated by trap catches. *Australian Journal of Zoology* **14,** 835-853.

Norris, K. R. (1969). Notes on the screening of buildings to reduce the blowfly nuisance inddors in southern Australia. *Architectural Science Review* **12,** 21-24.

Norris, K. R. (1973). Synonomy and status of some Australian Calliphoridae (Diptera). *Journal of the Australian Entomological Society* **12,** 1-2.

Norris, K. R. (1991). General biology. In 'The Insects of Australia'. (Eds I. D. Naumann, P. B. Carne, J. F. Lawrence, E. S. Nielsen, J. P. Spradbury, R. W. Taylor, M. J. Whitten and M. J. Littlejohn.) pp. 68-108. (Melbourne University Press: Carlton.)

Norris, K. R. (1994). Three new species of Australian 'Golden Blowflies' (Diptera: Calliphoridae: *Calliphora*), with a key to described species. *Invertebrate Taxonomy* **8,** 1343-1366.

Nuorteva, P. (1965). The flying activity of blowflies (Dipt., Calliphoridae) in subarctic conditions. *Annales Entomologici Fennici* **31,** 242-245.

O'Flynn, M. A. (1976). A study of blowflies breeding in sheep and in carrion. M.Ag.Sc. Thesis, University of Queensland, Brisbane.

O'Flynn, M. A. (1983). The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Journal of the Australian Entomological Society* **22,** 137-148.

O'Flynn, M. A., and Moorhouse, D. E. (1980). Identification of early immature stages of some common Queensland carrion flies. *Journal of the Australian Entomological Society* **19,** 53-61.

Ono, M., Igarashi, T., Ohno, E., and Sasaki, M. (1995). Unusual thermal defence by a honeybee against mass attack by hornets. *Nature* **377,** 334-336.

Ono, M., Okada, I., and Sasaki, M. (1987). Heat production by balling in the Japanese honeybee, *Apis cerana japonica* as a defensive behavior against the hornet, *Vespa simillima xanthoptera* (Hymenoptera: Vespidae). *Experientia* **43,** 1031-1032.

Palmer, D. H. (1980). Partitioning of the carrion resource by sympatric Calliphoridae (Diptera) near Melbourne. Ph.D. Thesis, La Trobe University, Melbourne.

Pape, T. (1992). Phylogeny of the Tachinidae family-group (Diptera: Calyptratae). *Tijdschrift voor Entomologie* **135,** 43-86.

Parkash, R., Jyoutsna, and Vandna (1994). Allozyme phylogeny and five species of *takahashii* species subgroup of *Drosophila. Korean Journal of Genetics* **16,** 187-196.

Patton, W. S. (1925). Diptera of medical and veterinary importance, II. The more important blowflies, Calliphorinae. *Philippine Journal of Science* **27,** 397-411.

Payne, J. A. (1965). A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* **46,** 592-602.

Peterson, J. L., and Murdock J. E. (1989). Forensic science ethics: developing an integrated system of support and enforcement. *Journal of Forensic Sciences* **34,** 749-762.

Piedrahita, O., Ellis, C. R., and Bogart, J. P. (1985). Electrophoretic identification of the larvae of *Diabrotica barberi* and *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* **78,** 537-40.

Pimental, D. (1958). Alteration of microclimate imposed by populations of flour beetles (*Tribolium*) *Ecology* **39,** 239-246.

Prins, A. J. (1982). Morphological and biological notes on six South African blow-flies (Diptera, Calliphoridae) and their immature stages. *Annals of the South African Museum* **90,** 201-217.

Putman, R. J. (1977). Dynamics of the blowfly, *Calliphora erythrocephala*, within carrion. *Journal of Animal Ecology* **46,** 853-866.

Putman, R. J. (1983). 'Carrion and Dung: Decomposition of Animal Wastes.' (Edward Arnold (Publishers) Limited: London.)

Randall, D., Burggren, W. and French, K. (1997). 'Eckert Animal Physiology. Mechanisms and Adaptations.' (W. H. Freeman and Company: New York.)

Ratcliffe, F. N. (1935). Observations on the sheep blowfly (*Lucilia sericata* Meig.) in Scotland. *Annals of Applied Biology* **22,** 742-753.

Reed, H. B. (1958). A study of dog carcase communities in Tennessee, with special reference to the insects. *The American Midland Naturalist* **59,** 213-245.

Richardson, B. J., Baverstock, P. R., and Adams, M. (1986). 'Allozyme Electrophoresis: A Handbook for Animal Systematics and Population Studies.' (Academic Press: Sydney.)

Robineau-Desvoidy, J. B. (1830). Essai sur les myodaires. *Mémoires Présentés par divers Savants à l'Académie Royal des Sciences l'Institut de France* **2,** 1-813.

Robineau-Desvoidy, J. B. (1863). 'Histoire naturelle des diptères des environs de Paris. Oeuvre posthume du Dr Robineau-Desvoidy. Publiée par les soins de sa famille, sous la direction de M.H. Monceaux.' Tome second. (V. Masson et Fils: Paris.)

Rodendorf, B. B. (1926). Morphologisches Studium an äusseren Genitalorganen der Calliphorinen (Diptera). *Zoologicheskii Zhurnal* **6,** 83-128.

Rodriguez, W. C., III, and Bass, W. M. (1985). Decomposition of buried bodies and methods that may aid in their location. *Journal of Forensic Sciences* **30,** 836-852.

Rogers, J. S. (1972). Measures of genetic similarity and genetic distance. *Studies in Genetics. VII. University of Texas Publication number* **7213,** 145-153.

Rognes, K. (1991). Blowflies (Diptera: Calliphoridae) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica* **24,** 1-272.

Rognes, K. (1997). The Calliphoridae (blowflies) (Diptera: Calliphoridae) are not a monophyletic group. *Cladistics* **13,** 27-68.

Saunders, D. S. (1987). Maternal Influence on the incidence and duration of larval diapause in *Calliphora vicina*. *Physiological Entomology* **12,** 331-338.

Schmidt-Nielsen, K. (1997). 'Animal Physiology. Adaptation and Environment.' (Cambridge University Press: Cambridge.)

Schumann, H. (1953/1954). Morphologisch-systematische Studien an Larven von hygienisch wichtigen mitteleuropäischen Dipteren der Familien Calliphoridae-Muscidae. *Wissenschaftliche Zeitschrift der Universität Greifswald. Matematisch-naturwissenschaftliche Reihe* **4/5,** 245-274.

Shean, B. S., Messinger, L., and Papworth, M. (1993). Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Sciences* **38,** 938-949.

Smith, K. G. V. (1986). 'A Manual of Forensic Entomology.' (Cornell University Press: Ithaca, New York.)

Smith, K. G. V. (1989). An introduction to the immature stages of British flies. Dolling, W. R. and Askew, R. R. (Eds), *Handbooks for the identification of British insects* **10 (14).** (Royal Entomological Society of London: London.)

Sneath, P. H. A., and Sokal, R. R. (1973). 'Numerical Taxonomy: The Principles and Practice of Numerical Classification.' (W. H. Freeman and Company: San Francisco.)

Sperling, F. A. H., Anderson, G. S., and Hickey, D. A. (1994). A DNA-based approach to the identification of insect specimens used for postmortem interval estimation. *Journal of Forensic Sciences* **39,** 418-427.

Spradbery, J. P. (1992). Studies on the prepupal and puparial stages of the old world screw-worm fly, *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae). *CSIRO Division of Entomology Technical Paper* **29,** 1-24.

Steck, G. J. (1991). Biochemical systematics and population genetic structure of *Anastrepha fraterculus* and related species (Diptera: Tephritidae). *Annals of the Entomological Society of America* **84,** 10-28.

Swofford, D. L. (1981). On the utility of the distance-Wagner procedure. In 'Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society'. (Eds V. A. Funk and D. R. Brooks.) pp. 25-43. (New York Botanic Gardens: New York.)

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. In 'Molecular Systematics'. (Eds D. M. Hillis, C. Moritz and B. K. Mable.) pp. 407-514. (Sinauer Associates: Sunderland.)

Tantawi, T. I., and Greenberg, B. (1993). The effect of killing and preservative solutions on estimates of maggot age in forensic cases. *Journal of Forensic Sciences* **38,** 702-707.

Teskey, H. J. (1981). Morphology and terminology - larvae. In 'Manual of Nearctic Diptera'. (Eds J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth and D. M. Wood.) pp. 65-88. (Research Branch, Agriculture Canada: Ottawa.)

Thomas, D. B. (1991). Time-activity budget of adult screwworm behavior (Diptera: Calliphoridae). *Journal of Medical Entomology* **28,** 372-377.

Tipple, S. (1986). Forensic science: the new trial by ordeal? *Law Society Journal* **August,** 44-52.

Tribe, M. A., and Bowler, K. (1968). Temperature dependence of "standard metabolic rate" in a poikilotherm. *Comparative Biochemistry and Physiology* **25,** 427-436.

Tullis, K., and Goff, M. L. (1987). Arthropod succession in exposed carrion in a tropical rainforest on O'ahn Island, Hawaii. *Journal of Medical Entomology* **24,** 332-339.

Turner, B., and Howard, T. (1992). Metabolic heat generation in dipteran larval aggregations: a consideration for forensic entomology. *Medical and Veterinary Entomology* **6,** 179-181.

Ullyett, G. C. (1950). Competition for food and allied phenomena in sheep blowfly populations. *Philosophical Transactions of the Royal Society of London (Series B)* **234,** 77-175.

Unwin, D. M., and Corbet, S. A. (1991). 'Insects, Plants and Microclimate.' (The Richmond Publishing Co. Ltd.: Slough.)

Walker, F. (1849). 'List of the specimens of dipterous insects in the collection of the British Museum'. Parts II-IV. pp. 231-1172. (British Museum: London.)

Walker, F. (1853). Diptera. Part IV. In 'Insecta Saundersiana: or characters of undescribed insects in the collection of William Wilson Saunders, Esq., F.R.S., F.L.S. &c'. Vol. 1. pp. 253-414. (Van Voorst: London.)

Wall, R. (1988). Analysis of the mating activity of male tsetse flies *Glossina m. morsitans* and *G. pallidipes* in the laboratory. *Physiological Entomology* **13,** 103-110.

Wall, R. (1993). The reproductive output of the blowfly *Lucilia sericata*. *Journal of Insect Physiology* **39,** 743-750.

Wall, R., and Warnes, M. L. (1994). Responses of the sheep blowfly *Lucilia sericata* to carrion odour and carbon dioxide. *Entomologia Experimentalis et Applicata* **73,** 239-246.

Wallman, J. F. (1990). Ecology of blowflies (Diptera: Calliphridae) in carrion in winter. BSc (Hons) Thesis, The University of Adelaide, Adelaide.

Waterhouse, D. F. (1947). The relative importance of live sheep and carrion as breeding grounds for the Australian sheep blowfly *Lucilia cuprina*. *Bulletin of the Council for Scientific and Industrial Research* **217,** 1-31.

Wells, J. D., and Greenberg, B. (1992). Laboratory interaction between introduced *Chrysomya rufifacies* and native *Cochliomyia macellaria* (Diptera: Calliphoridae). *Environmental Entomology* **21,** 640-645.

Wells, J. D., and LaMotte, L. R. (1995). Esimating maggot age from weight using inverse prediction. *Journal of Forensic Sciences*. **40,** 585-590.

White, A., and Doubleday, E. (1843). Fauna of New Zealand. Materials towards a fauna of New Zealand, Auckland Island, and Chatham Islands. List of the annulose animals hitherto recorded as foubnd in New Zealand, with the descriptions od some new species. In E. Dieffenbach, 'Travels in New Zealand; with contributions to the geography, geology, botany and natural history of that country'. Vol II. pp. 265-269. (Murray: London.)

Wigglesworth, V. B. (1967). 'The Principles of Insect Physiology.' (Methuen: London.)

Williams, H. (1987). The effects of competition and larval habitats on populations of the Australian sheep blowfly *Lucilia cuprina* (Wiedemann). Ph.D. Thesis, University of Tasmania, Hobart.

Williams, H., and Richardson, A. M. M. (1984). Growth energetics in relation to temperature for larvae of four species of necrophagous flies (Diptera: Calliphoridae). *Australian Journal of Ecology* **9,** 141-152.

Wilson, E. O. (1971). 'The Insect Societies.' (The Belknap Press of Harvard University Press: Massachusetts and London.)

Wobeser, G., and Galmut, E. A. (1984). Internal temperature of decomposing duck carcasses in relation to botulism. *Journal of Wildlife Diseases* **20,** 267-271.

Yurkiewicz, W. J. (1968). Flight range and energetics of the sheep blowfly during flight at different temperatures. *Journal of Insect Physiology* **14,** 335-339.

Yurkiewicz, W. J., and Smyth, T., Jr. (1966a). Effect of temperature on flight speed of the sheep blowfly. *Journal of Insect Physiology* **12,** 189-194.

Yurkiewicz, W. J., and Smyth, T., Jr. (1966b). Effects of temperature on oxygen consumption and fuel utilization by the sheep blowfly. *Journal of Insect Physiology* **12,** 403-408.

Zamudio, K. R., Huey, R. B., and Crill, W. D. (1995). Bigger isn't always better: body size, developmental and parental temperature and male territorial success in *Drosophila melanogaster*. *Animal Behaviour* **49,** 671-677.

Zar, J. H. (1984). 'Biostatistical Analysis.' (Prentice-Hall: New Jersey.)

Zumpt, F. (1965). 'Myiasis in Man and Animals in the Old World.' (Butterworth and Co.: London.)