APPLIED STUDIES OF SOME SOUTHERN AFRICAN BLOWFLIES (DIPTERA: CALLIPHORIDAE) OF FORENSIC IMPORTANCE

by

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Thesis submitted in fulfilment
of the requirements of the degree of

MASTER OF SCIENCE in ENTOMOLOGY

The Department of Zoology and Entomology
Rhodes University

November 2002

DECLARATION

This thesis is the result of the author's original work, except where acknowledged or specifically stated in the text. No part of this work has been submitted for any other degree or examination at any other university or academic institution.

Nicola Lunt, November 2002

ABSTRACT

Three major aspects of blowfly (Diptera: Calliphoridae) research were the focus of this study. Firstly, the phylogenetic relationships of 40 oestroid species from a variety of geographical localities were investigated using Cytochrome Oxidase b subunit I (COI) gene sequences. Maximum parsimony (MP) and Jukes-Cantor neighbor-joining (NJ) analyses both extracted a paraphyletic Calliphoridae, with the Calliphorinae-Luciliinae clade being sister to the Sarcophagidae. Short branch lengths within *Chrysomya* indicate a recent rapid radiation of this genus. *Phormia* and *Protophormia* either formed a sister clade to *Chrysomya*, or were embedded in this genus. Tree topologies were comparable between MP and NJ trees, but the positions of some genera were ambiguous.

Secondly, developmental parameters and behaviour were investigated for four southern African species of forensically important blowflies *viz. Chrysomya chloropyga, C. putoria, C. megacephala* and *Lucilia sericata*, and *ad hoc* observations were made for *Calliphora croceipalpis, Chrysomya marginalis* and the predatory *C. albiceps*. Choice of oviposition substrate differed between species, mirroring substrate preferences in the field. Sexual dimorphism and dwarfism within a cohort complicated ageing maggots using size, but the use of developmental events (e.g. ecdysis) allowed ages to be determined unambiguously. Separate species status was supported for the previously synonymised *C. chloropyga* and *C. putoria,* by differences in maggot behaviour, larval growth rates and temperature optima. The proportion of total development time assigned to each larval instar and pupariation was variable among temperatures, but similar between congeneric species.

Thirdly, since a negative linear relationship was found to occur between the developmental constant (K) and developmental zero (D_0) for both Calliphoridae and Sarcophagidae, the potential for predicting physiological parameters of unstudied taxa was investigated. Species and genera of Palaearctic origin generally had high K's and low D_0's, and the reverse was true for the tropical taxa. It was found that both K and D_0 can be estimated for "unknown" taxa using the Felsenstein's Independent Contrasts (FIC) method of PDTree (Garland *et al.* 2001), provided that branch lengths are relatively short and the phylogenetic position of the estimated taxon is unambiguous.

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ACKNOWLEDGEMENTS

There are a number of people who have provided much-needed assistance, inspiration, support and encouragement throughout this project. Firstly, thanks must go to my family who have supported my extended period of "studying gogos", and have sacrificed so much to help me through university.

On the technical side, I would especially like to thank my supervisors, Prof. Martin Villet and Dr. Nigel Barker, for their guidance, patience and calming influence throughout. Thanks also to everyone who helped with the provision of information, advice and translations – Dr. Mervyn Mansell, Prof. Pat Hulley, Mrs. Larissa Pakhomova, Prof. Knut Rognes and all of the staff in the Zoology and Entomology Department. Also, to my fellow students and friends who've assisted with laboratory work and colony maintenance (despite the stench!) - Kirstin Williams, Jackie-Ann Rapson, Claire Jackson, and James Clery – I am eternally grateful.

Thanks also go to the people who have assisted me with the collection of specimens from around Africa – Martin Villet, Kim Wilkins, Kirstin Williams, Chris Kelley, Kyle Smith, Tammy Smith and Mark Robertson, and the "Namaqualand botany trip" members.

This project was funded in part grants to Martin Villet by the Rhodes University Joint Research Council and the National Research Foundation, and partly by a Rhodes University Postgraduate Scholarship (Masters).

Chapter 1

General Introduction to Medico-criminal Forensic Entomology, With Reference to Southern Africa

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Forensic entomology involves the use of insects as evidence in legal investigations. The field is divided into a number of disciplines, including stored product, urban and medico-legal (or medico-criminal) forensic entomology (Smith, 1986; Catts & Goff, 1992; Hall, 2001). This classification is dependent primarily on the legal areas that they address (e.g. civil or criminal law), and secondarily on the nature of the entomology. Stored product forensic entomology is used largely in civil cases to determine liability for insect infestations of stored products such as grain, or insects in foodstuffs (Hall, 2001), while urban forensic entomology deals with insect damage to, or infestations of abodes, the misuse of pesticides and delusional parasitosis (Catts & Goff, 1992).

Medico-criminal forensic entomology is most often involved with the determination of the time of events in cases where felonies - for example, murder or physical abuse - have been committed, but may also be of use in determining the origin of narcotics such as marijuana (Smith, 1986; Catts & Goff, 1992). Although murder cases get the most exposure by virtue of their high profiles, medico-criminal forensic entomology also deals with the neglect or abuse of infants and the physically or mentally disabled (e.g. Benecke & Lessig, 2001), and determining the post-mortem interval (PMI) in non-criminal cases such as accidental death (Hall, 2001). Forensic entomologists may even assist with cases of wildlife poaching (e.g. Anderson, 1999) and stock theft. In addition, some of the flies that are of forensic importance are also myiasis agents (e.g. *Lucilia cuprina*, *Cochliomyia* spp.) or disease transmitters (Zumpt & Patterson, 1952) and are therefore of interest to the medical and veterinary fraternities (Zumpt, 1956; Pont, 1980).

In medico-criminal entomology, insects may be used to determine several parameters. Firstly, the time of infestation of a body (living or dead) by insects may be estimated by using either the community structure as a guide (Schoenly, 1992; LaMotte & Wells, 2000), or from the stage of development that has been reached by immature insects (e.g. maggots) at discovery (Smith, 1986; Catts, 1992; Higley & Haskell, 2001). Fly eggs and maggots are usually used for the latter determination in the early stages of decomposition (fresh, bloat and active decay) because the eggs are invariably laid on the substrate on which the maggots develop, and the maggots usually remain on this substrate until the end of their feeding period. Once the flies have left the carcass, the dominant insects tend to be beetles (e.g. Dermestidae, Silphidae), but a variety of arthropods may be present on the carcass throughout the decomposition process (Smith, 1986; Catts & Goff, 1992; Anderson, 2001). Apart from giving an indication of the PMI, insects may provide evidence of corpse relocation (Catts, 1992), especially if the habitat in which the corpse is found does not correspond to the known habitat of insects present on the body (Smith, 1986). Entomological evidence, such as insect remains on windscreens or the front grilles of cars, can potentially link suspects to the area of the crime scene. In one case, a suspect was irrefutably linked to the scene of a crime by chigger mite bites on his legs (Lord, 1990).

1.1 - Decomposition process of carcasses

Since the focus of this study is medico-legal, and more specifically using fly development to estimate PMI's, it is relevant to outline the decomposition process of a carcass. The decomposition process tends to follow a predictable sequence of events (Smith, 1986), but the nature of the process and the important necrophages differ depending on whether the body is exposed, buried or submerged in water (Rodriguez & Bass, 1985; Smith, 1986; Catts & Goff, 1992; Hobischak & Anderson, 1999; Galloway *et al.*, 2001; Merritt & Wallace, 2001). In addition, corpses exposed to warm, dry conditions may mummify and be unattractive to early colonisers (Smith, 1986).

The decomposition process of an exposed carcass can be divided into several stages (Smith, 1986; Catts, 1992; Higley & Haskell, 2001). Some authors view decomposition as a successional process (e.g. Higley & Haskell, 2001), but since no climax community is ever reached and the habitat is diminished in the process, this interpretation is specious (Braack, 1981). The sequence of decomposition consists of graded rather than distinct stages, so that determining the transition between two stages using only the physical characteristics of the corpse may be problematic. To complicate matters further, the duration of each stage may vary considerably depending on the environmental conditions, the habitat to which the corpse is exposed (Greenberg, 1973; Anderson, 2000), pre- or post-mortem damage to the body, for example, decapitation (Anderson, 1997), stab wounds, or dismemberment by scavengers (Catts, 1992), and other factors such as the body being clothed or covered, submerged in water, or buried (Catts, 1992; Galloway *et al.*, 2001; Merritt & Wallace, 2001).

When considering the decay process of an exposed body (as opposed to one that is buried or submerged in water), the body initially bloats as a result of aerobic and subsequent anaerobic bacterial action. Later, once insects (such as fly maggots) have colonised the resource, active decay takes place. It is towards the end of this phase that the greatest loss of biomass occurs, when post-feeding maggots migrate to pupate (Catts, 1992). With the loss of biomass and the concomitant removal of moisture from the body as a result of maggot activity and environmental desiccation, the carcass enters the advanced decay and finally the dry remains stages (Catts, 1992).

Although determining the time since death of the body using the physical characteristics of the corpse may be difficult, each stage in the process may attract a specific community of insects (e.g. Smith, 1986), and the analysis of the community present on the body can assist an investigator with the estimation of PMI's. Volatile chemical attractants appear to be the primary cue for colonisation. For example, blowflies are attracted to indole, skatole and trimethylamine, which are produced during early meat decomposition (Greenberg, 1973). Smith (1986) and Catts and Goff (1992) recognised four main guilds of arthropods that may utilise the decomposing carcass as a habitat. These are the necrophages (such as fly maggots) that feed directly on the tissues of the body, predators and parasites (e.g. beetles, ants and parasitoid wasps) that feed on other carcass visitors, omnivores (e.g. beetles) which may feed both on the body tissues and other associated organisms, and incidental taxa (e.g. spiders and springtails) which use the body as an extension of their usual habitat (e.g. using the body as a hunting site or refuge, or the liquid exudates that enter the soil as a source of moisture). Many species of fly also fit into this latter category - for example, some muscids will visit a carcass to obtain protein meals or feed on the exudates but do not usually oviposit on the resource (Mansell, pers. comm.).

The blowflies (Calliphoridae) are usually the first to colonize a carcass and are often the dominant insects present during the fresh, bloat and active decay stages (Smith, 1986; Catts, 1992). If females have mated and have mature eggs, they will oviposit on the body, usually near natural orifices such as the eyes and mouth, or at sites of damage such as puncture wounds (Anderson, 1997; Benecke, 1998; Benecke & Lessig, 2001). Once the maggots hatch, they feed on the decaying tissues and will usually only leave the carcass in their post-feeding stage, prior to pupariation; however, if food is limited or conditions become unsatisfactory, they may leave the medium earlier to go in search of supplementary food sources (Christopherson & Gibo, 1997). Fleshflies (Sarcophagidae) and muscids may also oviposit on the carcass during early decomposition, but usually arrive later than the blowflies (Smith, 1986). Hymenopterous parasitoids, and predators and scavengers such as ants (Formicidae) and beetles (e.g. Staphylinidae and Silphidae) may also visit a carcass during early decomposition to feed on fly eggs or larvae (Braack, 1981, 1987; Smith, 1986) or corpse tissues. From the onset of the active decay stage, fly larvae become less common, although there may be a second wave of fly colonization if the carcass is sufficiently moist (Smith, 1986). From advanced decay through to the dry remains stage, beetle (e.g. Dermestidae, Trogidae, Ptinidae) activity dominates (Catts, 1992).

1.2 - Utility and limitations of flies (Diptera) for PMI estimations

As a result of their dominance early in the decomposition process and our ability to determine the age of maggots with some accuracy and precision (Reiter, 1984; Wells & LaMotte, 1995), fly maggots are the most frequently used post-mortem indicators in the early stages of decomposition (Smith, 1986; Catts, 1992; Catts & Goff, 1992; Higley & Haskell, 2001). Fly larvae, being poikilotherms, are unable to physiologically control their body temperatures, so the environmental temperature regime to which they are exposed will affect their rates of development. All ectotherms have unique upper and lower thermal thresholds above and below which, respectively, no development will occur (Sharpe & DeMichele, 1977). Between the two thresholds, development rates mainly increase with temperature, but the rate increments are not linear for the entire temperature range (Sharpe & DeMichele, 1977; Higley & Haskell, 2001). Thus, for an ectotherm, a graph of growth rates against temperature will be skewed unimodal with a roughly linear segment in the region of common environmental temperatures (Allen, 1976).

The method for estimating the PMI or the time of corpse colonisation by insects, involves calculating a thermal constant (K), determining a lower thermal threshold (D_0) for development in the linear section of the developmental curve of the relevant species, and using a variation of the rate summation method to calculate the probable oviposition time (Sharpe & DeMichele, 1977; Higley *et al.,* 1986; Higley & Haskell, 2001). As described by Higley and Haskell (2001), there are several rate summation models that can be applied which differ in their ease of use, although there are apparently only minor disparities in their accuracy.

Despite the wide use of maggots for estimating PMI, there are in reality many environmental variables that may confound the estimate by affecting the rate of corpse decomposition, including maggot-generated heat, pre-mortem ingestion of toxins and drugs, the environment to which the carcass is exposed, dismemberment of the corpse and the time lapse between death and the first opportunity that flies have to oviposit on the body (Catts, 1992; Campobasso *et al.,* 2001). However, if due cognisance of these factors is taken, maggots can be used to determine a variety of additional information about the conditions to which a body has been exposed, both pre- and post-mortem (see Marchenko, 1988, 2001; Catts & Goff, 1992).

1.2.1 - Oviposition windows, habitat and evidence of corpse transport

The "oviposition window" (Catts, 1992) is the period during which a corpse is exposed to insects, and flies are stimulated to oviposit. This term refers to any period when oviposition can, or is likely to occur, i.e. between fresh and active decay. Often the window may be a brief period of sunny or warm weather during an inclement bout (Catts, 1992).

Catts (1992) commented that most homicides in the United States are committed at night; a time when blowflies are largely inactive and thus not ready to "start the clock". He argued, therefore, that an estimate of PMI based only on entomological evidence might underestimate the time of death by a substantial margin if the crime was committed in darkness. However, Greenberg (1990) and Singh and Bharti (2001) reported that blowflies will oviposit at night in urban environments, even in the absence of strong ambient light, and Green (1951) reported that *Calliphora vicina* oviposited nocturnally in slaughterhouses. Despite their ability to oviposit at night, blowflies generally require temperatures in excess of 10°C for oviposition (Catts, 1992) and in cold weather, sunny conditions tend to be stronger oviposition stimuli than warm temperatures (Green, 1951). It is probable then, that during winter or in inclement weather, nocturnal oviposition will not occur. Unfortunately, since the minimum nighttime temperatures in both Singh and Bharti (2001) and Greenberg's (1990) experiments exceeded 16°C, the combined effects of darkness and cold are, as yet, undetermined.

The habitat to which a body is, or has been, exposed can also be elucidated using insect evidence (Smith, 1986). Fly distribution is not spatially or temporally uniform, and flies may be localised in a particular habitat - for example in shaded areas or near water sources (Smit, 1931; Hightower & Alley, 1963; Cruickshank & Wall, 2002) - or may be more prevalent at specific times of the year (Ullyett, 1950; Meskin, 1986). Flies may have preferred habitats or localised distributions and can potentially provide clues to the conditions to which the body has been exposed since death (Smith, 1986; Anderson, 2000). In a case involving the discovery of a dead woman in full sun in a bare field, Mansell (pers. comm.) was intrigued to find *Calliphora croceipalpis* maggots in the corpse. This fly in South Africa tends to prefer cool, shaded habitats and is often found indoors (pers. obs.), which would indicate that the body had been moved or had recently been uncovered. After further queries, it was discovered that the field had, until recently, been covered in thatching grass, and the body would have been shaded at the time of fly oviposition.

Insect evidence may give a strong indication of corpse translocation if there are species present on the body that do not occur in the habitat or region where the corpse was eventually discovered. This may include predominantly urban flies on a rural corpse, but theoretically, it is possible to detect long-distance transport (e.g. cross country). A complication is that forensically important blowflies tend to be synanthropic, so they may hitchhike on long-distance transportation such as trains (Pead, 1910) or livestock (Baker *et al.*, 1968). Thus there is the potential that "exotic" flies may be present in corpses found along transport routes, but presumably they would comprise a small fraction of the total corpse fauna.

1.2.2 - Maggot generated heat, competition and final size

It is well documented that large maggot masses can increase the temperature of the substrate by metabolic and physical heat generation (Payne, 1965; Goodbrod & Goff, 1990; Catts, 1992; Byrd & Butler, 1998). In decomposing pigs kept at sub-freezing temperatures, Payne (1965) reported that the temperature inside maggot masses exceeded the ambient low and high temperatures by about 35°C to 45°C and 20°C, respectively. Since ambient (air) temperatures are most often used by investigators to determine the probable oviposition time, this disparity between corpse (or maggot mass) and air temperatures will result in an overestimation of the PMI (Catts, 1992). It has been shown that the corpse temperature and sub-carcass soil temperature are more strongly correlated than corpse and air temperatures, so investigators should be cautious about complete reliance on weather station data (Catts, 1992).

In addition to heat generation, high maggot densities may result in dwarf individuals (Ullyett, 1950), higher mortality rates (Ullyett, 1950) and, in species such as *Chrysomya rufifacies* predation and cannibalism (Goodbrod & Goff, 1990) due to intra- and interspecific competition. In mixed cultures of *Chrysomya rufifacies* and *Chrysomya megacephala*, Goodbrod and Goff (1990) illustrated that high maggot densities led to *C. rufifacies* outcompeting *C. megacephala*. Many investigators use the size of the largest maggots in a corpse as PMI indicators, assuming that these maggots are the oldest (Williams, 1984; Wells & LaMotte, 1995; Byrd & Butler, 1998, 2000; Grassberger & Reiter, 2001). However, dwarfism appears to be a fairly common phenomenon, even in the absence of overcrowding or food deprivation (Ullyett, 1950), for example, in cohorts of *Lucilia sericata* at high temperatures (pers. obs.) and in *Chrysomya megacephala* (Wells & Kurahashi, 1994). Failure to recognise that dwarf individuals may be the oldest may result in inaccurate PMI estimates (Wells & Kurahashi, 1994; Gaudry *et al.*, 2001).

1.2.3 - Temperature fluctuations and their effects on maggot growth rates

Most laboratory studies of development rates of insects are carried out at constant temperatures (Nawal *et al.*, 1982; Nishida *et al.*, 1986; Grassberger & Reiter, 2001) for simplicity and accuracy. Investigators then use mean daily temperatures to calculate the PMI (e.g. Anderson, 1997). However, fluctuating temperatures can affect development rates (Introna *et al.*, 1989; Worner, 1992; Davies and Ratcliffe, 1994; Byrd & Allen, 2001a; Higley & Haskell, 2001). For temperature fluctuations in the linear part of the development rate *vs.* temperature curve, the effects of averaging the high and low temperatures may not seriously affect the accuracy of PMI estimates (Sharpe & DeMichele, 1977; Higley & Haskell, 2001), but using the mean is often inaccurate especially if the temperature fluctuations are close to, or straddle, the thermal thresholds (Sharpe & DeMichele, 1977; Worner, 1992; Byrd & Butler, 1998; Byrd & Allen, 2001a). As benchmark studies, constant temperature rearing experiments are useful, but due cognisance should be given to temperature fluctuations when estimating actual PMI's.

1.2.4 - Pre-mortem ingestion of toxins and drugs

The field of "entomo-toxicology" (Pounder, 1991) has received much attention in recent years (e.g. Nuorteva & Nuorteva, 1981; Goff *et al.*, 1989, 1994, 1997; Introna *et al.*, 1990; Goff & Lord, 1994; Sadler *et al.*, 1995; 1997b; Musvasva *et al.*, 2001). Pre-mortem ingestion of drugs and toxins has been shown to have a variety of effects on the development rates of fly maggots. Goff *et al.* (1989) showed that the presence of cocaine in tissues significantly accelerated growth in the fleshfly *Sarcophaga peregrina* (Sarcophagidae), while Sadler *et al.* (1997b) determined that the growth rate of *Calliphora vicina* (Calliphoridae) was adversely affected by the presence of amitriptyline in tissues. Maggots that have been feeding on a corpse can also be used as toxicological indicators when insufficient tissue samples are available for drug analysis (Beyer *et al.*, 1980; Pounder, 1991; Goff & Lord, 1994). In addition, the effects of drugs are not necessarily uniform between species or families (e.g. Williams *et al.*, submitted), and the breakdown products of drugs may also differ in their effects on maggot development (e.g. Bourel *et al.*, 1999; Goff *et al.*, 1999).

With the return in popularity of maggot debridement therapy in medicine, it is necessary to know how drugs (such as antibiotics) affect maggot development, in order that appropriate treatment regimes can be followed. Sherman *et al.* (1995) reported that clinical dosages of seven antibiotics had no significant effect on the development rate of *Lucilia* (=*Phaenicia*) *sericata*, the primary species used for maggot debridement therapy. However, little is known about the effects of other commonly used drugs in this context and it is probable that drugs that show tropism (i.e. accumulate in certain organs such as the liver) may have a greater effect on the development of maggots feeding on corpses than those used in debridement therapy.

1.2.5 - Site of maggot infestation as an indicator of pre-mortem damage

Female flies tend to oviposit in places where larvae have ready access to tissues soon after hatching from their eggs. In an undamaged and uncovered corpse, such places would be the nose, mouth, eyes and genital or anal orifices (Anderson, 1999; Benecke & Lessig, 2001). However, in cases where the body has been damaged (e.g. by scavenger action post-mortem (Ellison, 1990) or by gunshot wounds or stabbing pre-mortem), flies may preferentially oviposit at wound sites (Catts & Goff, 1992). Thus, the investigator can determine if the body was damaged prior to fly oviposition, by taking the locality of the maggots into account (Catts & Goff, 1992).

1.2.6. – Precocious development of eggs in old females

Smith (1986) and Wells & King (2001) commented on the potential for overestimation of the PMI, as a result of embryonic development in retained eggs. A female blowfly generally matures a large number of eggs simultaneously, but will only oviposit if she can find a suitable substrate. Often, if a substrate is unavailable immediately, she will retain the eggs for some time - for at least three weeks in *Aldrichina grahami* (Aldrich) and *Calliphora nigribarbis* Vollenhoven (Wells & King, 2001). However, a single egg may pass into the common oviduct, and, since it is fertilised on the way, embryonic development may commence before oviposition occurs (Wells & King, 2001). As a result, the precocious larva may hatch some time before its siblings; in fact, it may hatch immediately after oviposition (Wells & King, 2001) if it has been retained for a sufficient period. Precocious egg development has been recorded most commonly in calliphorine blowflies, and has been observed in up to 62% of female *Calliphora terraenovae* Macquart (Wells & King, 2001). If, as is the common practice, the largest or oldest larva is employed to estimate the PMI, such precocious larvae will result in an overestimation of the time since death.

1.3 - Motivation

Forensic entomology in southern Africa has not been exhaustively studied (Braack, 1981, 1984, 1986, 1987, Prins, 1982, 1983, 1984a, 1984b; Meskin, 1986, 1991; Louw & van der Linde 1993), as it is still a relatively young discipline in this region of the world. For this field to progress, basic research is needed in several areas. Apart from ecological and successional studies, the life-histories and development rates of the forensically important taxa arguably require the most attention in order for accurate post-mortem interval estimations to be made. Several of the species present in the region have wide distributions (e.g. *Calliphora vicina*) and have been studied in other parts of the world (Davison, 1969; Reiter, 1984; Williams & Richardson, 1984), but there are still few data available for the most important species - *Chrysomya chloropyga* and *C. albiceps* (Mansell, pers. comm.).

There is also a need to provide accurate means of identification of both adults and early instar larvae, especially of taxa that are, like *Lucilia sericata* and *Lucilia cuprina,* morphologically similar (Ullyett, 1945; Zumpt, 1956; Holloway, 1991). Morphological and molecular methods of identification are both invaluable in this pursuit and should be given priority.

The last catalogue of the African Calliphoridae was published by Pont (1980), and the lack of uniformity in calliphorid systematics on a global scale is a cause for concern; there is an urgent need to test more recent classifications (e.g. Rognes 1991, 1997a) with independent data to elucidate relationships. Apart from the academic utility of a robust phylogeny, more applied uses may be possible, such as species identification (e.g. Wells & Sperling, 2001) and prediction of behavioural and developmental traits (see chapter 4). With the advent of global travel, anthropogenic introductions of flies to new regions are inevitable (Pead, 1910; Baumgartner & Greenberg, 1984). For example, species of the genus *Chrysomya* have already been introduced to North and South America from Africa (Baumgartner & Greenberg, 1984; Wells & Sperling, 2001), and *Calliphora vicina* has been introduced into South Africa from Europe (Pont, 1980). In addition, populations of the same species may exhibit different behavioural characteristics. For example, *Lucilia cuprina* is a primary myiasis agent in Africa and Australasia but not in North America (Stevens & Wall, 1996) and *L. sericata* populations differ in their oviposition response to sheep wool in different geographic regions (Stevens & Wall, 1997). Thus, an understanding of the biology and behaviour of blowflies throughout their distributions is relevant.

1.4 – The forensically important calliphorids of southern Africa

The Calliphoridae is a cosmopolitan family and is very diverse. The Afrotropical region is the most species-rich, with over 300 species in about 40 genera (Zumpt, 1956; Shewell, 1987). Historically, the Calliphoridae has been divided into two to fourteen subfamilies, depending on authors' interpretations of distinctive characters (e.g. Zumpt, 1956; Pont, 1980; Shewell, 1987; Rognes, 1991, 1997a). Following Rognes (1991), the family is divided into fourteen probably monophyletic subfamilies, several of which are not of immediate relevance to humans. In southern Africa, eight subfamilies are represented (Pont, 1980; Rognes, 1991). The Rhiniinae, Bengaliinae and Prosthetosomatinae are not synanthropic; the latter taxon's larvae are known exclusively from termitaria, and adults have never been recognised (Pont, 1980; Rognes, 1991). Members of the five remaining subfamilies - Luciliinae, Chrysomyinae, Calliphorinae, Auchmeromyiinae and Phumosiinae - are of medical and/or forensic importance.

For the purposes of this thesis, only the representatives of the forensically important African taxa will be considered. A simplified key for the identification of known or potential forensically important calliphorids is given (Appendix A). Further information can be acquired from Pont (1980) and Zumpt (1956) for the Afrotropical Calliphoridae, Rognes (1991, 1997) for the Scandinavian taxa, and Hall (1948) and Shewell (1981) for the Nearctic species.

1.4.1 - Subfamily Calliphorinae

Rognes (1991) questioned the monophyly of the diverse Calliphorinae (*sensu* Hall, 1948; Zumpt, 1956), which was united by a plesiomorphic character - the absence of setae on the stem vein. He thus split the Calliphorinae into several putatively monophyletic subfamilies, and a later morphological phylogenetic analysis indicated that this division was valid (Rognes, 1997). The Calliphorinae (*sensu* Rognes, 1991) is characterized by seven apomorphies, six of which are adult characters. In the Afrotropical region, the revised subfamily contains the genus *Calliphora* Robineau-Desvoidy, *Pericallimyia* Villeneuve, *Adichosina* Villeneuve, *Coganomyia* Dear, *Ochromelinda* Villeneuve, *Onesihoplisa* Villeneuve, *Termitocalliphora* Bauristhene, *Tricycleala* Villeneuve and *Zernyiella* Zumpt (Pont, 1980; Rognes, 1991). In a forensic context, only *Calliphora* is of immediate importance in the Afrotropics.

The genus *Calliphora* is represented by two species in southern Africa, *viz. C. croceipalpis* Jaennicke and *C. vicina* Robineau-Desvoidy (Appendix A). *Calliphora croceipalpis* is indigenous to the region, and is distributed widely throughout southern and East Africa. There are also records of this fly from the subantarctic islands and islands off the African mainland (Pont, 1980). By contrast, *C. vicina* is of Palaearctic origin and although it is widespread in India and the Australasian region, it is not widely distributed in Africa (Pont, 1980). Its presence on the Prince Edward Islands (Chown, pers. comm.) and in the Gauteng province of South Africa (pers. obs.) is probably a result of anthropogenic introduction, but it is likely that its range will expand further in the future.

In Europe, Australia and the United States, *Calliphora* spp. are often dominant flies in the forensic context (Williams & Richardson, 1984; Introna *et al.*, 1990; Wallman & Donnellan, 2001). Although *Calliphora* spp. do not appear to be of major forensic importance in southern Africa, they are occasionally encountered in protected corpses (Mansell, pers. comm.) and *C. croceipalpis* maggots have been recovered from small mammal and bird carcasses in Grahamstown (pers. obs.). This fly appears to be more common in late winter and spring (July to October) in Grahamstown (Williams, pers. comm.), and was absent from December to April in the Highveld (Meskin, 1986). *Calliphora vicina*, due to its current localized distribution, is likely to be less commonly encountered than *C. croceipalpis*.

1.4.2 - Subfamily Luciliinae

The Luciliinae was previously treated as a tribe of the Calliphorinae (e.g. Zumpt, 1956; Pont, 1980; Shewell, 1987) but was promoted to subfamily status by Rognes (1991). The subfamily is characterised by bare parafaciala, a setulose metakapepisternum, the elongate and entire ST8 of the ovipositor and a posteriorly positioned, metallic, setose sclerite on the suprasquamal ridge (Rognes, 1997a).

In the Afrotropical region, the genera *Hemipyrellia* Townsend and *Lucilia* Robineau-Desvoidy represent the subfamily. The two genera are easily confused (Appendix A) (Zumpt, 1956). *Lucilia* species are of forensic and medical or veterinary interest (Meskin, 1986), and *Hemipyrellia* species are cutaneous myiasis agents of wild animals (Pont, 1980), although *H. fernandica* has been found in low densities in carcasses (Meskin, 1986). There are four species of *Hemipyrellia* in the region, two of which are confined to tropical islands (Pont, 1980).

It is believed that the genus *Lucilia* is of Palaearctic origin, but increased its range and subsequently underwent further radiation in new habitats (Stevens & Wall, 1996). Four species of *Lucilia* are found in the Afrotropics, although *L. nigrocoerulea* Macquart is confined to Réunion, and *L. infernalis* Villeneuve tends to be more common in northern, West and East Africa (Pont, 1980). *Lucilia cuprina* Wiedemann and *L. sericata* Meigen are of economic interest as causative agents of cutaneous myiasis in sheep throughout the world (Hepburn, 1943a,b; Ullyett, 1945; Waterhouse & Paramonov, 1950; Zumpt, 1956; Vogt & Woodburn, 1979; Pont, 1980; Smith *et al.*, 1981; Stevens & Wall, 1995), in addition to being forensically important (Introna *et al.*, 1989; Bourel *et al.*, 1999; Grassberger & Reiter, 2001). In southern Africa, *L. sericata* tends to be more common in carrion than *L. cuprina* (pers. obs.) and is thus likely to be a more important forensic fly. Both species have extended their ranges to the extent that they are now virtually cosmopolitan, but *L. sericata* is of Palaearctic origin and *L. cuprina* originated in the Aftrotropical or Oriental region (Stevens & Wall, 1997a,b).

1.4.3 - Subfamily Chrysomyinae

Shewell (1981) grouped the Rhiniini, Phormiini and Chrysomyini in the subfamily Chrysomyinae. However, Rognes (1991) argued that the defining character of this taxon - a row of setae on the stem vein - might have evolved independently in the Rhiniini and Chrysomyini/Phormiini. Further, he raised doubts about the validity of Phormiini and Chrysomyini, since *Phormia* Robineau-Desvoidy 1830 is apparently more closely related to the latter tribe (Rognes, 1991). He therefore split the subfamily (*sensu* Shewell, 1987) into the Rhiniinae and Chrysomyinae and sank the chrysomyine tribes, subject to revision with future phylogenetic analysis. Such additional analysis has not to my knowledge been published, so for the sake of simplicity, Rognes' (1991) classification will be maintained.

In the Afrotropical region, the Chrysomyinae is represented by the genera *Chrysomya* Robineau-Desvoidy and *Chrysopyrellia* Séguy. The latter genus is confined to Madagascar and is not included in this analysis. *Chrysomya* historically has an Old World distribution, although a few species (see below) were introduced into, and have become established in the Americas since the 1970s and 1980s (Baumgartner & Greenberg, 1984; Wells & Kurahashi, 1994; Wells & Sperling, 1999, 2001).

Chrysomya species are the dominant necrophagous flies in South Africa, and are therefore frequently used by forensic investigators (Mansell, pers. comm.). The most commonly encountered flies in the forensic context tend to be *Chrysomya albiceps* (Wiedemann) and *C. chloropyga* (Wiedemann), but *C. megacephala* (Fabricius) and *C. marginalis* Wiedemann also occur in low densities (Mansell, pers. comm.). Three southern African species of *Chrysomya* are not of known forensic importance, although it is possible that adult female *C. inclinata* Walker and *C. laxifrons* Villeneuve have been confused with *C. marginalis* in forensic collections. Certainly, *C. inclinata* is present in low densities in the Grahamstown area, but although the females are attracted to rotting meat, they do not oviposit on this medium in the laboratory (pers. obs.). The Old World screw-worm blowfly (*Chrysomya bezziana* Villeneuve), which is morphologically similar to, and sympatric with, the forensically significant *C. megacephala* is an obligate mammalian parasite and is therefore not encountered in the forensic context (Zumpt, 1956; Baker *et al.*, 1968).

Chrysomya albiceps is distributed throughout Africa and into north-west India (Pont, 1980). It has also fairly recently been introduced into, and become established in South America (Baumgartner & Greenberg, 1984) and North America (Wells & Sperling, 2001).

Chrysomya putoria (Wiedemann) (the tropical latrine blowfly) and *C. chloropyga* (the coppery-tailed blowfly) have been synonymised in the past (e.g. Zumpt, 1956). Although potentially sympatric throughout their ranges, the past confusion over the validity of species-status of *C. putoria* (e.g. Zumpt, 1956; Pont, 1980) may have introduced error into the known distributions of both species. From trapping records throughout southern Africa and in Kenya, *C. putoria* appears to be more common in the tropics and subtropics, whilst *C. chloropyga* is commonly encountered in the subtropics and temperate zones of South Africa (pers. obs.). *Chrysomya putoria* prefers to oviposit on faeces (Zumpt & Patterson, 1952) although this fly does breed on decomposing meat in the laboratory (see chapter 3) and has occasionally been found in carcasses in the Grahamstown area (pers. obs.).

Chrysomya megacephala is a widespread fly, being found throughout Africa, the Oriental region, Australasia and the Pacific islands (Pont, 1980). Like *C. chloropyga* and *C. albiceps*, *C. megacephala* has been introduced to, and become established in the Americas (Pont, 1980; Baumgartner & Greenberg, 1984; Wells & Sperling, 2001).

Chrysomya marginalis is also fairly widespread, being commonly encountered throughout Africa, Madagascar and Pakistan (Pont, 1980). From personal observations and literature inferences (e.g. Braack, 1981, 1984, 1986, 1987; Meskin, 1986), *C. marginalis* appears to prefer large animal carcasses.

1.5 - Aims and scope

The ultimate objective of this study is to provide practical information for use in forensic investigations, although much of the information included will also have applications in other fields of study, such as medical and veterinary entomology, physiology, systematics and biogeography. To this end, the major aims of this study are itemised below:

i) To aid in the identification of species by providing molecular sequences and a morphological key to the adults of the southern African region.

ii) To elucidate systematic relationships of calliphorid genera and species, primarily those in the subfamilies Luciliinae, Calliphorinae and Chrysomyinae.

iii) To provide developmental data at a range of temperatures for four species of necrophagous Calliphoridae, *viz. Chrysomya chloropyga* Wiedemann, *Chrysomya putoria* Wiedemann, *Chrysomya megacephala* Fabricius and *Lucilia sericata* Meigen. Although some data for *Chrysomya albiceps* Wiedemann and *Chrysomya* *marginalis* Wiedemann are reported, difficulties involved in maintaining laboratory colonies precluded the comprehensive inclusion of these species.

iv) To provide a model for the prediction of developmental parameters (such as thermal thresholds and developmental constants) using systematic relationships.

This study focuses only on the southern African necrophagous Calliphoridae for all developmental, behavioural and morphological work, but extra-limital taxa, and taxa that have different feeding strategies have been included in the systematic studies for completeness of context. No attempt has been made to confirm controlled laboratory development experiments with field experiments in this study, since the major objective was to produce a baseline for further research and refinement. However it is believed that it will be possible to extrapolate the majority of the information provided in this thesis to real conditions.

Chapter 2

Phylogenetic Relationships Of Forensically Important Calliphoridae

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2.0 - Introduction

The higher-level systematics of the Calliphoridae is contentious. Historically, the family has been divided into anything from two to twelve subfamilies depending on authors' interpretations of uniting characters (e.g. Zumpt, 1956; Pont, 1980; Rognes, 1991; 1997a; Shewell, 1987). Rognes (1991), in his catalogue of the Scandinavian blowflies, refuted the monophyly of the diverse Calliphorinae (*sensu* Shewell, 1987; Zumpt, 1956), and split the subfamily into several putatively monophyletic subfamilies. Similarly, he removed the Rhiniini from the Chrysomyinae (*sensu* Shewell, 1987) and accorded it subfamilial status. He argued that the uniting character of the Calliphorinae (*sensu* Shewell, 1987; Zumpt, 1956) - the absence of setae on the dorsal surface of the stem vein - is plesiomorphic, and is not valid in a phylogenetic sense, and that the setulose stem vein of the Chrysomyinae (*sensu* Shewell, 1987) may have multiple origins (Rognes, 1997a). I agree with his view, and have adopted Rognes' (1991; 1997a,b) classification for the purposes of this paper.

A recent cladistic analysis of the Oestroidea indicated that the Calliphoridae is not monophyletic (Rognes, 1997a). However, because little has been published on the higher systematics of the Calliphoridae since Rognes' treatment, and since he used composite taxa (which implicitly assumed that the taxon groups that he used in his classifications were monophyletic), the final verdict is still wanting. Several recent lower-level phylogenetic analyses have been undertaken by a variety of authors (e.g. Rognes, 1997b; Stevens & Wall, 1996; 1997a,b; 2001; Wallman & Adams, 1997, Wallman & Donnellan, 2001; Wells *et al.*, 2001). However, most authors have focused primarily on the Nearctic and Palaearctic taxa (e.g. Stevens & Wall, 2001) although Wells and Sperling (2001) included several African taxa that have been introduced to the Nearctic region in their analysis of North American blowflies, and Wallman and Adams (1997) and Wallman and Donnellan (2001) concentrated on Australian taxa. As a result, little is known about the relationships between the Afrotropical Calliphoridae, despite the region's rich fauna (Zumpt, 1956).

In recent years, there have been several publications of molecular sequence data of calliphorids, especially focusing on infrageneric and infratribal relationships (Malgorn & Coquoz, 1999; Stevens & Wall, 1997a; Wallman & Adams, 1997; Wells & Sperling, 1999; 2001), but to date, no comprehensive molecular phylogeny of the family has been produced. Despite producing cladograms, Wells & Sperling (2001) and Wallman & Donnellan (2001) focused on the use of molecular data for identification rather than to produce phylogenies *per se*. Besides the obvious benefits of using molecular sequence data for the identification of problematic species or damaged specimens, it is also of great importance to understand the relationships of taxa to produce a natural classification and to predict likely behavioural and life-history tactics. The objective of this study is to produce a robust molecular phylogeny of the Calliphoridae, albeit with forensic and African biases.

2.1 - Methods

2.1.1 - Taxon sampling

Calliphorids from throughout southern Africa were collected in liver-baited traps or at carcasses and preserved whole in 95% ethanol. In some instances, more than one representative of each species was sequenced if flies from different localities were available. Male flies were used preferentially because identification keys for males are more lucid. However, in several instances, only female specimens were obtained from bait traps and these were used if their identities were unambiguous. The genitalia of the specimens sequenced in this study were cleared and mounted on slides or preserved in ethanol as vouchers, and are retained in the Entomology Department at Rhodes University.

Sequences of additional taxa were downloaded from GenBank (Table 2.1). These included members of the families Muscidae, Sarcophagidae, and Oestridae, which served as adjuncts to the outgroup (*Musca domestica*) in case taxon sampling became a problem.

2.1.2 - DNA extraction

Total cellular DNA was extracted from ethanol-preserved muscle tissue. Approximately $2mm^3$ of wing muscle were dissected from flies, macerated with a sterile scalpel blade, and placed in a Chelex ®100 extraction solution (150µl 20% Chelex 100, 450µl buffer containing 10mM Tris-HCl, 1mM EDTA and 0.1% sodium azide at pH=8). The samples were incubated at 60°C for one to two hours and then denatured at 95°C for 15 minutes. After a one-minute centrifugation at 14000 rpm, the supernatant was removed and aliquoted into two 200µl samples. The DNA extracts were frozen at -10°C until required.

In some instances, inadequate quantities of DNA were acquired from Chelex extractions, and in those cases, a modified hot CTAB (hexadecyltrimethylammonium bromide) extraction method was used (Doyle & Doyle, 1990). About $2mm^3$ of muscle tissue were ground with a pestle and mortar, in 600µl CTAB isolation buffer (3% CTAB, 1.4M NaCl, 0.2% 2-mercaptoethanol, 20mM EDTA, 100mM Tris-HCl, pH = 8.0) and incubated at 60°C for one to two hours. An equal volume of phenol/chloroform-isoamyl alcohol (25:24:1) was then added, and the sample was shaken for 2 minutes before a 10-minute centrifugation at 10000 rpm. The supernatant was retained, combined with 600µl of chloroform-isoamyl alcohol (24:1), shaken briefly, and spun for a further 10 minutes at 10000 rpm. Thereafter, the aqueous phase was removed and added to two volumes of cold absolute ethanol and 7.5M ammonium acetate, and the sample was incubated overnight at -5°C to precipitate the DNA. After a final centrifugation at 10000 rpm, the DNA pellet was washed with 70% isopropanol, air-dried and resuspended in sterile water.

2.1.3 - DNA amplification

A region of the Cytochrome Oxidase subunit 1 gene (COI), approximately 500 base pairs (bp) long was amplified by means of PCR in the region between positions 1718 and 2191 of the *Drosophila yakuba* genome, using the primers C1-J-1718 and C1-N-2191 (Simon et al., 1994). COI was used because it has been found to adequately resolve species-level relationships (e.g. Sperling *et al.*, 1994; Wells & Sperling, 2001). (The 50µl reactions comprising 5µl template, 5µl 10x buffer, 0.34nM each primer, 100µM dNTP, 1 unit *Taq* polymerase (Promega) and between 1 and 4mM $MgCl_2$ were subjected to 35 cycles of denaturation at 95°C, 45s annealing at 52°C and 3 min extension at 72°C, and a final 10 min extension. All PCR reactions were carried out in a Hybaid PCR Sprint thermocycler. Amplification efficiency was checked by electrophoresing 10µl of amplified product on a 1% agarose gel stained with ethidium bromide. 200µl amplified product were acquired prior to DNA purification.

2.1.4 - DNA purification

Amplified DNA was purified using a QIAquick kit and protocol (QIAgen). However, the DNA was eluted with 20µl of water rather than the manufacturer's specification of 40µl.

2.1.5 - Sequencing

Cycle sequencing of the purified PCR products was carried out using an ABI PRISM BigDye Terminator Cycle Sequencing Kit Version 2, and later Version 3 (Perkin-Elmer) following the manufacturer's protocol. Sense and antisense strands were sequenced in all cases. The final sequencing product was precipitated with a 75% solution of isopropanol or with sodium acetate and ethanol, according to the manufacturer's protocols. Sequencing was carried out on an ABI 3100 autosequencer. Sequence traces were edited using Sequencher (Gene Codes Corporation) and aligned by eye using DAPSA (DNA and Protein Sequence Alignment, E. Harley, Department of Chemical Pathology, University of Cape Town, South Africa).

2.1.6 - Analysis

Aligned sequences were imported into PAUP* version 4.0b8 (Swofford, 2001). Because not all of the sequences included in the analysis were of the entire COI gene (Appendix B), the data set was subdivided into three subsets: a conservative set which incorporated only the region that included the southern African sequences (i.e. positions 1 to 701 of the alignment), a semi-conservative set, which included all information from position 1 to position 801, and the complete data set which included all sequence data. The latter two data sets contained large proportions of missing data due to incomplete sequences (Table 2.2). Missing data were coded separately to gaps (Appendix B).

Both maximum parsimony (MP) and Jukes-Cantor neighbor-joining (NJ) analyses were carried out using *Musca domestica* as the outgroup. Maximum likelihood analyses were also run, but the trees were omitted in the results due to poor resolution and bootstrap support, and ambiguous relationships within genera. Maximum parsimony analyses were done using simple stepwise addition and the tree-bisection-reconnection (TBR) branch-swapping algorithm. Uninformative characters were excluded. Branches of zero length were collapsed, and the MulTrees option was maintained. Node support was established by 1000 full heuristic bootstrap replicates.

2.2 - Results

Of the total coding fragment of 2322 bases, 1741 bases were uninformative, 1511 of which were constant across all taxa. Of the 168 informative characters in the conservative data set, 37, 3 and 128 were from the first, second and third codon positions, respectively. To allow for the consistency between data sets to be determined, data sets within analytical methods (MP or NJ) were compared.

2.2.1 - Maximum Parsimony (MP) analysis

Although MP analysis of the semi-conservative data set produced the fewest trees (Table 2.2), the support statistics for the trees were lower than those produced for the conservative data set's analysis (Table 2.2). The consistency and retention indices were low (< 0.4 and 0.6 respectively) in the analyses of all three data sets, which suggest that there may be high levels of homoplasy in the data set. However, as indicated by the common nodes on each consensus tree (Fig. 2.1.1 – 2.1.2), there was a large number of clades that were consistently extracted by MP analysis. This indicates that there is a strong underlying phylogenetic signal; much of the homoplasy may be accounted for by the problematic taxa discussed below. Despite a large number (>10) of trees being produced by MP analysis of each of the three data sets, the strict consensus trees were fairly well resolved at the genus level and node collapse occurred primarily at terminals in the genus *Chrysomya* (Fig. 2.1.1 - 2.1.3). To facilitate comparisons of distinct clades across trees, and to highlight nodal disparities at lower phylogenetic levels, each family and subfamily has been treated separately below.

i) Sarcophagidae

All of the consensus trees had some deeper nodes that were intuitively problematic (i.e. differed widely from accepted monophyletic groups). Firstly, the Sarcophagidae was consistently paraphyletic, with *Brachicoma* basal to *Gasterophilus* (Oestridae: Gasterophilinae) and *Wohlfahrtia* (Fig. 2.1.1 - 2.1.3). The latter sister-group had moderate bootstrap support in the analysis of the conservative and semi-conservative data sets (61% and 66% in Fig. 2.1.1; 2.1.2 respectively). The *Brachicoma-Gasterophilus-Wohlfahrtia* clade was distinct from the remainder of the Sarcophagidae in all but the analysis of the conservative data set (Fig. 2.1.1). Parsimony analysis of the semi-conservative data set placed the clade basal to the Luciliinae-Calliphorinae clade (Fig. 2.1.2), and analysis of the total data set extracted the clade as a basal group to the Calliphoridae and the remainder of the Sarcophagidae (Fig. 2.1.3). However, none of these relationships received bootstrap support >50% (Fig. 2.1.1 - 2.1.3).

Apart from the positions of *Wohlfahrtia* and *Brachicoma*, the relationships of the remainder of the sarcophagids were similar in all three consensus trees as evidenced by the

25 comparable nodes on each tree (Fig. 2.1.1 - 2.1.3), but the resolution and bootstrap support were greatest in the tree derived from the semi-conservative data set (Fig. 2.1.2). Within the main sarcophagid group, *Peckia chrysostoma* was embedded in the genus *Sarcophaga* in the analysis of the conservative data set, but this placement had bootstrap support <50% (Fig. 2.1.1). From analysis of the other two data sets, the basal position of *Peckia* to *Sarcophaga* had poor to moderate bootstrap support of 59% and 62% (Fig. 2.1.2; 2.1.3).

Within *Sarcophaga*, the sister groupings of *S. ruficornis* and *S. crassipalpis* had strong bootstrap support (70-93%), while *S. bullata* and *S. cooleyi* were extracted as sister taxa with support values of 87% and 88% in the analysis of the conservative and semi-conservative data sets (Fig. 2.1.1; 2.1.2). The positions of *S. peregrina* and *S. africa* within *Sarcophaga* varied with the data sets, and their positions were not well supported by bootstrapping (Fig. 2.1.1-2.1.3).

ii) Calliphorinae and Luciliinae

The analysis of each of the three data sets produced identical luciliine and calliphorine topologies, although the bootstrap support values for the terminal nodes in the Calliphorinae were variable (Fig. 2.1.1 - 2.1.3); only in the analysis of the semi-conservative data set was there >50% bootstrap support for the arrangement *Calliphora* (*Eucalliphora, Cynomyopsis*) (Fig. 2.1.2). The extraction of the two subfamilies (Luciliinae and Calliphorinae) as sister groups had weak (51%) to moderate (68%) bootstrap support in the analysis of the semi-conservative and complete data sets, respectively (Fig. 2.1.2; 2.1.3), but this arrangement was not supported by the bootstrap analysis of the conservative data set (Fig. 2.1.1).

In all cases, *Lucilia illustris* was basal to the remainder of the genus, and there was poor (57%) to good (83%) support for this species' position in the semi-conservative and complete data sets' consensus trees (Fig. 2.1.2; 2.1.3), but <50% bootstrap support in the analysis of the conservative data set (Fig. 2.1.1). In the analysis of all three data sets, there was strong support (84% to 96%) for the sister grouping of *L. sericata* and *L. cuprina* (Fig. 2.1.1 - 2.1.3).

iii) Chrysomyinae

The generic relationships within the Chrysomyinae differed depending on the data set analysed. In all cases, *Chrysomya, Phormia* and *Protophormia* were extracted together, but the relative positions of the three genera varied (Fig. 2.1.1 - 2.1.3). In the analysis of the complete data set, the consistent *Phormia-Protophormia* clade was sister to *Chrysomya* (Fig. 2.1.3), but the analyses of the other two data sets resulted in a paraphyletic *Chrysomya. Chrysomya norrisi* was sister to *Phormia* and *Protophormia* in both the conservative and semi-conservative trees (Fig. 2.1.1; 2.1.2), and this clade was embedded within *Chrysomya* in the analysis of the semi-conservative data set (Fig. 2.1.2) and sister to *Chrysomya* in the analysis of the conservative data set (Fig. 2.1.1). Bootstrap support within the Chrysomyinae was generally poor (<50%), and only some terminal nodes (uniting conspecifics or previously synonymised congenerics) had >60% bootstrap support (Fig. 2.1.1 - 2.1.3).

The positions of the genera *Cochliomyia, Protocalliphora* and *Compsomyiops* varied dramatically depending on the data set analysed (Fig. 2.1.1 - 2.1.3). In all three cases, the positions of one or all of these genera rendered the Chrysomyinae paraphyletic (Fig. 2.1.1-2.1.3). Parsimony analysis of the conservative data set resulted in an unresolved basal polytomy including *Compsomyiops, Cochliomyia* and the remainder of the ingroup. (Fig. 2.1.1). The result of the analysis of the semi-conservative data set was that all three genera were removed from the Chrysomyinae; *Protocalliphora* and *Compsomyiops* were basal to the oestrid clade (*Hypoderma-Ovis*), while *Cochliomyia* was basal to the Sarcophagidae (Fig. 2.1.2). However, bootstrap support values for the positions of these genera in both cases were <50%. The analysis of the complete data set also yielded a paraphyletic Chrysomyinae, but in this case only *Protocalliphora* was removed from the chrysomyine clade; *Compsomyiops* and *Cochliomyia* were extracted as sister taxa (bootstrap support 89%) and were a sister clade to the *Phormia-Protophormia-Chrysomya* clade (Fig. 2.1.3).

Within *Chrysomya*, poor to moderate bootstrap support (59% to 76%) was found for the sister-groupings of *C. chloropyga* and *C. putoria* together with the South American "*C. chloropyga putoria*" which is most probably *C. putoria*. Similarly, the morphologically similar *C. rufifacies* and *C. albiceps* were consistently extracted as sister species, and bootstrap support for this grouping was >98% in all trees (Fig. 2.1.1 - 2.1.3). Despite being present in all three MP trees, the sister species relationship of *C. bezziana* and *C. megacephala* only had moderate bootstrap support (62%) in the analysis of the complete data set (Fig. 2.1.3); this node received <50% bootstrap support in the analyses of the other two data sets (Fig. 2.1.1; 2.1.2).

2.2.2 - Jukes-Cantor Neighbor Joining (NJ) analyses

The topologies of the neighbor-joining trees produced from the analysis of the three data sets were superficially similar to the MP consensus trees, especially within genera. However, several important differences were evident in the NJ trees, primarily in the placements of genera within subfamilies.

i) Sarcophagidae

The NJ analysis of all three data sets produced a paraphyletic Sarcophagidae, with *Gasterophilus* embedded within the family as a sister genus to *Wohlfahrtia* (Fig. 2.2.1 - 2.2.3). This arrangement had >60% support in the conservative and semi-conservative bootstrap consensus trees (Fig. 2.2.1; 2.2.2). However, the phylograms show that *Gasterophilus* is positioned on a long branch, which may explain its unexpected placement. The analysis of the conservative data set yielded the clade *Brachicoma-Ravinia-Gasterophilus-Wohlfahrtia* that was sister to *Sarcophaga, Peckia* and *Blaesoxipha* (Fig. 2.2.1). In the analysis of the semi-conservative and complete data sets, the *Brachicoma-Wohlfahrtia-Gasterophilus* clade formed a sister clade (along with the oestrids, *Hypoderma* and *Oestrus*) to the remainder of the Sarcophagidae (Fig. 2.2.1 - 2.2.3). In none of these cases did bootstrap support exceed 50%. The analysis of the complete data set placed the chrysomyine *Protocalliphora* basal to the oestrid-paramachronychiine sarcophagid clade, but this arrangement was not supported by bootstrapping (Fig. 2.2.3).

The relationships within the main sarcophagid group, as seen from the analysis of the semi-conservative and complete data sets, were identical to the corresponding MP consensus trees (Fig. 2.1.2; 2.1.3; Fig. 2.2.2; 2.2.3). The conservative data set yielded a paraphyletic *Sarcophaga*, but whereas *Peckia* rendered the genus paraphyletic in the MP analysis of this data set, *Blaesoxipha* had a similar effect in the NJ analysis. With the exception of the trees derived from the MP and NJ analyses of the conservative data set (Fig. 2.1.1; Fig. 2.2.1), both of which had few supported sarcophagine nodes, the bootstrap support values for the Sarcophaginae in the NJ trees were greater than the corresponding MP trees (Fig. 2.1.2; 2.1.3; Fig. 2.2.2; 2.2.3).

ii) Luciliinae and Calliphorinae

The Luciliinae and Calliphorinae were extracted as sister taxa, as in the MP analysis, and this grouping had good bootstrap support of 84% to 94% (Fig. 2.1.1 –2.1.3; Fig. 2.2.1 – 2.2.3). As in the MP analysis, the Calliphorinae-Luciliinae clade was sister to the Sarcophaginae, albeit with non-existent (<50%) bootstrap support (Fig. 2.2.1 – 2.2.3).

In the analyses of the conservative and complete data sets, the Calliphorinae and Luciliinae were monophyletic; by contrast, analysis of the semi-conservative data set resulted in a paraphyletic Calliphorinae, with *Calliphora vicina* basal to the luciliine clade (Fig. 2.2.2). In all three NJ trees, *C. vicina* was on a long branch and its placement varied (Fig. 2.2.1 – 2.2.3). However, the analyses of the complete and semi-conservative data sets placed *Eucalliphora* and *Cynomyopsis* as sister genera, with moderate (61%) bootstrap support in both cases (Fig. 2.2.2; 2.2.3). The complete data set's NJ tree placed *Calliphora* basal to this clade, with poor bootstrap support (53%) (Fig. 2.2.3) whilst the *Eucalliphora* (*Calliphora, Cynomyopsis*) arrangement in the conservative tree was not supported by bootstrapping (Fig. 2.2.1).

Within *Lucilia* the branch-lengths were short, especially between conspecifics (Fig. 2.2.1 - 2.2.3). The basal position of *Lucilia illustris* to the remainder of *Lucilia* had moderate to good (62% to 84%) bootstrap support, and the majority of the remaining luciliine nodes had >70% bootstrap support in all trees (Fig. 2.2.1 - 2.2.3).

iii) Chrysomyinae

Neighbor-joining produced a monophyletic Chrysomyinae in the analyses of the conservative and semi-conservative data sets, although bootstrap support was below 50% in both cases (Fig. 2.2.1 - 2.2.2). By contrast, the analysis of the complete data set resulted in *Protocalliphora* being basal to the oestrid-paramachromyiine sarcophagid clade but the monophyly of the remainder of the Chrysomyinae was reasonably well supported by bootstrapping (71%) (Fig. 2.2.3). In all three NJ trees, the genera *Compsomyiops* and *Cochliomyia* were basal to the remainder of the clade, and these two genera were consistently extracted as sister genera with variable bootstrap support - <62% to 99% (Fig. 2.2.1 – 2.2.3).

The positions of *Phormia* and *Protophormia* relative to each other and *Chrysomya* varied between trees (Fig. 2.2.1 – 2.2.3). The two *Phormia regina* specimens were always placed together (bootstrap support 100%), and *Phormia* and *Protophormia* were extracted as sister genera in the conservative (bootstrap support <50%) and complete (bootstrap support 77%) NJ trees (Fig. 2.2.1 – 2.2.3). In both cases, the *Phormia-Protophormia* clade was embedded in *Chrysomya* by virtue of *Chrysomya semimetallica* being basal to the entire clade (Fig. 2.2.1; 2.2.3). The analysis of the semi-conservative data set placed *Phormia* as sister to *Protocalliphora*, and *Protophormia* sister to *Chrysomya norrisi* within the main *Chrysomya* clade (Fig. 2.2.2).

In all three NJ trees, the common nodes within the *Chrysomya* clade tended to have reasonable bootstrap support (Fig. 2.2.1 – 2.2.3). *Chrysomya bezziana* was extracted as sister to *C. megacephala* in the conservative and semi-conservative NJ trees, although this arrangement was only supported by bootstrapping (71%) in the former tree (Fig. 2.2.1 – 2.2.2). The analysis of the complete data set placed *C. bezziana* basal to the remaining Chloropyga species (Fig. 2.2.3).

Notably, the hairy-maggot blowflies, *C. albiceps* and *C. rufifacies* were consistently extracted as sister species (bootstrap support 85% to 100%), and the morphologically-similar *C. chloropyga* and *C. putoria* were placed together, with reasonable to good bootstrap support (64% to 94%) (Fig. 2.2.1 – 2.2.3). *Chrysomya inclinata* and *Chrysomya marginalis* were also consistently grouped as sister species (86% to 94%) and placed as a sister clade to the *C. chloropyga* and *C. putoria* clade (Fig. 2.2.1 – 2.2.3).

2.3 – Discussion

2.3.1 – Familial and subfamilial relationships in the Oestroidea

With the exception of the *Gasterophilus-Wohlfahrtia* grouping, all of the nodes that had good bootstrap support were congruent with previous studies (e.g. Rognes, 1991 for Calliphoridae; Stevens & Wall, 1996; 1997b for *Lucilia*; Wells *et al.*, 2001 for Sarcophagidae; Wells & Sperling, 2001 for Chrysomyinae). At the higher levels, the results from this study mirror some of the problems encountered by previous authors with respect to the monophyly of families and subfamilies within the Oestroidea (e.g. Rognes, 1997a). None of the trees produced in this study were entirely unambiguous, although most of them had elements that converged on a likely scenario.

As a result of the consistent sister-grouping of the Luciliinae-Calliphorinae with the Sarcophagidae, the Calliphoridae was rendered paraphyletic, regardless of the data set or analytical method considered. This grouping supports the classification of the Sarcophagidae as a subgroup within the Calliphoridae, as followed by Zumpt (1956), and reinforces Rognes' (1997a) proposal that the Calliphoridae is paraphyletic and that the Oestroidea requires revision. However, bootstrap support for the sister status of Sarcophaginae and Calliphorinae-Luciliinae was low in all cases, and further investigation including more data and/or more taxa is required to determine if this arrangement is realistic or a result of homoplasy. By contrast, analysis of morphological characters resulted in the Oestridae instead of the Sarcophagidae being embedded within the Calliphoridae (s.l.), and the Sarcophagidae was sister to the Rhiniinae (Rognes, 1997a). Unfortunately, molecular data for many of the taxa included in Rognes' (1997a) analysis were not included in this study, so the position of the Oestridae with respect to the entire Calliphoridae could not be unambiguously determined.

The results of this study also cast doubt on the monophyly of both the Oestridae and Sarcophagidae, since the gasterophiline *Gasterophilus* consistently grouped with the paramachronychiine sarcophagids *Brachicoma* and *Wohlfahrtia*. Wells *et al.* (2001), in their analysis of the Sarcophagidae, showed that the Paramachronychiinae was basal to the Sarcophaginae, with the Oestridae outgroup to the Sarcophagidae. This suggests that the placement of the Paramachronychiinae and *Gasterophilus* as basal to the Sarcophaginae (not Oestridae) is the most likely arrangement (Fig. 2.1.1; 2.1.2). Long branch attraction may account for the grouping of *Gasterophilus* with *Wohlfahrtia* and *Brachicoma* in all the trees, since the rapid evolution (or lack of close relatives in the ingroup) of *Gasterophilus* and *Wohlfahrtia* could result in them both being on long branches, and *Gasterophilus* would be attracted to the latter genus because of this artefact (Philippe, 2000). Random inclusion of taxa in the study may also account for this arrangement, thus, the inclusion of more taxa may produce a more intuitively correct tree. However, if *Gasterophilus* continues to be grouped with the paramachronychiine sarcophagids when other sources of data are analysed (e.g. different genes, or a combination of data) and more gasterophiline taxa are included, then the status of both families will require further scrutiny.

Sarcophaga was monophyletic in all but the MP and NJ analyses of the conservative data set (Fig. 2.1.1; 2.2.1), where *Peckia* and *Blaesoxipha*, were embedded in the genus. However, in the remainder of the trees (Fig. 2.1.2; 2.1.3; Fig. 2.2.2; 2.2.3), the relationships were congruent with those produced by Wells *et al.* (2001) – (*Ravinia* (*Blaesoxipha* (*Peckia* ((*S. bullata, S. cooleyi*) *S. africa, S. peregrina*) ((*S. crassipalpis, S. ruficornis*) *S. argyrostoma*)))). In addition, the subgeneric classification of *Sarcophaga* (Pape, pers. comm.) – *S. (Liopygia) argyrostoma, S. (L.) ruficornis, S. (L.) crassipalpis, S. (Neobellieria) bullata, S. (N.) cooleyi, S. (Boettcherisca) peregrina* and *S. (Bercaea) africa* - was reflected in the trees produced by the semi-conservative and complete data sets (Fig. 2.1.2; 2.1.3; Fig. 2.2.2; 2.2.3).

2.3.2 – Relationships within the Calliphoridae

The Chrysomyinae was not consistently monophyletic, but this may have been an artefact of taxon sampling at the generic level. The three genera *Compsomyiops, Cochliomyia* and *Protocalliphora* were problematic in the parsimony analyses of all three data sets, but the NJ analyses of the conservative and semi-conservative data sets produced a monophyletic Chrysomyinae. Wells & Sperling (2001), using MP analysis of the COI, tRNA-Leucine and COII genes, produced a similar result to the NJ analyses of the conservative data set (Fig. 2.2.1), with *Protocalliphora, Compsomyiops* and *Cochliomyia* basal to *Phormia, Protophormia* and *Chrysomya.* The bootstrap support within the subfamily was low in the MP analyses; only the groupings of sister species were well supported, and the closely related *Protophormia* and *Phormia* were also consistently allied (Fig. 2.1.1 - 2.1.3; 2.2.1 - 2.2.3), as found by Wells & Sperling (2001). Rognes (1991) stated that the preservation of the tribe Phormiini was probably invalid, since examination of aedeagal and ovipositor morphology suggested that *Phormia* and *Protophormia* are more closely related to *Chrysomya* than some of the other genera in the "Chrysomyini". He therefore suggested that the tribal classification be suspended until further investigations were carried out. Both the NJ and MP analyses of the COI gene in this study, and the MP analysis carried out by Wells & Sperling (2001) support the discontinuation of Phormiini as a formal rank, since *Phormia* and *Protophormia* were either embedded within, or were sister to *Chrysomya,* and the other Chrysomyini were basal to this clade.

Although the African members of *Chrysomya* were well sampled (with seven of the twelve indigenous species included in the study), the infra-generic relationships were generally not well supported by bootstrapping, and the relationships between species were inconsistent. This implies either that random taxon sampling (i.e. insufficient generic representatives in the data set) outside the Afrotropical region is having an effect, that the gene used is limited in its ability to resolve relationships at this level, or that a recent rapid radiation occurred within the genus. The short internal branch lengths evident in the NJ trees (Fig. 2.2.1 - 2.2.3) indicate that rapid radiation is the most likely cause. The COI gene has been successfully used to resolve subfamilial and generic relationships in a variety of insect groups (e.g. Sperling & Harrison, 1994; Gleeson & Sarre, 1997; Wallman & Adams, 1997; Dobler & Müller, 2000; Wells *et al.*, 2001; Wells & Sperling, 2001), which indicates that the choice of gene is unlikely to be the cause of the poor bootstrap support within the genus.

There are several groups of species in the genus *Chrysomya* that exhibit morphological similarities and would be expected to form distinct clades. However, not all of these *a priori* assumptions were met. Based on wing morphology and chaetotaxy (Zumpt, 1956), it would have been expected that *C. marginalis* and *C. inclinata* would be closely allied, but these two species were only extracted as sister species with any support (bootstrap values >84%) in the NJ trees. Only partial sequences were available for either species (*c.* 500bp; Appendix B), so the large number of missing characters in the semi-conservative and complete data sets may account for their ambiguous positions in the MP trees (Fig. 2.1.1 – 2.1.3). The Australian *C. varipes* would similarly be expected to group with the other "hairy maggot" flies, *C. albiceps* and *C. rufifacies*. However, it was repeatedly placed in the same clade as, or basal to, *C. chloropyga* and *C. putoria* (Fig. 2.1.1 – 2.1.3; 2.2.1) or basal to the majority of the *Chrysomya* clade (Fig. 2.2.2 – 2.2.3). Assuming that this arrangement approximates the true specific relationships, it indicates that the cuticular tubercles that produce the "hairy" appearance of maggots, evolved twice in *Chrysomya*.

Although allopatric, the Afrotropical *C. albiceps* and the Oriental-Australasian *C. rufifacies* are morphologically and behaviourally similar both as adults and larvae, and the systematic statuses of the taxa were ambiguous until recently (Wells & Sperling, 1999). In this study, they were consistently extracted as sister species with a divergence of 3.1% across the gene, which is comparable to the divergence between *C. chloropyga* and *C. putoria* (3.5%). The latter pair have also been synonymised in the past (e.g. Zumpt, 1956; Pont, 1980) but have been shown to be distinct by the structure of the genitalia (Rognes, pers. comm.), cross-breeding experiments (Paterson, 1965; Laurence, 1988), oviposition preferences (Hulley, pers. comm.; Meskin, 1986), development rates and temperature optima (Chapter 3, this study) and larval behaviour and puparial structure (Chapter 3, this study).

Indications are that, as in *Lucilia* (Stevens & Wall, 1996; 1997b) the evolution of a parasitic lifestyle occurred more than once in *Chrysomya*. This is evidenced by the fact that the clade incorporating the obligate primary myiasis fly, *C. bezziana*, is either on a separate branch to the other parasitic members of the genus, or is basal to the remainder of the genus (Fig. 2.1.1 - 2.1.3; Fig. 2.2.1 - 2.2.3). The other parasitic species are facultative (usually secondary) myiasis flies (e.g. *C. albiceps, C. rufifacies, C. chloropyga*) and all are morphologically readily distinguishable from *C. bezziana* by their white - rather than orange or brown - prothoracic spiracles (Zumpt, 1956).

The relationships within the Luciliinae in this study mirror those produced by Stevens and Wall (1996; 1997b; 2001). However, as a result of the poor sampling within the Luciliinae and Calliphorinae, the monophyly of both of these taxa cannot be confirmed from this study, but it is evident that the two taxa are distinct. Stevens & Wall (1996; 1997b; 2001) have dealt with some European representatives of both subfamilies using molecular and morphological data, and indications are that, as proposed by Rognes (1991; 1997a), the splitting of the Calliphorinae into several subfamilies is valid.

2.3.3 – Systematic summary and forensic implications

The phylogenies produced by this study are fairly robust, as illustrated by the good level of congruence between trees produced from each of the data sets and analytical methods. Similar results produced by alternative algorithms indicate that the phylogeny is probably close to reality. However, the data sets in this study are by no means independent of one another since they are subsets of the same gene. In addition, gene trees and species trees are not necessarily congruent (de Queiroz *et al.*, 1995). Based on bootstrap support and the relationships of taxa in unambiguously accepted monophyletic groups, the neighbor-joining tree derived from the complete data set (Fig. 2.2.3) is probably the best estimate of calliphorid relationships at this time. However, the paramachronychiine sarcophagid and oestrid relationships were ambiguous in this study. In light of this, the current phylogeny should be tested using a variety of independent sources of data and analytical methods. It has been argued that combining data (rather than measuring congruence between trees) maximizes cladistic parsimony, and will therefore lead to more reliable phylogenies (Nixon & Carpenter, 1996). Often, weak phylogenetic signals that are drowned by homoplasy in individual data sets come to the fore when data are combined (e.g. de Queiroz *et al.*1995; Eernisse & Kluge, 1993; Olmstead & Sweere, 1994; Page, 1996).

To date, a molecular phylogeny incorporating members of all of the forensically important oestroid subfamilies has not been undertaken, and the only recent comprehensive oestroid phylogeny (based on morphological data) was aimed at higher-level relationships and utilized combined characters from a number of species (Rognes, 1997a). In addition, most authors involved in necrophagous insect systematics have focused on using molecular data to identify species within a biogeographical region (e.g. Sperling *et al.*, 1994; Wallman & Adams, 1997; Benecke, 1998; Malgorn & Coquoz, 1999; Wallman & Donnellan, 2001; Wells *et al.*, 2001; Wells & Sperling, 2001) rather than to understand the evolutionary relationships between taxa (e.g. Stevens & Wall, 1996; 1997a,b; 2001; Taylor *et al.*, 1996; Wells & Sperling, 1999). Although this study has been aimed at producing a robust oestroid phylogeny that is not restricted by geography, it has also produced sequence data for eight southern African calliphorids which are known to be, or are potentially, forensically important. Restricting study to a particular geographic region has several drawbacks, the most serious of which is that species are constantly expanding their ranges with human and livestock transport (e.g. Pead, 1910; Wijesundara, 1957a; Vogt & Woodburn, 1979; Baumgartner & Greenberg, 1984; Wells & Sperling, 2001), and misidentifications are possible if investigators are unaware of recent establishments of introduced species.

More comprehensive taxon sampling using a variety of data types will result in more robust (and reliable) phylogenies. Although by no means comprehensive, this study has highlighted the fact that the Oestroidea (and Calliphoridae in particular) requires revision. Further, it illustrates that taxa from different geographic regions may be closely allied, and that the current system of classifying geographic "blocks" of taxa is specious; a global classification system - especially of the forensically important taxa - is necessary.

TABLE 2.1 – Taxon list and sources of data.

Family	Subfamily	Species	Source	Accession Number
Calliphoridae	Calliphorinae	*Cynomyopsis cadaverina*	Wells & Sperling, 2001	AF259505
Calliphoridae	Calliphorinae	*Eucalliphora latifrons*	Wells & Sperling, 2001	AF295557
Calliphoridae	Luciliinae	*Lucilia cuprina*	Gleeson, unpub.	U60008
Calliphoridae	Luciliinae	*Lucilia illustris*	Vincent, unpub.	AF022369
Calliphoridae	Luciliinae	*Lucilia sericata 2*	This study	
Calliphoridae	Luciliinae	*Lucilia sericata*	Sperling et al., 1994	L14947
Calliphoridae	Luciliinae	*Lucilia 17*	This study	
Calliphoridae	Luciliinae	*Lucilia 41*	This study	
Calliphoridae	Luciliinae	*Lucilia 34*	This study	
Calliphoridae	Chrysomyinae	*Cochliomyia macellaria*	Wells & Sperling, 2001	AF295555
Calliphoridae	Chrysomyinae	*Compsomyiops callipes*	Wells & Sperling, 2001	AF295549
Calliphoridae	Chrysomyinae	*Protocalliphora sialia*	Wells & Sperling, 2001	AF295559
Calliphoridae	Chrysomyinae	*Protophormia terraenovae*	Wells & Sperling, 2001	L14946
Calliphoridae	Chrysomyinae	*Protophormia atriceps*	Wells & Sperling, 2001	AH010010
Calliphoridae	Chrysomyinae	*Phormia regina1*	Wells & Sperling, 2001	AF295550
Calliphoridae	Chrysomyinae	*Phormia regina2*	Wells & Wagner, unpub.	AF262956
Calliphoridae	Chrysomyinae	*Chrysomya norrisi*	Wells & Sperling, 2001	AF295552
Calliphoridae	Chrysomyinae	*Chrysomya varipes*	Wells & Sperling, 2001	AF295556
Calliphoridae	Chrysomyinae	*Chrysomya semimetallica*	Wells & Sperling, 2001	AF295562
Calliphoridae	Chrysomyinae	*Chrysomya chloropyga*	This study	
Calliphoridae	Chrysomyinae	*Chrysomya putoria*	This study	
Calliphoridae	Chrysomyinae	*Chrysomya "chloropyga putoria"*	Wells and Sperling, 2001	AF295554
Calliphoridae	Chrysomyinae	*Chrysomya marginalis*	This study	
Calliphoridae	Chrysomyinae	*Chrysomya megacephala1*	Wells & Sperling, 2001	AF295551
Calliphoridae	Chrysomyinae	*Chrysomya megacephala*	This study	
Calliphoridae	Chrysomyinae	*Chrysomya bezziana*	Wells & Sperling, 2001	AF295548
Calliphoridae	Chrysomyinae	*Chrysomya albiceps1*	Wells & Sperling, 1999	AF083657
Calliphoridae	Chrysomyinae	*Chrysomya rufifacies*	Wells & Sperling, 1999	AF083658
Oestridae		*Oestrus ovis*	Otranto *et al.*, unpub.	AF257118

TABLE 2.1 continued – Taxon table and data sources

Family	Subfamily	Species	Source	Accession Number
Oestridae		*Hypoderma bovis*	Otranto *et al.*, unpub.	AF257115
Oestridae		*Hypoderma lineatum*	Otranto *et al.*, unpub.	AF257116
Oestridae		*Przhevalskiana silenus*	Otranto *et al.*, unpub.	AF257119
Oestridae		*Gasterophilus intestinalis*	Otranto *et al.*, unpub.	AF257117
Sarcophagidae		*Brachicoma devia*	Wells, Pape & Sperling, 2001	AF259517
Sarcophagidae		*Wohlfarhtia vigila*	Wells, Pape & Sperling, 2001	AF259516
Sarcophagidae		*Peckia chrysostoma*	Wells, Pape & Sperling, 2001	AF259515
Sarcophagidae		*Sarcophaga africa*	Wells, Pape & Sperling, 2001	AF259508
Sarcophagidae		*Sarcophaga ruficornis*	Wells, Pape & Sperling, 2001	AF259511
Sarcophagidae		*Sarcophaga crassipalpis*	Wells, Pape & Sperling, 2001	AF259510
Sarcophagidae		*Sarcophaga peregrina*	Wells, Pape & Sperling, 2001	AF259509
Sarcophagidae		*Sarcophaga cooleyi*	Wells, Pape & Sperling, 2001	AF259507
Sarcophagidae		*Sarcophaga bullata*	Wells, Pape & Sperling, 2001	AF259506
Sarcophagidae		*Sarcophaga argyrostoma*	Wells, Pape & Sperling, 2001	AF259512
Sarcophagidae		*Blaesoxipha plinthopyga*	Wells, Pape & Sperling, 2001	AF259514
Muscidae		*Musca domestica*	Wells, Pape & Sperling, 2001	AF259518

TABLE 2.2 – Informative character content and maximum parsimony tree statistics for the three data sets.

Data set	**# characters**	**Informative characters**	**# Trees**	**Tree length**	**CI**	**RI**
Conservative	701	168	24	893	0.301	0.591
Semi-conservative	801	248	18	1261	0.297	0.555
Complete	2322	581	78	2353	0.342	0.522

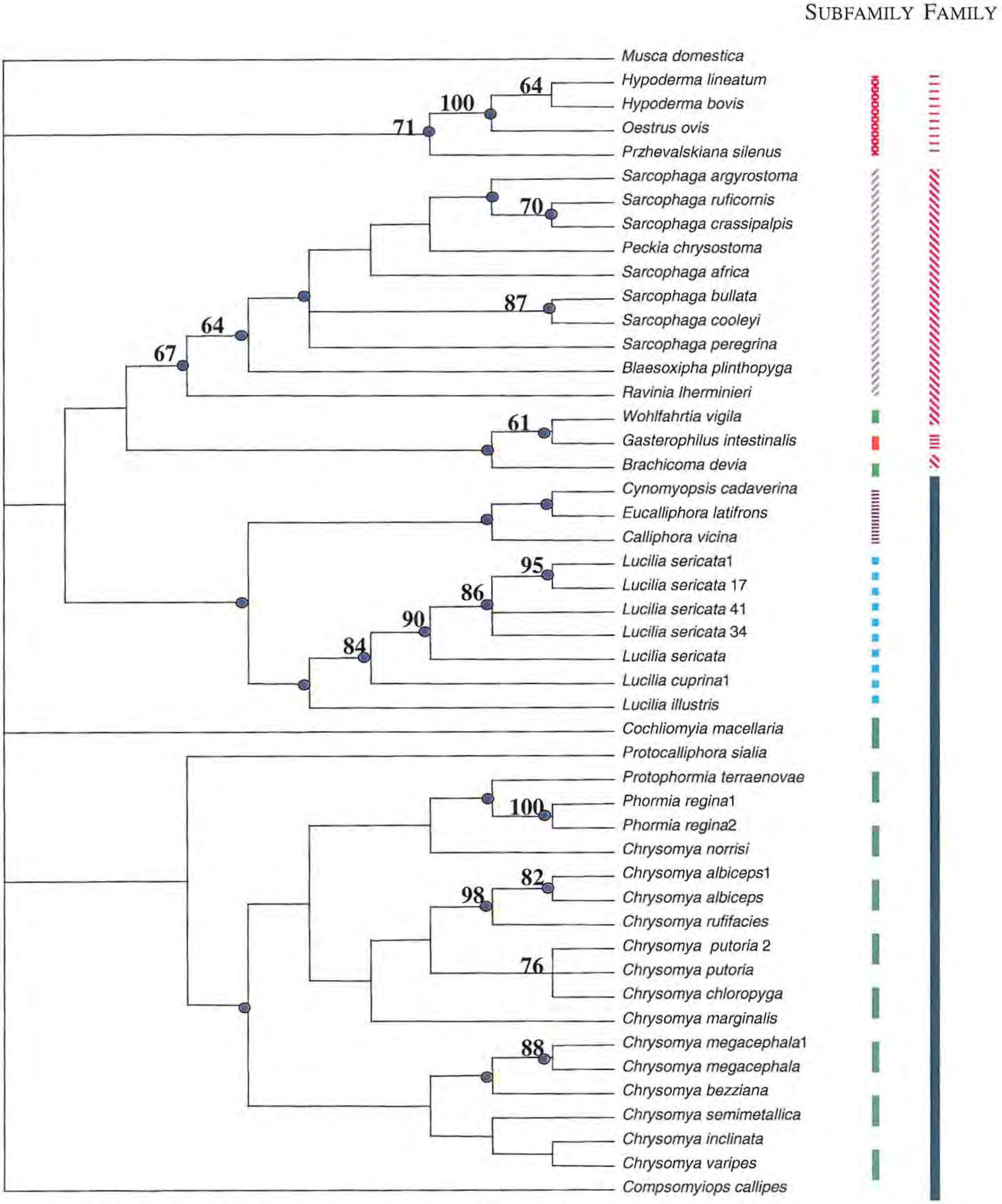

FIGURE 2.1.1 – Strict consensus tree derived from parsimony analysis of the conservative data set. Nodes common to all three MP trees are denoted by ●.
CI = 0.301; RI = 0.591; # Trees = 24.

Subfamily codes: – – Chrysomyinae; ...... Luciliinae; ,,,,,,,,, Calliphorinae;

▬ Oestrinae ▬ Paramachronychiinae; ...... Sarcophaginae; ▬ Gasterophilinae;

Family codes: ▬ Calliphoridae; \\\\\ Sarcophagidae; ,,,,,,,,, Oestridae

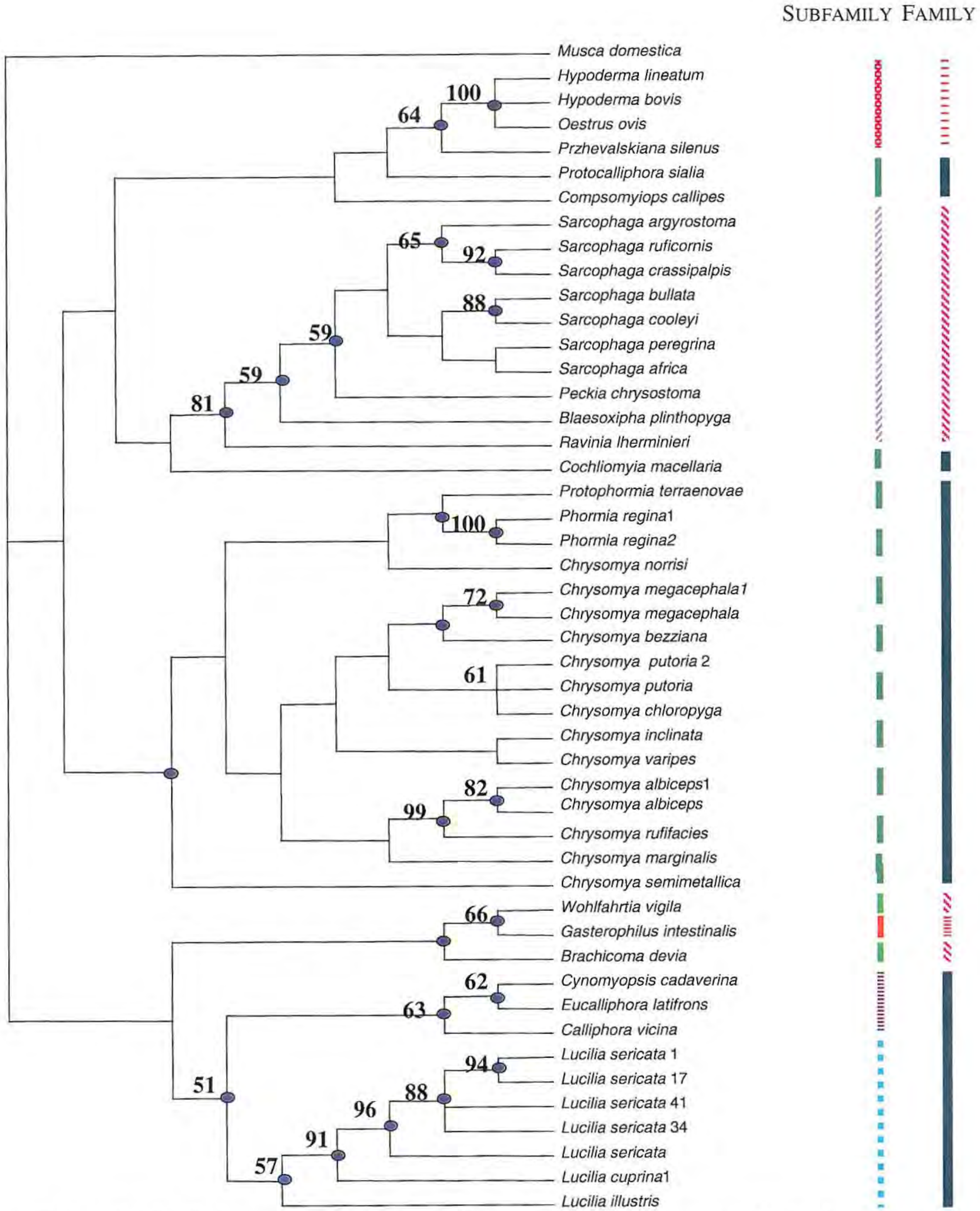

FIGURE 2.1.2 – Strict consensus tree derived from Maximum Parsimony analysis of the semi-conservative data set. Nodes common to all three MP trees are denoted by ●. CI = 0.297; RI = 0.555; # Trees = 18.

Subfamily codes: — — Chrysomyinae; ...... Luciliinae; .... Calliphorinae; ~~~~~ Oestrinae —— Paramachronychiinae; ~~~~~ Sarcophaginae; —— Gasterophilinae;

Family codes: —— Calliphoridae; ~~~~~ Sarcophagidae; ||||||||| Oestridae

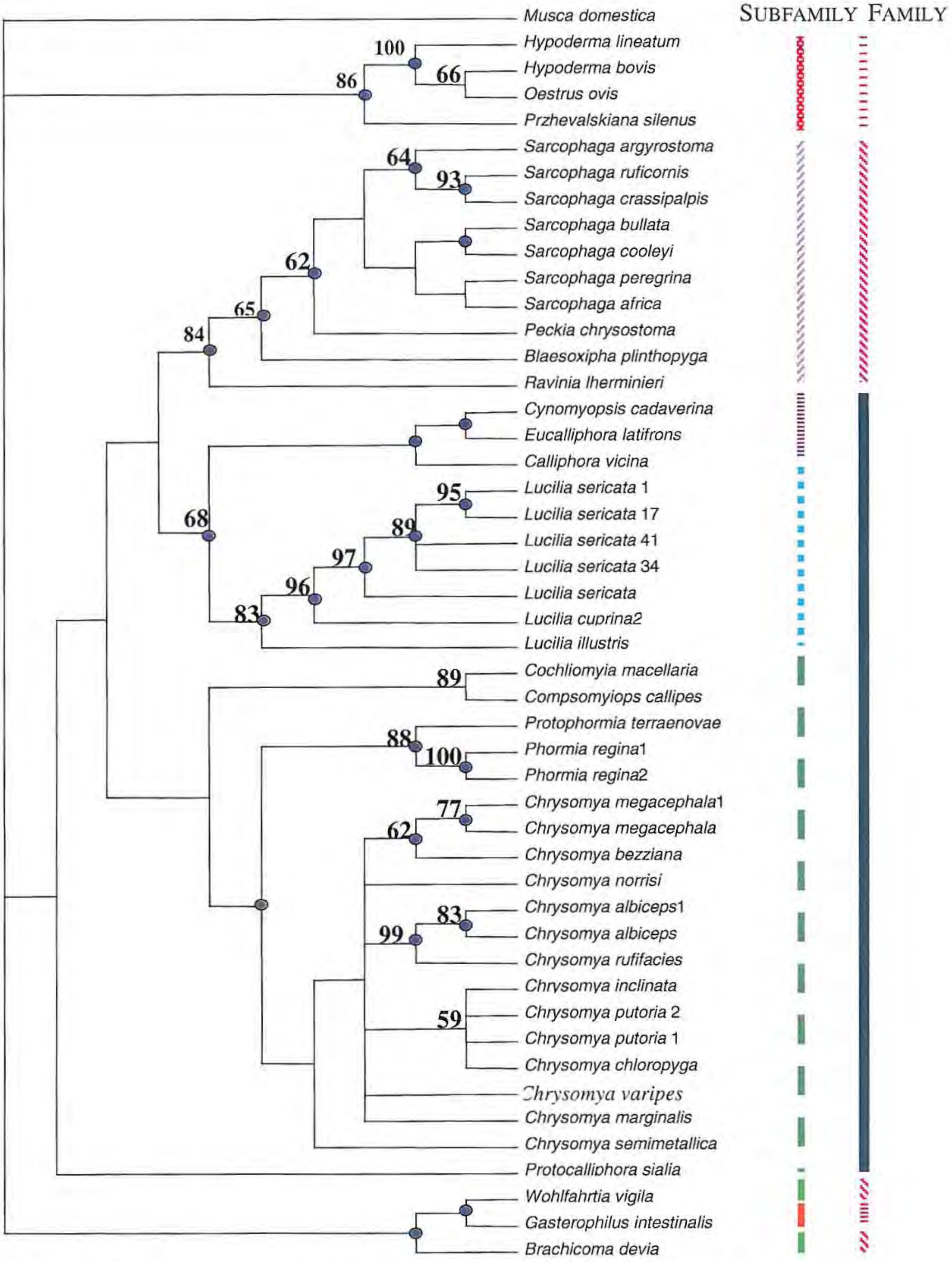

FIGURE 2.1.3 – Strict consensus tree derived from Maximum Parsimony analysis of the complete data set. Nodes common to all three MP trees are denoted by ● CI = 0.342; RI = 0.522; # Trees = 78.

Subfamily codes: — — Chrysomyinae; ...... Luciliinae; .... Calliphorinae;

...... Oestrinae —— Paramachronychiinae; ...... Sarcophaginae; —— Gasterophilinae;

Family codes: —— Calliphoridae; ∧∧∧∧ Sarcophagidae; .... Oestridae

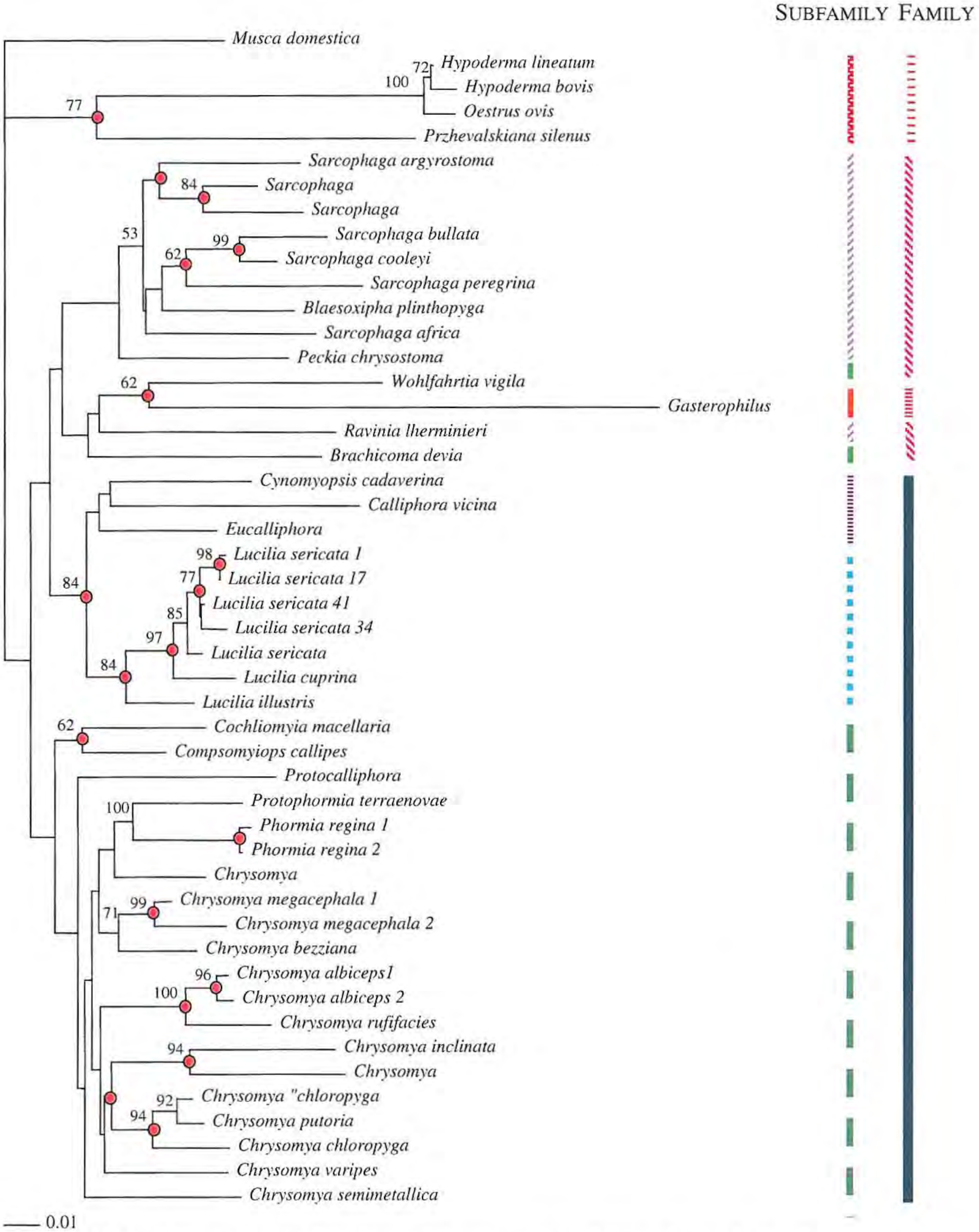

FIGURE 2.2.1 – Jukes-Cantor Neighbor-Joining tree derived from the analysis of the conservative data set. Nodes common to all three NJ trees denoted by red circles.

Subfamily codes: – – Chrysomyinae; ...... Luciliinae; ,,,,,,,,,, Calliphorinae;

⁓⁓⁓ Oestrinae ——— Paramachronychiinae; ...... Sarcophaginae; ——— Gasterophilinae;

Family codes: ——— Calliphoridae; ⁓⁓⁓ Sarcophagidae; ,,,,,,,,, Oestridae

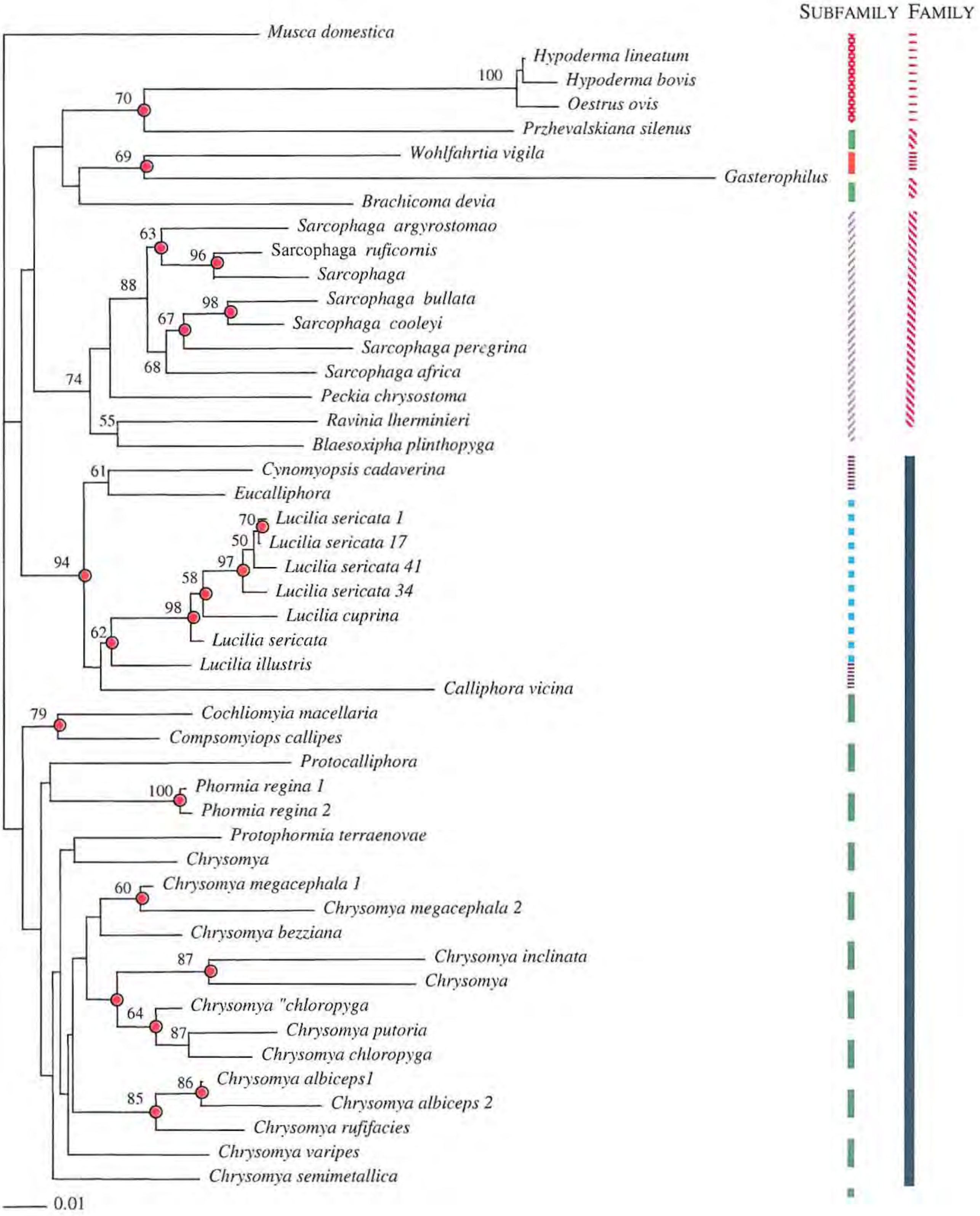

FIGURE 2.2.2 – Jukes-Cantor Neighbor-Joining tree derived from the analysis of the semi-conservative data set. Nodes common to all three NJ trees denoted by red circles.

Subfamily codes: ‒ ‒ Chrysomyinae; ⋯⋯ Luciliinae; ⦙⦙⦙⦙ Calliphorinae;

⌇⌇⌇ Oestrinae ▬ Paramachronychiinae; ⑊⑊⑊ Sarcophaginae; ▬ Gasterophilinae;

Family codes: ▬ Calliphoridae; ⑊⑊⑊ Sarcophagidae; ⦙⦙⦙⦙ Oestridae

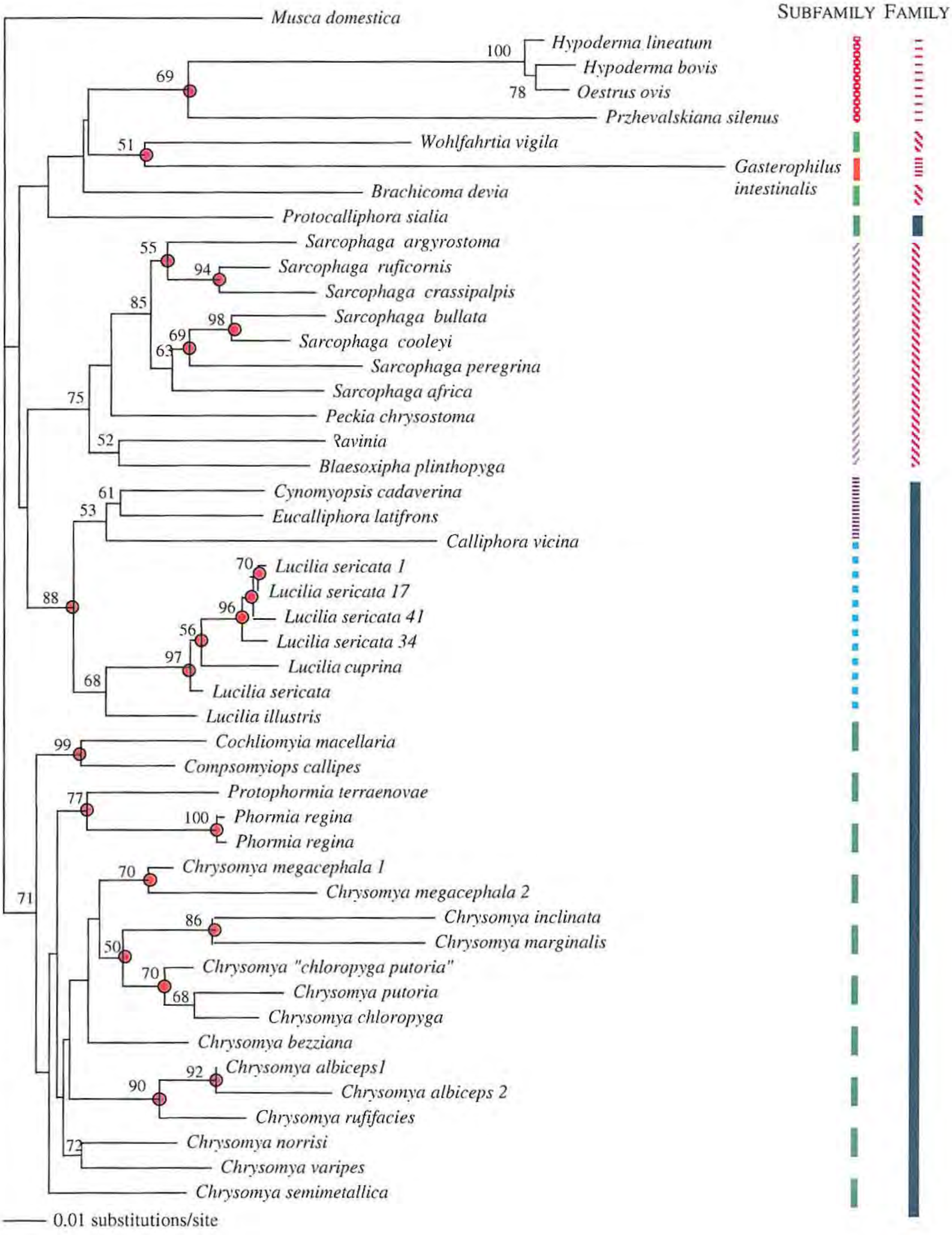

FIGURE 2.2.3 – Jukes-Cantor Neighbor-Joining tree derived from the analysis of the complete data set. Nodes common to all three NJ trees denoted by ●

Subfamily codes: Chrysomyinae; Luciliinae; Calliphorinae;

Oestrinae Paramachronychiinae; Sarcophaginae; Gasterophilinae;

Family codes: Calliphoridae; Sarcophagidae; Oestridae

Chapter 3

Development Rates Of Five Calliphorid Species Over A Range Of Temperatures

3.0 - Introduction

Metabolic functions are largely enzyme-mediated, and since enzymes are sensitive to factors such as pH, temperature and substrate concentration, it becomes immediately obvious that the internal environment of an organism will have a substantial effect on the efficiency of enzymes, and indirectly on the overall rate of metabolism. Sharpe and DeMichele (1977) suggested that the rate of metabolism (and thus growth) of poikilotherms at a given temperature is determined by the slowest enzyme-mediated - or rate-limiting - step of the process. Enzyme action approximately doubles with every 10°C increase in temperature between the lower (T_L) and upper (T_U) thermal thresholds (i.e. the minimum and maximum temperature, respectively, at which an enzyme can function), with a peak at the thermal optimum (T_{opt}) (Campbell, 1990). Near the thermal thresholds, the gradient of the curve decreases, giving rise to the typical skewed unimodal distribution observed in rate *vs.* temperature plots. Such graphs have an approximately linear portion that occurs in the functional range for metabolic enzymes, and incidentally spans the commonly encountered environmental temperatures.

In a homeotherm, the internal thermal environment is maintained at a fairly constant level, usually within the optimal range for metabolic enzymes. By contrast, a heterotherm is a "slave" to the external environmental temperature, and although it may thermoregulate by behavioural means, it has little other control over its core temperature. Not surprisingly, the range of temperatures experienced is much larger for heterotherms than for homeotherms and the thermal thresholds of enzymes are more likely to be governing features of heterotherm development. The lower thermal threshold of metabolism of a heterotherm is defined by the metabolic enzyme that has the highest T_L, and the opposite is true for the upper thermal threshold (Sharpe and DeMichele, 1977). It is likely that at a heterotherm's optimal developmental temperature (T_{opt}), the metabolic rate is more strongly influenced by substrate concentration than by non-optimal temperatures.

Fly larvae are typical heterotherms, and the rate of larval development is greatly influenced by environmental temperature. Maggots tend to aggregate, and in densities greater than one maggot per 2 g of substrate, metabolic heat and maggot activity may increase the temperature of the substrate by several degrees (Payne, 1965; Goodbrod & Goff, 1990; Catts, 1992). Due to their habit of remaining immersed in their food source for a large portion of their life, maggots are able to exercise some control over the temperature and humidity to which they are exposed by adjusting their positions within the maggot mass. However, this control is constrained by the absolute condition of the substrate.

In the forensic context, the estimation of post-mortem interval (PMI) in cases of recent death is largely dependent on accurately determining the daily rate of development of the maggots in the corpse, and this in turn is reliant on knowing the temperature to which the maggots have been exposed (Byrd & Allen, 2001a; Higley & Haskell, 2001; Marchenko, 2001). Several thermal summation models have been devised for use in agricultural entomology to predict pest outbreaks (Higley *et al.*, 1986), and the models can easily be adapted to forensic entomology to retrospectively predict the time of ovi- or larviposition on a corpse (Byrd & Allen, 2001a; Marchenko, 2001). Briefly, a thermal constant, expressed in degree-days, is calculated by fitting a regression line to the linear portion of a growth-rate *vs.* temperature curve. The inverse of the gradient is the developmental constant (k) and the *x*-axis abscissa is the lower thermal threshold or "developmental zero" (D_0).

A large number of developmental and succesional studies have been carried out on a variety of forensically important flies (e.g. Ullyett, 1950; Kamal, 1958; Buri, 1959; Norris, 1965; Ash & Greenberg, 1975; Levot *et al.*, 1979; Subramanian & Raja Mohan, 1980; Prins, 1982; O'Flynn, 1983; Reiter, 1984; Nishida *et al.*, 1986; Introna *et al.*, 1989; Goodbrod & Goff, 1990; Wells & Kurahashi, 1994; Byrd & Butler, 1996, 1997, 1998, 2001; Davies & Ratcliffe, 1994; Anderson, 2000; Byrd & Allen, 2001b; Grassberger & Reiter, 2001). Unfortunately, the developmental durations reported in some of the earlier papers (e.g. Hobson, 1932a,b; Hafez, 1940; Buri, 1959) cannot be utilised directly, as the authors did not have access to controlled environment chambers and did not take the effects of larval density into account, but their papers contain invaluable behavioural information.

Davies and Ratcliffe (1994) and Byrd and Butler (1999) have illustrated that the mean of fluctuating temperatures is not necessarily comparable to an identical constant temperature, especially if the fluctuations are at or near the thermal thresholds (i.e. lie predominantly to one side of the thermal optimum). Although external environmental conditions are rarely constant, constant-temperature rearings provide useful baseline data for determining the thermal thresholds and developmental constants.

The majority of published studies have concentrated on Nearctic, Palaearctic or cosmopolitan taxa, and the majority of the indigenous southern African blowfly fauna has received little or no attention (Ullyett, 1950; Buri, 1959; Subramanian & Raja Mohan, 1980). With the growth of forensic entomology in this region of the world, it is imperative that developmental data are made available to local forensic investigators. The aim of this paper is to provide baseline developmental data for five southern African species of Calliphoridae at constant temperatures that are characteristic of conditions in or around corpses in the region.

3.1 - Methods

3.1.1 - Stock cultures and maintenance

Adult blowflies were trapped on rotting chicken livers placed in Red-top® fly traps (Miller Methods, Johannesburg) or were caught by sweep netting at carcasses and rubbish dumps in the Grahamstown area. Single-species cultures of *Lucilia sericata* Meigen, *Chrysomya chloropyga* Wiedemann, *Chrysomya putoria* Wiedemann and *Chrysomya megacephala* Fabricius, and a mixed culture of *Chrysomya albiceps* Wiedemann, *Calliphora croceipalpis* Jaenicke and *Chrysomya marginalis* Wiedemann, were established in wood and gauze cages (80 x 40 x 40 cm). The flies were maintained on a diet of powdered milk, sugar and water *ad libitum*. Chicken livers or pork chops were provided as a protein source and oviposition medium. In each generation, new stocks of flies were intermittently introduced to the fly cultures to prevent inbreeding depression. Cultures were maintained in a controlled environment (CE) room at 20°C with a photoperiod of 12L : 12D. Observations of the time of oviposition, fly activity and fecundity were made throughout the study.

3.1.2 - Egg and larva collection

Larvae were reared at temperatures (mean ± SE) of 20.2 ± 0.03°C; 24.7 ± 0.01°C; 30.0 ± 0.03°C; 34.9 ± 0.01°C in constant environment rooms or a KBW 240 precision growth chamber (WTB Binder Labortechnik GmbH, Tuttlingen, Germany). Preliminary experiments indicated that the flies oviposited most often either during the night or soon after lights-on in the morning, so fresh meat was provided at 17h00 (one hour before lights-out), and the eggs were harvested by 08h00 (two hours after lights-on) the following morning. The advantage of harvesting the eggs in the morning was that the larvae then hatched during the light hours.

Additionally, the meat was checked at approximately two-hour intervals during the day and new egg batches were harvested as necessary. Females of *C. chloropyga* and *L. sericata* tended to lay communal egg masses if given enough time, but individual batches that could be identified by the parallel orientation of the eggs were also present. Individual egg batches were placed on chicken livers that had been allowed to reach the appropriate rearing temperature and were monitored at one- to four-hour intervals during the light period until hatching was noticed. At 30°C and 35°C, egg batches were placed in closed Petri dishes that contained liver and water-soaked cotton wool, to prevent desiccation.

3.1.3. Treatments

To establish the effects of gregariousness on larval growth, and to follow growth rates of individual maggots, experiments were arranged as follows. Once at least 80% of the eggs in a clutch had hatched, the larvae were randomly assigned to one of two treatments. In treatment 1, eight to ten larvae were placed individually in 6.3 cm^3 liver-filled tinfoil cups. In treatment 2, two replicates of 30 to 35 larvae were placed communally in 250 cm^3 polystyrene containers, at a maximum density of 1 larva/ 2 g liver to prevent maggot-generated heat from warming the substrate (Byrd & Butler, 1998; Goodbrod & Goff, 1990). At random times, the temperature of the medium was measured with a thermometer to establish if maggot-generated heat was accumulating and affecting the substrate temperature. Fresh liver was added as necessary to maintain the minimum substrate-to-maggot ratio, or to prevent excessive substrate desiccation.

3.1.4 - Development of L. sericata

First instar larvae of *L. sericata* were weighed to the nearest 0.001 mg on a Sartorius MC5 microbalance, within twelve hours of hatching. All isolated larvae that could be located in the liver, and five grouped larvae were weighed daily. To establish the range of sizes of gregarious maggots, the largest and smallest maggots that could be located and three randomly-picked individuals, were chosen. The larvae were rinsed in water and gently rolled in paper towel to clean them prior to weighing. The ages of the larvae at each weighing, and also at each of several observation times during the day, were recorded to the nearest hour. Post-feeding (or migrating) isolated larvae that left the liver were transferred to 6.5 cm diameter Petri dishes that were filled to a depth of 1 cm with clean river sand. Each replicate of grouped larvae was allowed to wander in a 15 x 21 x 8 cm plastic container that was filled to a depth of 1.5 to 2 cm with river sand. At the onset of migration, the larvae were no longer weighed, as preliminary experiments indicated that physical disturbance during wandering delayed pupariation by up to 48 hours.

Tanned puparia were collected at least once daily, and were transferred to 6.5 cm diameter Petri dishes until emergence. The length of each puparium was measured to the nearest 0.1 mm. A maximum of 20 group-reared puparia were kept in a single Petri dish; greater densities resulted in late-eclosing adults being damaged by other flies prior to cuticle hardening. Isolated larvae were placed individually in Petri dishes. At higher temperatures (30°C and 35°C), the insides of the Petri dish lids were sprayed daily with fresh water to prevent the puparia from desiccating. Puparia were observed daily during the first few days of pupariation, and with increasing frequency towards the end of the puparial period. Thus, eclosion was generally known to within five hours unless the adults emerged during the dark hours. The sex ratio of the eclosed flies and the mortality rate of each replicate were recorded. The majority of the eclosed flies were returned to the stock colonies, but two specimens of each sex were mounted on pins and retained as vouchers. Voucher material is retained in the Department of Zoology and Entomology, Rhodes University.

3.1.5 - Development of Chrysomya *spp. and* Calliphora croceipalpis

Experimental procedures for *Chrysomya* spp. were identical to those described for *Lucilia sericata,* with the exception that maggot length rather than mass was recorded. The standard lengths of live larvae were measured once daily to the nearest 0.1 mm using a modification of the Vernier system (Villet, pers. comm.). *Chrysomya putoria* and *C. megacephala* were not reared at 35°C due to a halt in laboratory oviposition, and *C. marginalis* was reared at 20°C and 25°C only, for the same reason. Statistical analyses of developmental parameters were not done for *C. megacephala, C. marginalis* or *C. croceipalpis* due to the low number (<3) of replicates carried out at any temperature. Only *ad hoc* observations of *C. albiceps* and *C. croceipalpis* development were made, as females rarely oviposited in the laboratory.

3.1.6 – Statistics, and D_0 and K calculations

The total, larval and puparial durations, and puparium and maggot size, were compared using ANOVA, with relatedness (i.e. siblings vs. non-siblings), treatment (i.e. group-reared and isolated maggots) and temperature as factors. Age and instar were found to have significant effects on puparial and maggot sizes, respectively and were therefore included as covariates in the relevant analyses. Wald-Wolfowitz runs tests were used to test for protandrous larval exodus and adult eclosion, and departure from a 1:1 sex ratio was tested with 1-sample *t*-tests. The proportion of time spent in each stage of the lifecycle for each species was arcsine-transformed, and a 1-way ANOVA with temperature as a factor, was carried out.

In species where more than two temperatures were investigated, the lower thermal threshold (D_0) and the developmental constant (K) were calculated for each landmark (first ecdysis, second ecdysis, larval exodus, pupariation and adult eclosion) using the thermal summation method. A regression line was fitted through the linear portion of the rate *vs.* temperature graph; K was calculated as the inverse of the slope and D_0 as the ratio of the *y*-axis intercept to the slope (i.e. intercept/slope).

3.1.7 - Terminology

The terminology used in fly development literature varies dramatically depending on authors' preferences. In this study, feeding larvae that had food in their crops were recorded according to their instar. First, second and feeding third instar larvae are referred to as L1, L2 and L3f respectively. Once feeding had stopped, the larvae were recognised as post-feeding or wandering larvae (L3w). Wandering larvae were usually active, left the feeding medium, voided their crops and found a place to pupariate. They had a characteristic creamy-pink or orange colour at this time. Once the wandering larvae had ceased to move and begun to pupariate, they were recognised as prepupae. The onset of pupariation (P) was determined by the tanning of the puparial skin (Fraenkel and Bhaskaran, 1973). The time at which pupation was initiated could not be determined, since it occurred within the puparium.

3.2 - Results

3.2.1 - Oviposition and larval behaviour

Despite being provided with protein-rich food (whole milk powder), gravid females fed on blood or meat exudates for some hours before ovipositing. During mating, the female flies rapidly vibrated their wings, producing a characteristic buzzing sound. Individual adult female flies laid eggs in batches of about 60 to more than 300. Except for *Calliphora croceipalpis* and *C. marginalis,* which usually laid few eggs at a time, small egg batches (fewer than 80 eggs) often exhibited high levels of mortality or abnormality, with few larvae hatching or dwarf larvae being produced. It is possible that such "abnormal" egg batches were produced by old females, but this issue was not investigated further. Unless the female was disturbed, eggs were laid in parallel rows, usually one or two eggs deep. *Calliphora* *croceipalpis* females scattered eggs across the upper surface of the meat, and when gravid *C. marginalis* females were provided with chicken livers, they exhibited a similar behaviour. If media were provided to several gravid females, communal egg masses were deposited by two or more females, usually with only one or two females ovipositing at any given time. Individual egg batches within the communal mass could be recognised by the orientation of the eggs, but were not used in experiments because eggs from several females inevitably adhered to each other. Only gravid wild-caught *C. albiceps* oviposited in the laboratory; laboratory-reared individuals did not oviposit even when maintained in mixed colonies with *C. marginalis* or *C. putoria.*

Lucilia sericata and *C. chloropyga* laid eggs readily both on moist substrates (chicken liver) and drier media (pork meat), but *C. megacephala*, *C. marginalis* and *C. putoria* preferred to oviposit in muscle folds under pieces of pork meat, or on transversely cut bone. *Calliphora croceipalpis* similarly preferred drier media on which to oviposit, but eggs were laid on the upper surface of the meat, and larval mortality was typically high (exceeding 60%). *Chrysomya marginalis* females could only be induced to oviposit on liver if they were isolated and heavily gravid.

The duration of the hatching period of each batch of larvae did not exceed two hours for any species. On hatching, larvae initially remained near to the egg mass, and then spread out in a variety of directions until finally forming one or several maggot masses in the substrate more than an hour later. Thus, both size and behaviour could be used to determine the approximate hatching time of the larvae.

Group-reared maggots of all species remained in aggregations throughout their feeding period. Even soon after hatching, the larvae did not spend all their time feeding; at any time, an estimated 10% to 20% of the larvae could be found traversing the feeding medium and even those in the maggot mass spent an appreciable amount of time "jostling" rather than feeding. In all cases, the liver in the containers of treatment 2 (group-reared larvae) was liquefied to a much greater extent than that in treatment 1 (isolated larvae), and liver was added to increase the amount of food available rather than to counteract desiccation in these cases. By the onset of the third instar, the meat became frothy near the maggot mass as a result of larval activity; however, substrate temperature did not significantly increase. *Chrysomya chloropyga* and *Lucilia sericata* maggots tended to position themselves close to the surface of the meat so that their posterior spiracles were immediately noticeable from above, whereas *C. putoria* and *C. marginalis* maggots were usually deeply embedded in the substrate and could only be located by removing the meat from the container. Of all the species reared, *C. chloropyga* maggots were by far the most active throughout their development, and the insides of the rearing containers above the meat were usually covered in liquid exudates as a result of the maggots climbing up the sides of the cups. Although not quantified, the speed with which these maggots moved was also faster than the other species. Conversely, *C. albiceps* maggots were typically sluggish, and were rarely seen out of the feeding medium.

Most maggots left the feeding medium prior to pupariation, but pupariated beneath the liver container. Maggots wandered for some time before burying themselves and starting to contract into prepupae. The majority of maggots left the feeding medium during the dark period or soon after lights on. The puparia of *C. albiceps* were readily recognised by the spiny projections arising from the puparial cases, while those of *C. chloropyga* were typically covered with sand. The puparia of the remaining species were difficult to differentiate, except by size (Fig. 3.6.1 - 3.6.5).

The approximate ages of adult flies that eclosed were determined by observing the general form of the fly, and the colouration and hardness of the cuticle. A recently eclosed fly had unexpanded wings, was pale whitish-grey in colour and the ptilinum was inflated. As the fly hardened, the cuticle became darker, but passed through several shades of grey and purple before acquiring its characteristic metallic blue or green sheen up to two hours after emergence, depending on the temperature. Although not quantified, it was observed that female flies did not begin to lay eggs until five or six days after emergence, and oviposition would not occur until some days later if protein meals were not made available to the flies soon after emergence.

3.2.2 - Comparison of development rates (total, and per stadium) between species (C. chloropyga, C. putoria and L. sericata) and treatments over a range of temperatures

The total developmental durations (hatching to eclosion) were superficially similar within *Chrysomya*, but *C. putoria* consistently developed more rapidly than *C. chloropyga* (Table 3.1). At all temperatures, *Chrysomya* spp. development was faster than *Lucilia sericata,* and the greatest disparity in development time occurred at 20°C, where the latter species eclosed more than five days later than *C. putoria* (Table 3.1). The durations of the immature stages decreased by six (*C. putoria*) to eight (*L. sericata* and *C. chloropyga*) days with increasing temperatures up to 30°C, but the overall development rates decreased thereafter (Table 3.1; Fig. 3.1.1 - 3.1.3). Mean adult eclosion of *L. sericata* occurred some three days later at 35°C than at 30°C, and the development of *C. chloropyga* puparia was completely inhibited at the higher temperature (Table 3.1).

The wandering stage and puparial period accounted for more than 60% of the total development time in all species at all temperatures (Figure 3.2.1 - 3.2.3). Analyses of variance (ANOVAs) of arcsine-transformed data indicated that, in all species, the proportion of time spent in each stadium was significantly different between temperatures (Table 3.2.1 - 3.2.3). Closer inspection indicated that the greatest variation in stadial durations between temperatures occurred in the wandering and puparial stages (Fig. 3.2.1 – 3.2.3), but when these stages were excluded, stadial durations were still disproportionate among temperatures in *L. sericata* and *C. chloropyga* (Table 3.2.1; 3.2.2). By contrast, only the duration of the first instar as a proportion of the feeding period of *C. putoria* differed among temperatures (Table 3.2.3). Among species, MANOVA showed that the proportions of the developmental period spent in each stadium at each temperature were statistically similar in *C. chloropyga* and *C. putoria*, but *Lucilia sericata* exhibited a significantly different pattern of development (Table 3.3).

Analyses of variance revealed that stadial durations of *Lucilia sericata* were significantly affected by temperature (Table 3.2.1). Development rates for second instar maggots and puparia of this species increased more or less linearly from 20°C to 30°C (Fig. 3.3.1b, e). However, the rate decreased from 25°C to 30°C in all other stadia, which is possibly an artefact of the sampling system overestimating the durations of those stages (Fig. 3.3.1). The feeding stages (L1, L2 and L3f) exhibited a further increase in development rate to 35°C, and puparial development showed the same trend (Fig. 3.3.1). By contrast, the durations of the wandering stages at 30°C and 35°C were markedly increased from 25°C, resulting in a reduction in the total development rate at 35°C (Fig. 3.3.1f). At higher temperatures, wandering larvae were restless, and although they burrowed into the sand, they took up to 382 hours to pupariate at 35°C.

In general, all stadia of group-reared *Lucilia sericata* developed more slowly than isolated individuals (Fig. 3.3.1), but treatment had a statistically significant effect on the third instar and pupariation rates only (Table 3.2.1). Additionally, there were significant interactions between temperature and treatment in feeding third instar larvae at 20°C and wandering larvae at 25°C (Table 3.2.1).

The rate of development of each stage in the life cycle of *C. chloropyga* increased with temperature up to 30°C (Fig. 3.3.2 a-f). The development rate increased from 30°C to

35°C for second and third instar larvae only; in the remainder of the larval stages, the rate decreased (Fig. 3.3.2 a, d), and puparia did not survive at 35°C. Puparia dissected after two weeks at 35 °C contained fully formed flies, but the puparial cases were hard and probably prevented adult eclosion. By three weeks from pupariation, the specimens had completely desiccated, and the contents of the puparia had the appearance of crystalline sugar.

Between treatments and within temperatures, no significant differences in development rate were observed for first or second instar maggots or puparia of *C. chloropyga*. However, the durations of the third instar and wandering stages were statistically longer in grouped maggots than in isolated maggots at all temperatures (Fig. 3.3.2), and there was a significant interaction between treatment and temperature in larvae reared at 35°C (Table 3.2.2; 3.4.2). The disparities in stadial durations between treatments were especially noticeable in third instar larvae at lower temperatures (Fig. 3.3.2c), and in wandering larvae at 30°C (Fig. 3.3.2d).

In common with *C. chloropyga* and *L. sericata*, ANOVAs showed that the development rates of all immature stages of *C. putoria* increased significantly with temperature increments up to 30°C (Table 3.2.3). The increase in rate was approximately linear for the second instar and total development, but the rate accelerated greatly from 25°C to 30°C in the other stadia. The duration of the third instar was similar at 25°C and 30°C, resulting in a nominal increase in development rate with increasing temperature (Fig. 3.3.3).

Treatment had a significant effect only on the duration of the wandering stage of *C. putoria* (Table 3.2.3), where group-reared larvae wandered for a substantially longer period than did isolated larvae (Fig. 3.3.3). There were significant interactions between temperature and treatment for the puparial stage and the total development rate (Table 3.2.3), illustrating some variability between replicates. These differences were evident at 20°C and 25°C (Fig. 3.3.3 e, f), with group-reared puparia developing faster at 20°C and 30°C and slower at 25°C, than isolated puparia.

3.2.3 - Comparison of larval and puparial size between temperatures, sexes and treatments for C. chloropyga, C. putoria and L. sericata

Lucilia sericata maggots attained maximum masses, in the range of 10 mg to 68 mg, several hours before the onset of wandering (Fig. 3.4.1). The smaller maggots in a cohort usually died before leaving the feeding medium. In *C. putoria,* the maximum larval lengths for isolated and group-reared maggots were 12.8 mm and 13.7 mm respectively (Fig. 3.4.3). By comparison *C. chloropyga* larvae grew larger, with the largest individual reaching 15.7 mm at 30°C (Fig. 3.4.2). In all three species, the range of larval sizes (mass or length) increased with age (Fig. 3.4.1 - 3.4.3), but there was a trend of decreasing variability in L3f maggot size with increasing temperature (Fig. 3.4.1 - 3.4.3). Despite this general trend, the large variation in the masses of *L. sericata* maggots at 20°C can be largely attributed to a replicate that contained many dwarf individuals (Fig. 3.4.1) rather than to the effects of temperature alone.

When categorised by instar, the larval masses of *L. sericata* differed significantly between temperatures, with larvae reared at higher temperatures attaining greater sizes (Fig. 3.4.1; Table 3.2.1). Treatment did not have a significant effect on larval mass (Table 3.2.1), but group-reared maggots tended to grow larger than individually-reared maggots at the same temperature (Fig. 3.4.1). ANOVAs with temperature and treatment as factors and instar as a covariate indicated that the sizes of *C. chloropyga* and *C. putoria* larvae were affected by treatment but not temperature (Table 3.2.2; 3.2.3). Group-reared maggots of both species were shown to grow significantly larger than isolated maggots (Fig. 3.4.2; 3.4.3), and this phenomenon was especially distinct in *C. putoria* maggots reared at 20°C (Fig. 3.4.3).

Despite a lack of statistical significance (Table 3.2.1; 3.2.2), it can be seen that the largest individually-reared maggots of *L. sericata* and *C. chloropyga* at all temperatures tended to be female and, except for the dwarf *L. sericata* females reared at 20 °C, the smallest individuals were typically male (Fig. 3.5.1; 3.5.2). This sexual dimorphism in size was especially apparent late in the third instar. *Chrysomya putoria* did not exhibit such a clear trend of large females, but the smallest individuals tended to be male (Fig. 3.5.3; Table 3.2.3).

Puparium size differed greatly between treatments and temperatures (Table 3.2.1 - 3.2.3). The lengths of puparia of *C. chloropyga* and *L. sericata* showed the opposite trend to larval length, with the mean size decreasing with increasing temperature (Fig. 3.6.1- 3.6.3). In contrast to these two species, the sizes of *C. putoria* puparia increased with increasing temperature, with the mean length of puparia maintained at 20°C being between 1 and 2 mm shorter than puparia kept at 30°C (Fig. 3.6.3).

In *L. sericata* and *C. putoria,* the mean lengths of group-reared puparia were greater than isolated individuals at all temperatures (Fig. 3.6.1- 3.6.3), despite the longer wandering period of group-reared maggots (Fig. 3.3.1 - 3.3.3). The same general trend was evident in *C. chloropyga*, although at 30°C, the mean puparial length of group-reared individuals was marginally lower than isolated individuals (Fig. 3.6.2). The mean length of group-reared puparia of *L. sericata* exceeded 7.6 mm at all temperatures, but the mean size of isolated individuals never exceeded 7.8 mm.

Spjotvoll-Stoline tests of homogeneity indicated that within temperatures, treatment had significant effects on puparium size of *C. chloropyga* at 25°C, *L. sericata* at all temperatures except 30°C, and *C. putoria* at 20°C (Figure 3.6.1 – 3.6.3). In parallel with larval characteristics, sex did not have a significant effect on the sizes of puparia (Table 3.2.1 - 3.2.3). As expected, large larvae tended to form large puparia, so the larger female maggots would necessarily also belong to the largest size-class of puparia.

3.2.4 - Sex ratios, protandry, and mortality rates of larvae and puparia

In all three species, the first larvae to leave the feeding medium were usually male. However, males did not exclusively begin to wander before females, so there was no statistically significant difference in the timing of larval exodus or adult eclosion between the sexes for any species (runs test; $p > 0.05$).

Sex ratios of *Lucilia sericata* did not significantly vary from equality at any temperature (*t*-test; $p > 0.05$), although males slightly outnumbered females at 30°C (Table 3.1). *Chrysomya chloropyga* also exhibited sex ratios that did not diverge statistically from 1:1 at any temperature (*t*-test; $p > 0.05$), although there was a non-significant male bias at 30°C, and females outnumbered males at 20°C (Table 3.1). In *C. putoria*, males outnumbered females by almost 3:1 at 30°C (Table 3.1), but at 20°C and 25°C, the ratio did not differ significantly from 1:1 (*t*-test; $p > 0.05$). In all three species and in each replicate, the first specimens to eclose tended to be male, but since a mix of sexes emerged later (i.e. males did not exclusively eclose first), protandry was not statistically supported (Table 3.4).

Larval and puparial mortality rates of *L. sericata* were greatest at 20°C and 35°C, respectively, exceeding 50% in both cases (Table 3.1). The highest larval survivorship was attained at 30°C, and puparial mortality was at a minimum at 25°C (Table 3.1). In *C. chloropyga*, larval mortality varied greatly between replicates at 25, 30 and 35°C. When replicates were combined, it became apparent that larval mortality at 35°C was over five times higher than at 25°C (Table 3.1). Puparial mortality increased from 20°C to 35°C, and no puparia survived at the latter temperature (Table 3.1).

At all temperatures, the larval and puparial mortalities of *C. putoria* were high, exceeding 10% (Table 3.1). The larval mortality rate was lowest (14.1%) at 30°C and highest at 20°C, and puparial survivorship was greatest at 25°C (Table 3.1).

3.2.5 - Observations on the development of Chrysomya albiceps, C. megacephala, C. marginalis and Calliphora croceipalpis

First and second instar *Chrysomya albiceps* maggots developed normally on chicken livers, despite their mothers' reluctance to oviposit in the laboratory. Isolated maggots were not very active, and their food substrate had to be macerated manually in order for the larvae to survive. Isolated maggots were apparently obligate predators and died unless they were provided with live maggots to feed on in their third instar. The predatory maggots wrapped themselves around their prey, pierced them with their mouthhooks and ingested the fluids. The preys' larval cuticles were discarded thereafter. Third instar *C. albiceps* did prey on smaller (first and second instar) conspecifics, but unfortunately, due to the difficulties involved in maintaining a laboratory culture of this species, it could not be ascertained whether maggots would overpower similar-sized conspecifics, or whether they preferentially preyed on unrelated individuals or other species. In the field, *C. albiceps* larvae were observed in a wide variety of carcasses, but were most frequent in large mammal carcasses.

Although a number of *C. albiceps* specimens pupariated, none eclosed in the laboratory. A large number of wandering larvae failed to pupariate, but simply shrivelled and desiccated. The high mortality during the wandering stage may have been a result of low ambient humidity in the CE rooms, or possibly premature larval exodus due to suboptimal rearing conditions. At 20°C, the L1, L2, L3f and L3w stages lasted a mean of 63, 66, 48 and 71 hours respectively. At 25°C, no individuals successfully pupariated, but the feeding stage lasted a total of 87 hours and the larvae wandered for up to 25 hours before dying.

Chrysomya megacephala females oviposited reasonably readily on pork, provided that the piece of meat had an area of more than 80cm^2 and the flies were able to crawl underneath to oviposit. Slightly putrid meat (> 1 day old) was preferred in most cases. Despite the apparent ease of rearing this species in the laboratory, maintaining and augmenting a laboratory culture was complicated by the fluctuating field population; the fly was virtually absent from carcasses and traps in the winter months and at no time was the species abundant in the field. Inbred populations that had spent more than about five generations in the laboratory exhibited a high mortality rate and reduced fecundity.

In common with *L. sericata, C. chloropyga* and *C. putoria*, group-reared *C. megacephala* maggots attained a greater size and took longer to reach maturity than did isolated maggots (Fig. 3.1.4; Fig. 3.4.4). However, L1 and L2 development by group-reared maggots was more rapid than isolated maggots (Fig. 3.3.4), but the L3f and L3w stages of the group-reared maggots were substantially longer than those of the isolated maggots. At 20°C, the group-reared L1, L2 and L3f stages accounted for 41.5% of the total development from hatch to eclosion. The wandering period ranged from 20.5 to 116.75 hours in both treatments, with a peak in pupariation at about 60.5 hours after exodus from the feeding medium. At 25°C, the L1, L2 and L3f stages comprised 44% (group-reared) to 45% (isolated) of the total development time (Fig. 3.2). Post-feeding larvae wandered for at least 18 hours before the initiation of pupariation. Adult eclosion occurred some 115 (group-reared) or 109 (isolated) hours after the puparial skin tanned. Thus, the total hatch to eclosion developmental period of group-reared maggots exceeded that of the isolated maggots by a mean of 33 hours at 20°C, and 22 hours at 25°C.

Isolated *C. megacephala* maggots exhibited a high level of mortality, with >55% dying before pupariation. The puparia of group-reared individuals were larger than those of isolated individuals at 25°C ($t_{22} = -3.64019$; $p = 0.001444$) (Fig. 3.5.4). No isolated larvae pupariated at 20°C, but the puparia of group-reared individuals at this temperature were not dissimilar in size to group-reared puparia at 25°C ($t_{37} = -0.300111$; $p = 0.765772$) (Fig. 3.5.4). Sex ratios did not diverge from equality at either temperature, although females slightly outnumbered males (0.52 : 0.48) at both 20°C and 25°C.

Chrysomya marginalis larvae developed normally on chicken livers, but isolated maggots did not survive past the second instar. At 25°C, group-reared specimens spent a mean of 34.38, 11.88 and 74.38 hours respectively in the three feeding instars, and the wandering period ranged from 24 to 72 hours, with peak pupariation at about 36 hours after exodus from the feeding medium. The puparial period lasted a maximum of 167 hours (mean = 122.82 hours), bringing the total mean hatch to eclosion duration to 12.1 days. Maximum length (mean = 15.1mm) was attained 104 to 117 hours after hatching (Fig. 3.4.5). Mortality in the group-reared treatment was high in both the larval and puparial phases (Table 3.1). Males slightly outnumbered females (0.52 : 0.48) in the two successful replicates, but the small sample size prevents firm conclusions about population sex ratios from being reached.

Calliphora croceipalpis adult females were reluctant to lay eggs in the laboratory; in fact, only two females were induced to oviposit, and high egg mortality in one case resulted in just one set of larvae being successfully reared at 25°C. The egg batches consisted of fewer than 20 eggs that were scattered singly or in pairs across the upper surface of the oviposition medium. Only three adults eclosed, more than 20 days from hatching, at 20°C. Although not measured, the larvae were of comparable size to *C. marginalis* and *Sarcophaga* spp. From field observations, *C. croceipalpis* were frequently reared from small mammal (e.g. rat) and bird carcasses in the cooler months of the year, but this species was absent from larger (e.g. cat and dog) carcasses year-round.

3.2.6 – Developmental parameters (K and D_0) for Chrysomya chloropyga, C. putoria and Lucilia sericata

Chrysomya chloropyga and *C. putoria* had similar developmental constants (K) and developmental zeroes (D_0) at all stages of development (Table 3.5). The K value for development to first ecdysis was some four times greater in *L. sericata* than in either *Chrysomya* sp. (Table 3.5), but thereafter, the K values for all three species were comparable until pupariation. Puparial development was substantially longer in *L. sericata* (6021°h) than either *C. putoria* (2799°h) or *C. chloropyga* (2109°h), resulting in the former species having a greatly increased total K. All three species showed a general trend of decreasing D_0 with development (Table 3.5), and in particular, the exposed stages (i.e. wandering larva and pupa) had D_0s of less than 11°C.

3.3 - Discussion

3.3.1 - Oviposition and larval behaviour

Many flies exhibit preferences in oviposition substrate, as documented by several authors (e.g. Zumpt & Patterson, 1952; Meskin, 1986; Laurence, 1988). Fly eggs are extremely sensitive to desiccation because they are small and immobile, and desiccation is probably one of the primary sources of egg mortality in natural conditions. However, excess moisture can also be detrimental and high mortality can result if eggs became too moist (Smit, 1931; Hobson, 1932a). From the literature and personal observations of animal carcasses, it has been found that the majority of calliphorids tend to oviposit in natural orifices, in fur, or under the animal (Smit, 1931; Meskin, 1986; Smith, 1986). Such oviposition sites are moist (but not excessively so) and well-protected from the sun, but afford newly-hatched larvae easy access to tissues. Female flies duplicated this behaviour in the laboratory; apart from *Lucilia sericata* and *Chrysomya chloropyga* - both early colonisers (Meskin, 1986) - flies rarely oviposited on very moist substrates (e.g. chicken livers), preferring to crawl under a firm medium and deposit eggs in sheltered muscle folds. These oviposition preferences may account for the difficulties involved in maintaining laboratory colonies of *C. albiceps, C. marginalis* and *C. croceipalpis*. Only gravid wild-caught females of these three species would readily oviposit in the laboratory, but larvae developed normally on chicken livers, even if mortality was high. From observations of a variety of carcasses maintained in natural conditions and part-shade, *C. croceipalpis* maggots were only collected on two occasions (from a bat-eared fox and a bird carcass), which suggests that carrion is not a primary oviposition site for this species. When heavily gravid *Chrysomya marginalis* females were provided with liver only, they oviposited, but scattered their eggs over the surface of the meat as opposed to their usual tendency to deposit large, cohesive batches of eggs under the substrate (Meskin, 1986; pers. obs.). This behaviour suggests that, although the oviposition stimulus was strong, the flies were reluctant to deposit eggs on a sub-optimal substrate.

Smit (1931), Ullyett (1950) and Marchenko (1984) published accounts of the predatory and cannibalistic behaviour of *Chrysomya albiceps* in natural conditions. Similarly, Byrd & Butler (1997) reported that *C. rufifacies*, a close relative of *C. albiceps,* is a facultative predator but is rarely cannibalistic if food is not limiting. Unfortunately, the latter authors did not report on the mortality rates of *C. rufifacies,* which makes comparisons with other species impossible. Meskin (1986) reported that *C. albiceps* was usually one of the last flies to oviposit on a carcass, and the female flies typically burrowed into the fur and laid several widely-scattered batches of eggs that were intermingled with the eggs of other species. Such behaviour would ensure that predatory larvae had ample access to prey, and indicates that the species is an obligate predator. In this study, despite the availability of excess food, isolated *C. albiceps* larvae died in their third instar unless they were provided with other maggots to feed on, lending further weight to the obligatory predatory nature of this species. Larval mortality was high in the group-reared treatment, but separating the effects of cannibalism from other sources of mortality was not possible. The maggots were not seen to attack larvae that were of a similar size or larger than themselves, but third instar larvae fed readily on second instar conspecifics. *Ophyra capensis* (Muscidae) larvae have been shown to be predatory on other fly larvae, specifically attacking larvae smaller than themselves (Olckers & Hulley, 1984). It is likely that cannibalism was rarely observed by Byrd & Butler (1997) in *C. rufifacies* colonies because of the similarity in the sizes of the larvae in each cohort. Both *C. rufifacies* and *C. albiceps* have cuticular tubercles which become apparent in the second instar, and it is possible that these are in some way associated with the maggots' predatory lifestyles.

Previous studies have indicated that larval density and concomitant competition for resources affects the development rates and final sizes of maggots (Ullyett, 1950; Payne, 1965; Meskin, 1986; Braack, 1987; Goodbrod & Goff, 1990). At high maggot densities, the temperature of the substrate may be increased (Payne, 1965; Goodbrod & Goff, 1990), resulting in more rapid larval development, but increased competition for resources (including position in the maggot mass) results in smaller individuals (Ullyett, 1950). In this study, larval densities were maintained at a level below that at which maggot-generated heat would be significant (Goodbrod & Goff, 1990), and the provision of excess food precluded competition for that resource. It would be expected, therefore, that isolated and group-reared maggots would exhibit similar developmental characteristics, but this was not the case. In fact, group-reared larvae fed for a longer period than isolated larvae, grew larger and exhibited lower mortality rates at all temperatures (Fig. 3.3.1-3.3.4; Fig. 3.4.1-3.4.4; Table 3.1), all of which suggest that substantial competition for resources did not occur.

Hobson (1932a) reported that in an alkaline environment, maggot excreta and the mechanical breakdown of meat by maggots resulted in almost total liquefaction of muscle tissue. In the present study, gregarious maggots appreciably liquefied the liver tissue, but isolated individuals did not. Since maggots can ingest only semi-liquid food, the disparity in size of gregarious and isolated larvae may have been a result of the isolated individuals being unable to ingest comparable quantities of food due to their inability to substantially liquefy the tissue. Since it is unlikely that the group-reared maggots were competing for resources, differences in the timing of larval exodus may have been due to premature wandering by isolated individuals as a result of suboptimal conditions. The failure of isolated *C. albiceps* and *C. marginalis* larvae to pupariate further indicates that laboratory conditions were inadequate for these species, and the mass oviposition behaviour exhibited by females of the latter species (Braack, 1981) may be a mechanism to ensure maggot survivorship. Christopherson & Gibo (1997) reported that food-deprived larvae of *Sarcophaga (Neobellieria) bullata* (Sarcophagidae) searched for alternative food sources. Therefore, although the quantity of food provided to isolated larvae was not limited, its quality may have encouraged premature larval exodus in this study.

The timing of larval exodus in *L. cuprina* is apparently controlled by a circadian clock, with the majority of maggots migrating during the night or at dawn (Smith et al., 1981; Smith, 1985). Although not specifically investigated in this study, larval exodus in all species was usually initiated during the dark hours, and migration was generally synchronous between similar-aged cohorts. Denlinger & Zdarek (1994) drew attention to the effect of physical disturbance of wandering larvae and its impact on pupariation. Pupariation was delayed in some species, whilst in others it was initiated, if the larvae were handled prior to puparium formation. In pilot studies for this experiment, it was found that physical disturbance (i.e. handling) delayed pupariation by up to 48 hours. In addition, although space was not limited, wandering larvae tended to aggregate, and disturbance by siblings may account for the substantially longer wandering period of group-reared larvae (Fig. 3.3.1-3.3.4). Smit (1931) reported a similar effect in *Chrysomya chloropyga* – in large aggregations of maggots, jostling by conspecifics substantially delayed pupariation.

3.3.2 – Developmental optima, sex ratios and mortality rates

It would be expected that the optimal developmental temperature for a species would be that at which development is most rapid, but it is logical that the optimum temperature would be coincident with maximum survival (Villet & Mackenzie, in prep.). In temperature-preference experiments, Byrd & Butler (1996, 1997) and Byrd & Allen (2001b) illustrated that larvae of three genera (*Chrysomya, Cochliomyia* and *Phormia*) moved down a temperature gradient as they matured, despite a reduction in the rate of development, until they finally pupariated at temperatures that were 5°C to 10°C cooler than those preferred by first instar larvae. Assuming that preferred and optimal temperatures are comparable, this is an indication that optimal temperatures vary with developmental stage. In this study, the increase in development rate with temperature became less marked as the larvae matured, and the duration of the wandering stage of *L. sericata* was significantly extended at 30°C. Unlike L3f and L3w development rates, the pupariation rates of *L. sericata, C. putoria* and *C. chloropyga* increased almost linearly from 20°C to 30°C which suggests that puparia are more tolerant of a wide range of temperatures than are larvae (Fig. 3.1.1 - 3.1.3). However, the puparial survival rates contradicted this expectation - the lowest mortalities of *C. putoria, C. chloropyga* and *L. sericata* puparia occurred at temperatures 5°C lower than the peak maggot survivorship temperature. Further evidence that high temperatures (35°C) are sub-optimal for puparial development comes from the extremely extended and variable wandering period of *L. sericata* and *C. chloropyga* at this temperature. Humidity may confound the effects of temperature, and puparia, being immobile, are more prone to environmental fluxes in temperature and humidity than maggots, which can behaviourally thermoregulate to some extent. However, Davison (1969) illustrated that puparia were more tolerant of high temperatures than either adult flies or larvae of *Calliphora vicina,* and indications from this study are that puparia have the lowest developmental zero (D_0) temperatures of any of the immature stages.

Another indicator of temperature optima would be final maggot size. It has been well documented that the body size of a female heterotherm is correlated with her fecundity

(Ullyett, 1950). Putatively, then, the temperature at which individuals are reproductively fittest is that at which they attain their greatest sizes. In *Sarcophaga ruficornis*, larvae were small when reared at low temperatures, but the opposite trend was evident in pupae (Amoudi *et al.*, 1994). However, the maximum sizes of both larvae and pupae were attained at the species' thermal optimum (Amoudi *et al.*, 1994). A similar result was found in this study. The sizes of *C. chloropyga, C. putoria, C. megacephala* and *L. sericata* larvae increased with temperature, but puparial length showed an opposite trend in *C. chloropyga* and *L. sericata.* The probable reason for this disparity in the relative sizes of larvae and puparia is that the wandering and prepupal periods were significantly longer than expected at high temperatures. Unfortunately, because temperature preferences were not investigated, it cannot be determined if, given the choice, wandering larvae from the 35°C experiment would migrate to a lower temperature and develop earlier into larger puparia. It would be expected that *L. sericata* and *C. putoria* larvae would maintain their temperatures close to 30°C, and *C. chloropyga* larvae would seek temperatures of 25°C. At the onset of wandering, all three species would migrate to cooler habitats to pupariate.

The geographical distributions of *Chrysomya* spp. included in this study mirror their apparent temperature preferences. *Chrysomya chloropyga*, which displayed maximum survival at 20°C to 25°C and which has D_0s in excess of 8°C at all stages of development, is frequently encountered in the subtropical and temperate regions of South Africa throughout the year, but was absent from bait traps set in warmer, tropical habitats in summer (pers. obs.; Villet, pers. comm.). By contrast, *C. putoria,* which is more tropical in distribution (Zumpt, 1956; Paterson, 1968; pers. obs.) developed most favourably at 25°C to 30°C and has a minimum D_0 of about 7°C in the puparial stage (Table 3.5). From mortality rates and larval and puparial sizes, it would be expected that *C. megacephala* – a historically tropical fly - would show a similar trend to *C. putoria*, but since the former species was only reared at two temperatures, firm conclusions cannot be made. Perhaps surprisingly, *L. sericata*, which is historically Palaearctic, exhibited similar temperature optima to *C. putoria* (Fig. 3.1.1; 3.1.3; Table 3.1), but this species is a facultative mammalian parasite (Stevens & Wall, 1995), so it must necessarily be tolerant of higher environmental temperatures. *Chrysomya rufifacies*, a warm-climate fly from the Oriental/Australasian region exhibited a pattern of increasing puparium size with increasing temperature (Byrd & Butler, 1997), as seen in *C. putoria* in this study. This provides additional support for the argument that flies from warmer climates have higher temperature optima.

3.3.3 – Applicability of developmental data and rearing methods to PMI estimations

Maggot size is popularly used as a determinant of age by many forensic investigators (e.g. Anderson, 1999), but has several shortcomings. Reiter (1984) and Grassberger & Reiter (2001) produced "isomegalendiagrams" with which investigators can determine maggot age from standard length. However, their approach has inherent inaccuracies, primarily because mean lengths of maggots were used, and no indication of variability was given. As shown by Wells & LaMotte (1995) and in this study, there is potentially a large variability in maggot size, especially at lower temperatures; the greatest disparity occurred in *L. sericata* at 20°C, where dwarf third instar maggots were the same size as some individuals that were about 40 hours younger (Fig. 3.3.1). Most forensic investigators use the largest maggots present in a corpse, assuming that the largest maggots are the oldest (e.g. Anderson, 1997, 1999), so the use of the isomegalendiagram system in such instances will potentially result in an overestimation of the PMI. Byrd & Butler (1996, 1997) overcame this problem by selecting the largest maggots from a cohort in their laboratory experiments. Although this method is more accurate, a large amount of information is lost when the range of sizes of maggots of a given age is not considered, and the sample will be biased towards females, which may have slightly different development rates. Additionally, if food is limited or of poor quality, maggots may be stunted or may leave the substrate earlier (Christopherson & Gibo, 1997). Thus, the use of isomegalendiagrams or maximum sizes will result in an underestimate of the PMI. In fact, the method is not used by the French gendarmerie (Gaudry *et al.*, 2001) and Dadour (2001) pointed out that it is not a measure of physiological age. Another major shortcoming of measuring maggots up to pupariation is that, as mentioned earlier, the handling of maggots in their post-feeding stage may delay pupariation (Denlinger & Zdarek, 1994).

An alternative method of determining maggot age (or corpse age) is to determine the time taken for maggots to reach a particular developmental landmark (e.g. Grassberger & Reiter, 2001). Despite the obvious difficulties involved in determining the timing of developmental events, this method is preferable in many ways to determining age from size, because landmark data are categorical. In addition, the problem of dwarfism is circumvented since the age at an event does not appear to be subject to effects of size (Fig. 3.1.1; Fig. 3.4.1) (Ullyett, 1950). However, as shown by Byrd & Butler (1997) and this study, there is still some variability in the development rates of individuals, especially at higher temperatures where the durations of the L3f and L3w stages are plastic. Again, the use of mean (or preferably median) data (e.g. Grassberger & Reiter, 2001) is specious, but the inclusion of an error term (SE, SD or range) will provide a confidence interval for any estimate. Alternatively, if range data are not available, then the minimum age at which a landmark is reached should be used in preference to the mean, but the method of determining PMI is ultimately dependent on the case.

If the proportions of total development spent in each developmental stadium are similar across temperatures, it may be possible to calculate the age of a cohort from the duration of a single developmental stage. *Lucilia sericata*, *C. chloropyga* and *C. albiceps* exhibited significantly different stadia apportionments across temperatures (Fig. 3.2.1-3.2.3), in common with results published by Anderson (2000). However, as found by Anderson (2000), trends within genera were similar. In this study, the alterations in proportions with temperature changes were not significantly different between *C. chloropyga* and *C. putoria*, but *L. sericata* differed significantly from both of these species (Table 3.3). Patterns of development such as this, that apparently have a phylogenetic link, are potentially useful for predictive purposes (see chapter 4).

3.3.4 – Conclusions

Results of this study illustrate that maggot density has a profound effect on the development rates of maggots in carrion. The increase in the development rates of feeding maggots in maggot masses is not necessarily solely attributable to metabolic heat generation increasing the ambient temperature, but also to the physical and chemical characteristics of the medium. Mechanical breakdown, alkali excretion and enzyme action combine to liquefy the medium and facilitate ingestion by maggots. Contrary to popular belief, groups of maggots – provided the maggot to substrate ratio is below that at which metabolic heat production is measurable (Goodbrod & Goff, 1990) - may develop at slower rates (especially from the L3f to P stages) than isolated maggots or very small maggot masses. Therefore, it can be expected that when a very low maggot density is found in a corpse, development rates may vary substantially from published reports.

For PMI estimations, maggot size – either mass or standard length - is not a good indicator of maggot age, since dwarfism, sexual dimorphism and maggot density may confound the estimate. However, stadial durations appear to be independent of body size, the range of times at which a given landmark is reached tends to be relatively small, and confidence intervals can be readily calculated. Using the proportion of total development spent in each stadium as an estimator of maggot age is also problematic in cases where temperatures fluctuate greatly, as the proportions vary substantially with temperature.

There are a great number of variables that may confound a PMI estimate, and many of these may not be easily compensated. Additionally, field studies should be carried out to test the validity of laboratory inferences. However, recognition of the potential sources of error can assist investigators with accurately calculating PMIs and open up new areas for blowfly research.

TABLE 3.1 – Summary of total development, sex ratios and mortality rates of *Lucilia sericata, Chrysomya chloropyga* and *Chrysomya putoria* at each rearing temperature.

Species	Temperature (°C)	Total developmental duration (days) from hatch to eclosion		Larval mortality (%)	Puparial mortality (%)	Proportion male (mean ± SE)
		Mean ± SE	range			
Lucilia sericata	20	**20.55 ± 0.09**	19.08-23.00	55.3	13	0.491 ± 0.013
	25	**14.37 ± 0.07**	12.4-17.27	34.6	1.2	0.515 ± 0.011
	30	**12.61 ± 0.09**	10.6-17.58	12.4	10.7	0.495 ± 0.015
	35	**14.98 ± 0.32**	10.88-19.40	47.1	66.1	0.448 ± 0.057
Chrysomya chloropyga	20	**17.08 ± 0.06**	15.02-19.95	30.1	2.75	0.326 ± 0.036
	25	**11.79 ± 0.05**	8.85-13.78	5.83	5.86	0.440 ± 0.029
	30	**9.10 ± 0.07**	7.81-10.71	25.4	37	0.651 ± 0.032
	35	N/A	N/A	47.4	100	N/A
Chrysomya putoria	20	**15.23 ± 0.15**	14.44-18.38	52.6	64.9	0.545 ± 0.104
	25	**11.28 ± 0.23**	10.31-11.85	47	54.3	0.724 ± 0.053
	30	**8.88 ± 0.00**	N/A	14.1	77.6	0.607 ± 0.706

TABLE 3.2.1 – ANOVAs of the effects of temperature, treatment and sex on aspects of *Lucilia sericata* development. *p*-values in **bold** are significant at $\alpha = 0.05$

Parameter	**Factors**	**df$_{\text{effect}}$**	**df$_{\text{error}}$**	F	**p**
Stadial proportions	Temperature	5	141	141.18990	**0.000000**
L1 rate	Temperature	3	181	123.53900	**0.000000**
	Treatment	1	181	0.34020	0.560449
	Interaction	3	181	0.15200	0.928272
L2 rate	Temperature	3	180	173.35880	**0.000000**
	Treatment	1	180	2.02580	0.156371
	Interaction	3	180	0.54470	0.652310
L3f rate	Temperature	3	182	107.76790	**0.000000**
	Treatment	1	182	35.34620	**0.000000**
	Interaction	3	182	8.22760	**0.000037**
L3w rate	Temperature	3	849	24.15950	**0.000000**
	Treatment	1	849	2.26637	0.132581
	Interaction	3	849	3.70239	**0.011503**
P rate	Temperature	3	706	196.80730	**0.000000**
	Treatment	1	706	6.13480	**0.013488**
	Interaction	3	706	2.24560	0.081758
Total development rate	Temperature	3	706	192.32400	**0.000000**
	Treatment	1	706	3.61350	0.057719
	Interaction	3	706	1.04150	0.373535
Larva mass	Temperature	3	657	24.85033	**0.000000**
	Treatment	1	657	0.18977	0.663252
	Interaction	3	657	2.42525	0.064593
Larva mass (Isolated only)	Temperature	3	188	12.61885	**0.000000**
	Sex	1	188	3.03558	0.083093
	Interaction	3	188	1.07702	0.360066
Puparium length	Temperature	3	471	8.84582	**0.000000**
	Treatment	1	471	37.51119	**0.000000**
	Interaction	3	471	3.69220	**0.024165**
Puparium length	Temperature	2	57	0.108025	0.108025
	Sex	1	57	0.000073	**0.000073**
	Interaction	1	57	15952.380000	**0.000000**

TABLE 3.2.2 – ANOVAs of the effects of temperature, treatment and sex on aspects of *Chrysomya chloropyga* development. *p*-values in **bold** are significant at $\alpha = 0.05$

Parameter	**Factors**	**df**$_{effect}$	**df**$_{error}$	F	**p**
Stadial proportions	Temperature	10	64	8.10000	**0.000000**
L1 rate	Temperature	3	116	202.7757	**0.000000**
	Treatment	1	116	0.0128	0.910030
	Interaction	3	116	0.3145	0.814901
L2 rate	Temperature	3	116	165.4448	**0.000000**
	Treatment	1	116	8.0382	**0.005405**
	Interaction	3	116	1.2665	0.289195
L3f rate	Temperature	3	116	17.70660	**0.000000**
	Treatment	1	116	76.15125	**0.000000**
	Interaction	3	116	4.83700	**0.003296**
L3w rate	Temperature	3	804	34.04813	**0.000000**
	Treatment	1	804	31.97582	**0.000000**
	Interaction	3	804	5.82749	**0.000611**
P rate	Temperature	2	669	179.3958	**0.000000**
	Treatment	1	669	0.1093	0.741031
	Interaction	2	669	1.1396	0.320557
Total development rate	Temperature	2	669	504.580	**0.000000**
	Treatment	1	669	29.3682	**0.000000**
	Interaction	2	669	2.8819	0.056725
Larva length	Temperature	3	523	0.50426	0.679494
	Treatment	1	523	17.50820	**0.000000**
	Interaction	3	523	2.09929	0.099353
Larva length (Isolated only)	Temperature	2	167	0.903044	0.407304
	Sex	1	167	1.344101	0.247967
	Interaction	2	167	1.242598	0.291288
Puparium length	Temperature	3	141	7.91243	**0.000065**
	Treatment	1	141	19.89400	**0.000017**
	Interaction	3	141	4.56404	**0.004398**
Puparium length	Temperature	2	27	1.03600	0.287406
	Sex	1	27	0.17800	0.676508
	Interaction	2	27	1.43900	0.254846

TABLE 3.2.3 – ANOVAs of the effects of temperature, treatment and sex on aspects of *Chrysomya putoria* development. *p*-values in **bold** are significant at $\alpha = 0.05$

Parameter	**Factors**	$\mathbf{df_{effect}}$	$\mathbf{df_{error}}$	F	p
Stadial proportions	Temperature	3	3		
L1 rate	Temperature	2	53	8621.326	**0.000000**
	Treatment	1	53	0.097	0.756773
	Interaction	2	53	0.175	0.839893
L2 rate	Temperature	2	53	33.03262	**0.000000**
	Treatment	1	53	0.86870	0.355542
	Interaction	2	53	0.18811	0.829073
L3f rate	Temperature	2	53	7.239441	**0.001661**
	Treatment	1	53	3.395163	0.070984
	Interaction	2	53	0.081101	0.922215
L3w rate	Temperature	2	322	45.15944	**0.000000**
	Treatment	1	322	92.11166	**0.000000**
	Interaction	2	322	3.87449	**0.021740**
P rate	Temperature	2	87	28.81240	**0.000000**
	Treatment	1	87	0.57241	0.451347
	Interaction	2	87	5.41807	**0.006059**
Total development rate	Temperature	2	87	267.0901	**0.000000**
	Treatment	1	87	1.0029	0.319381
	Interaction	2	87	8.5298	**0.000414**
Larva length	Temperature	2	369	2.901954	0.056170
	Treatment	1	369	7.431906	**0.006713**
	Interaction	2	369	0.222953	0.800260
Larva length (Isolated only)	Temperature				
	Sex				
	Interaction				
Puparium length	Temperature	2	100	26.55886	**0.000000**
	Treatment	1	100	8.42815	**0.004546**
	Interaction	2	100	2.15296	0.121493
Puparium length	Temperature	2	13	8.14700	**0.005088**
	Sex	1	13	1.52200	0.239118
	Interaction	1	13	2711.60900	**0.000000**

TABLE 3.3 – MANOVA results of the proportion of total development attributed to each developmental stage, in and between *L. sericata, C. chloropyga* and *C. putoria* at each rearing temperature. Proportions were arcsine transformed. *P*-values in **bold** are significant at $\alpha = 0.05$.

Species	**Factors**	**df_{effect}**	**df_{error}**	**F**	**p**
L. sericata	Temperature	3			
C. chloropyga	Temperature	6.5	10	72	**0.000000**
C. putoria	Temperature	10.825	10	14	**0.000056**
L. sericata/C. chloropyga	Temperature	15	284.74	212	**0.000000**
	Species	5	103	133.7	**0.000000**
	Interaction	5	103	122444	**0.000000**
L. sericata/ C. putoria	Temperature	10	68	14.5	**0.000000**
	Species	5	68	135.6	**0.000000**
	Interaction	5	68	183294	**0.000000**
C. chloropyga/C. putoria	Temperature	10	98	8.6	**0.000000**
	Species	5	49	2.3	0.058231
	Interaction	5	49	137757	**0.000000**

TABLE 3.4 – Wald-Wolfowitz tests for protandrous larval exodus and adult eclosion. *p*-values in **bold** are significant at $\alpha = 0.05$.

Species / Temperature (°C)	Stage	N (male)	N (female)	z-adjusted	p
L. sericata					
20	W	6	6	-0.303	0.762
	E	6	6	-0.303	0.762
25	W	10	11	0.908	0.364
	E	12	10	-0.180	0.857
30	W	16	13	0.323	0.747
	E	15	13	-0.166	0.868
35	W	4	1	-0.204	0.838
	E	4	1	-0.204	0.838
C. chloropyga					
20	W	10	9	0.486	0.627
	E	10	9	0.486	0.627
25	W	7	9	-0.198	0.844
	E	7	9	-0.198	0.844
30	W	6	2	-0.540	0.589
	E	6	2	-0.540	0.589
C. putoria					
25	W	3	8	-0.113	0.910
	E	3	8	2.364	**0.018**
30	W	2	2	-0.612	0.540
	E	2	2	0.612	0.540
C. megacephala					
25	W	3	2	0.109	0.913
	E	3	2	0.109	0.913

TABLE 3.5 – Developmental parameters for *Chrysomya chloropyga, C. putoria and Lucilia sericata* to developmental landmarks. Stadial abbreviations: E1 = first ecdysis, E2 = second ecdysis, W, P and E as in text.

Species	Stadium	K (°h)	D_0 (°C)	R^2	d.f. (n-1)
C. chloropyga	E1	128	15.5	0.8673	5
	E2	665	12.7	0.9094	5
	W	1894	8.4	0.9362	7
	P	2161	10.5	0.9238	3
	E	4270	9.5	0.9046	5
C. putoria	E1	120	16.3	0.8142	5
	E2	836	11.8	0.9449	5
	W	1874	9.0	0.9839	5
	P	2277	9.2	0.8764	5
	E	5076	6.9	0.9520	5
L. sericata	E1	560	10.2	0.8265	7
	E2	636	13.0	0.9792	7
	W	1286	11.3	0.9441	7
	P	2298	9.8	0.9826	3
	E	8823	3.0	0.9447	5

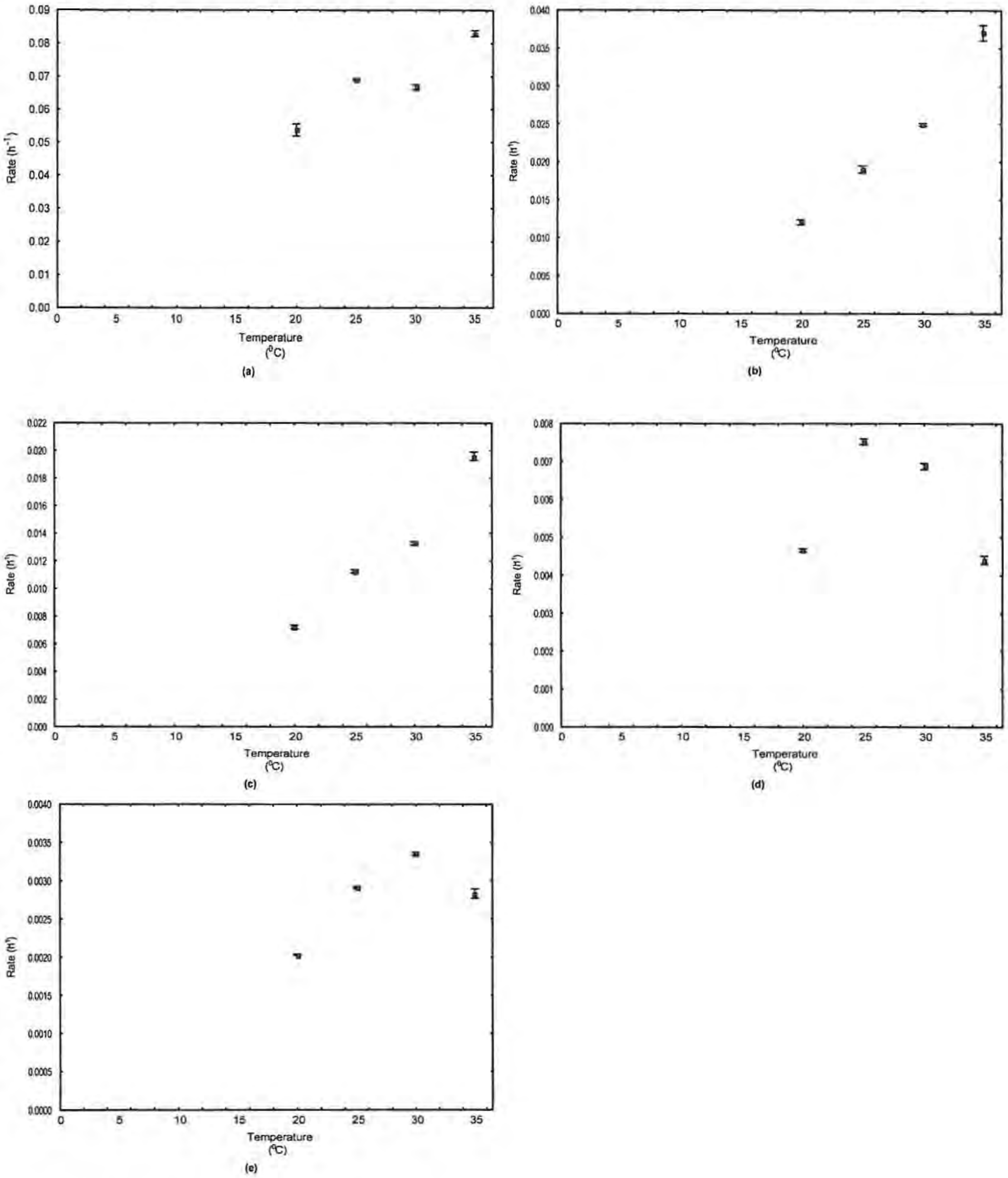

FIGURE 3.1.1 – Cumulative development rates to landmarks for *Lucilia sericata* (a) first ecdysis, (b) second ecdysis, (c) wandering, (d) pupariation, (e) eclosion.

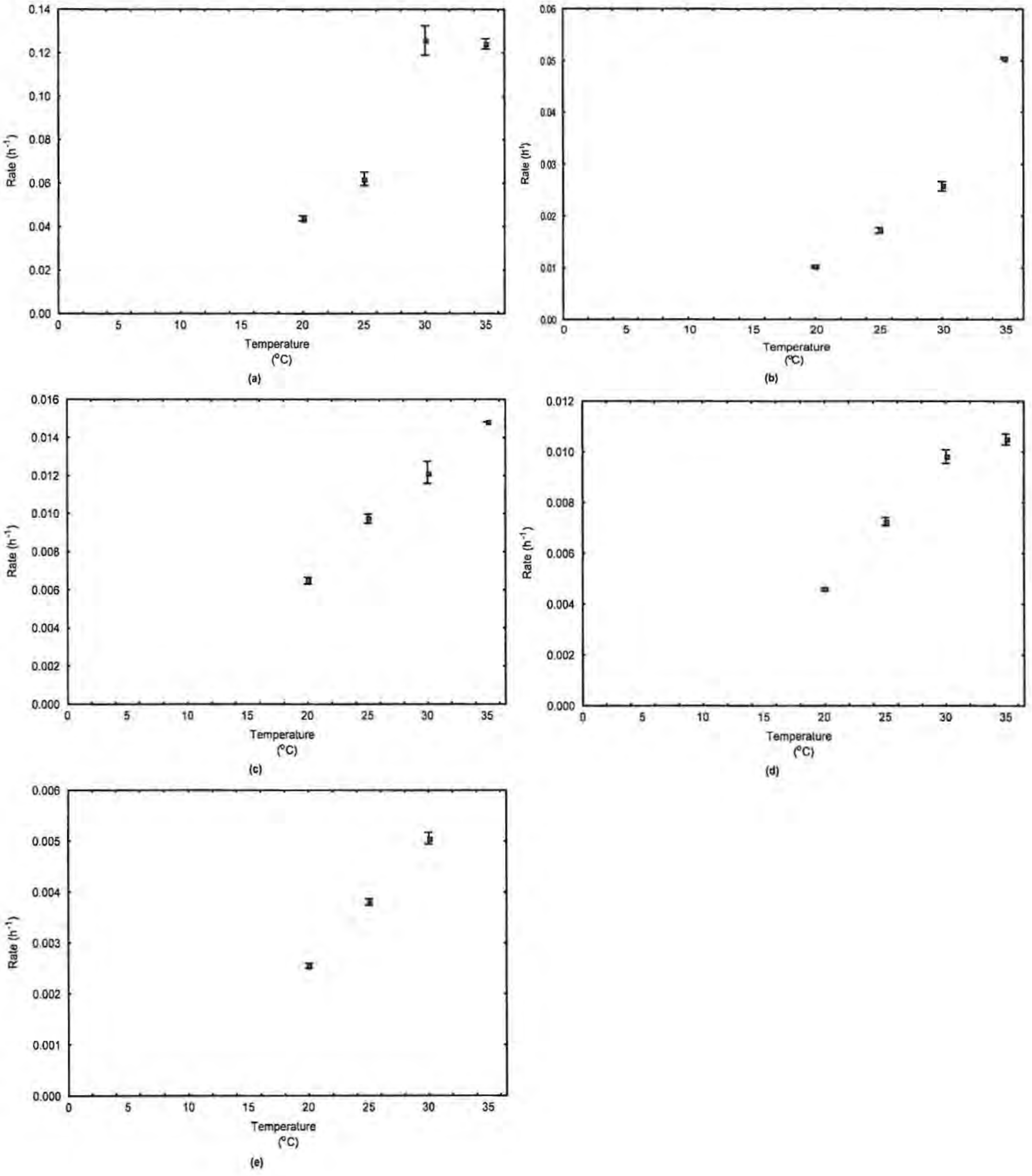

FIGURE 3.1.2 – Cumulative development rates to landmarks in the lifecycle of *C. chloropyga*. (a) first moult; (b) second moult; (c) larval exodus; (d) pupariation; (e) adult eclosion.

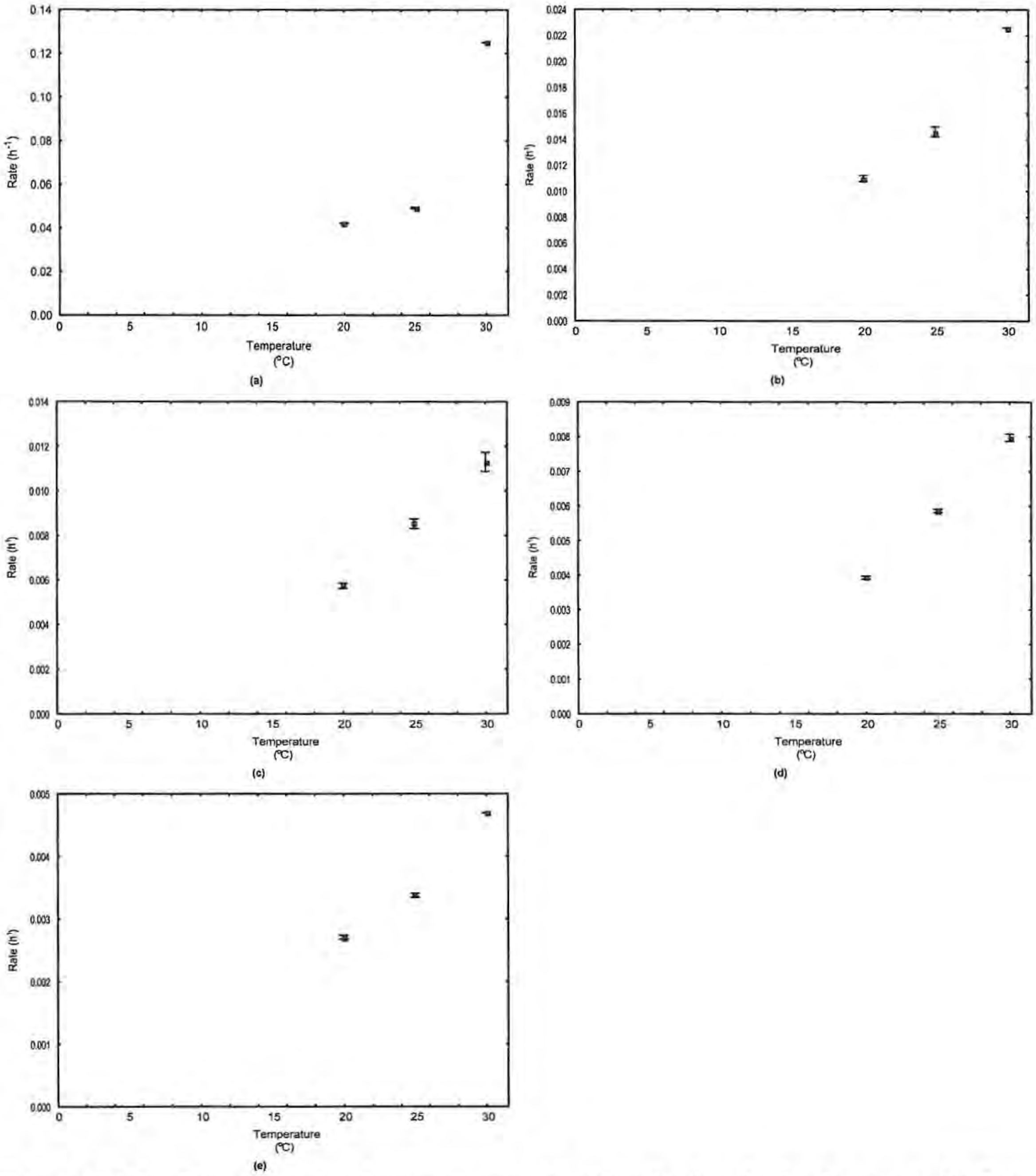

FIGURE 3.1.3 – Cumulative development (mean +/- SE) to landmarks for *C. putoria* (a) first ecdysis, (b) second ecdysis, (c) wandering, (d) pupariation (e) eclosion.

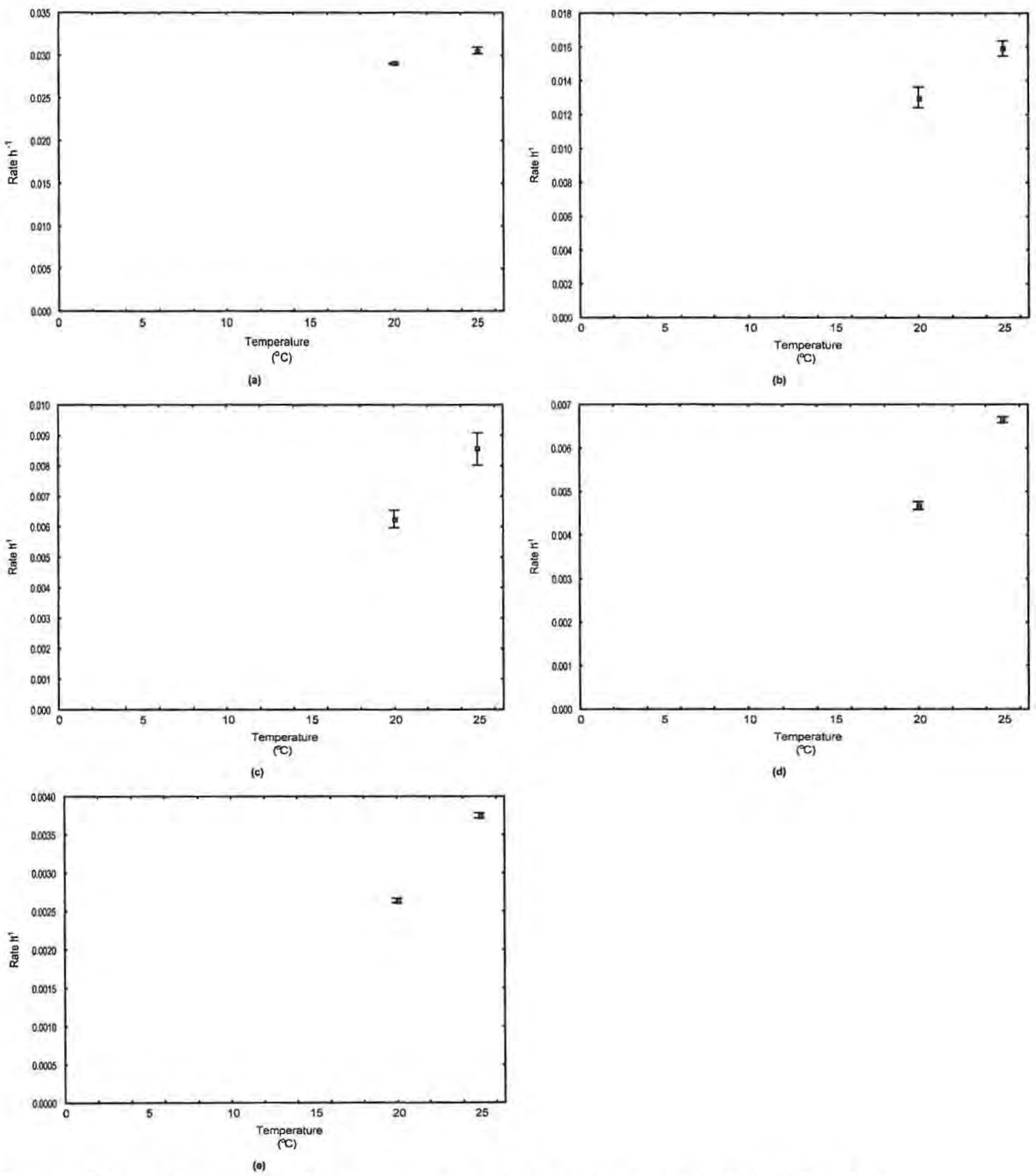

Figure 3.1.4 - Cumulative development rates to landmarks in the lifecycle of *C. megacephala*. (a) first moult; (b) second moult; (c) larval exodus; (d) pupariation; (e) adult eclosion.

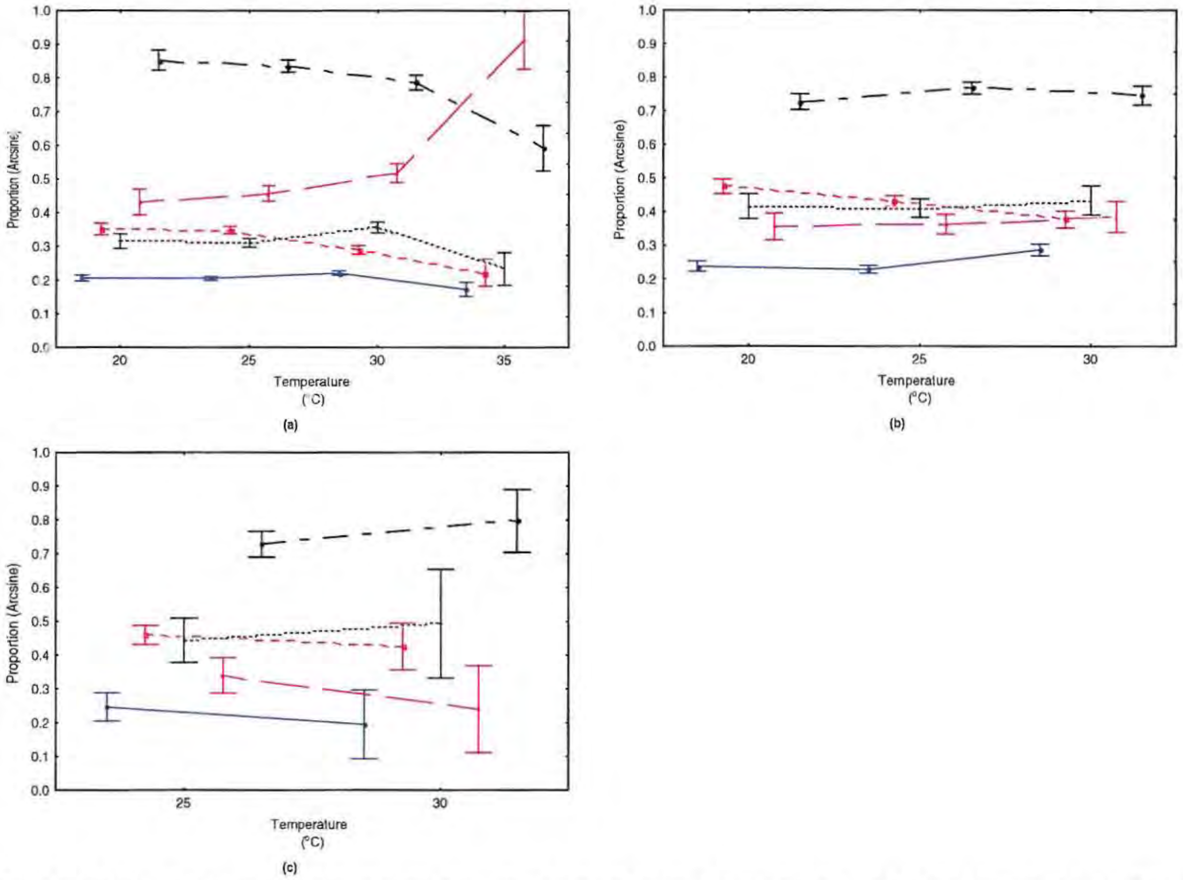

FIGURE 3.2 – Interaction plots of the arcsine-transformed proportions of development spent in each developmental stage for (a) *Lucilia sericata*; (b) *Chrysomya chloropyga*; (c) *Chrysomya putoria*. • L1;   L2; ◊ L3f; Δ L3w; o P.

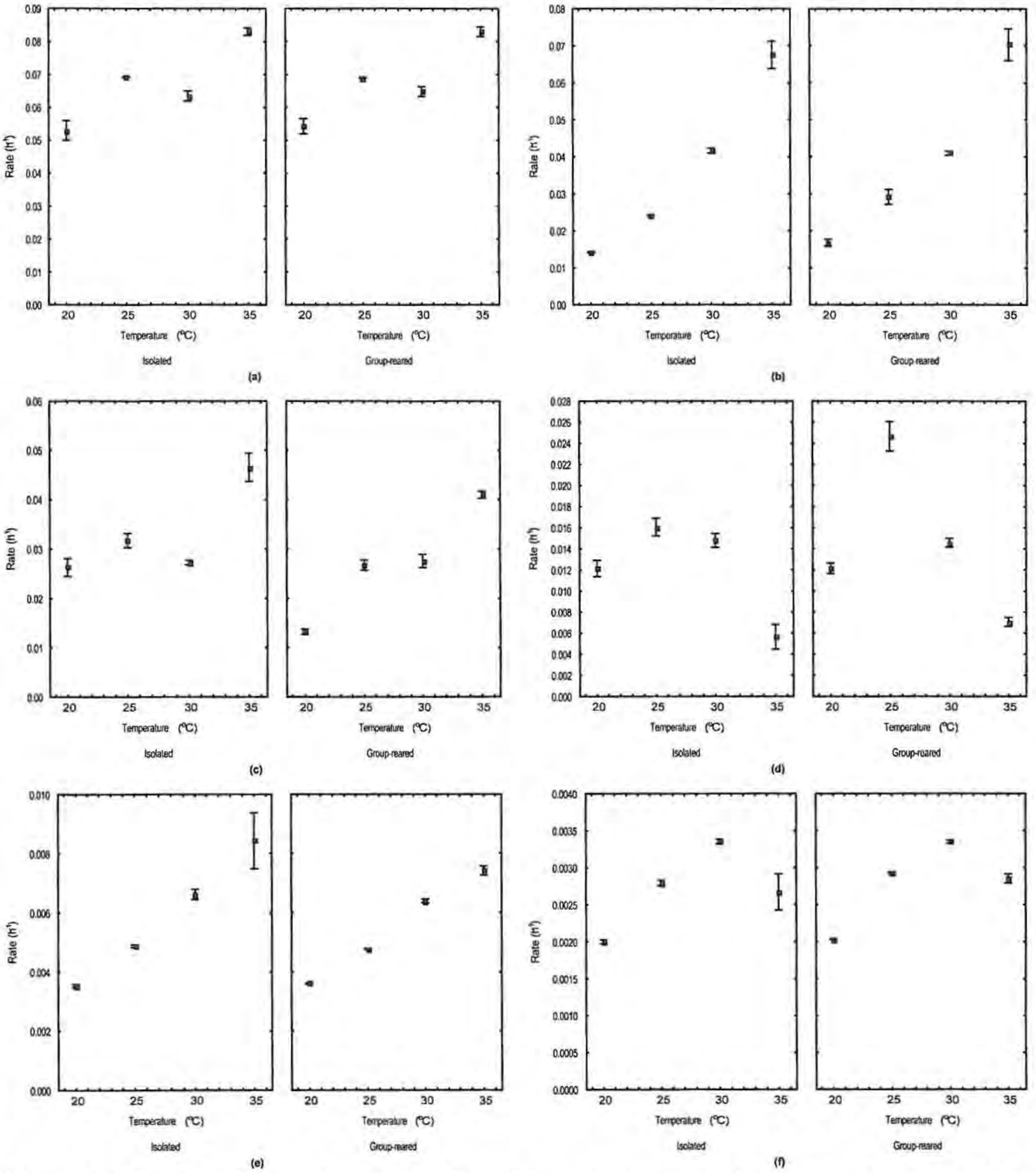

FIGURE 3.3.1 – Development of stadia of *Lucilia sericata* categorized by treatment. (a) L1, (b) L2, (c) L3f, (d) L3w, (e) P, (f) Total development.

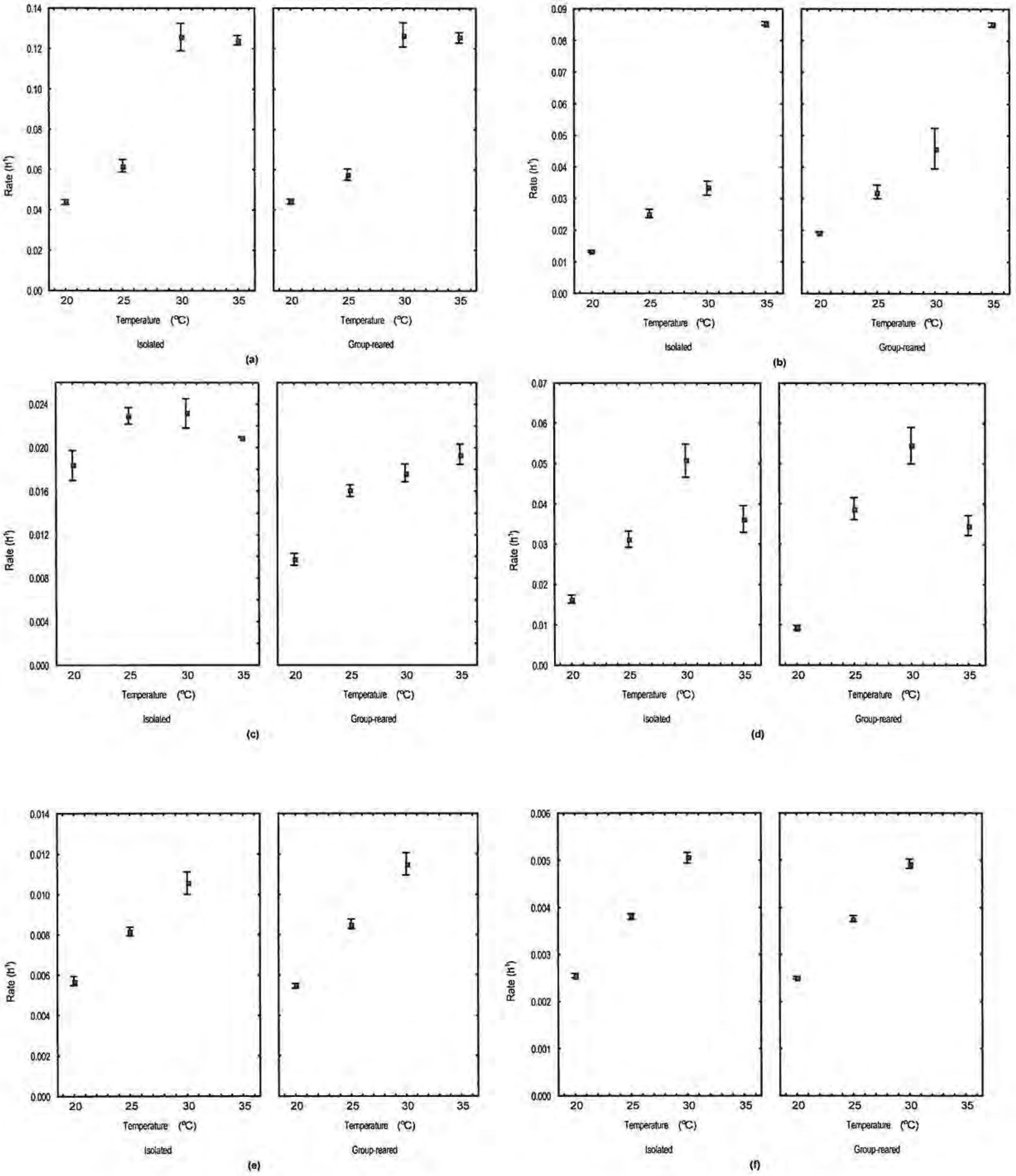

FIGURE 3.3.2 – Development rates of stadia of *C. chloropyga* categorized by treatment. (a) L1; (b) L2; (c) L3f; (d) L3w; (e) P; (f) total development.

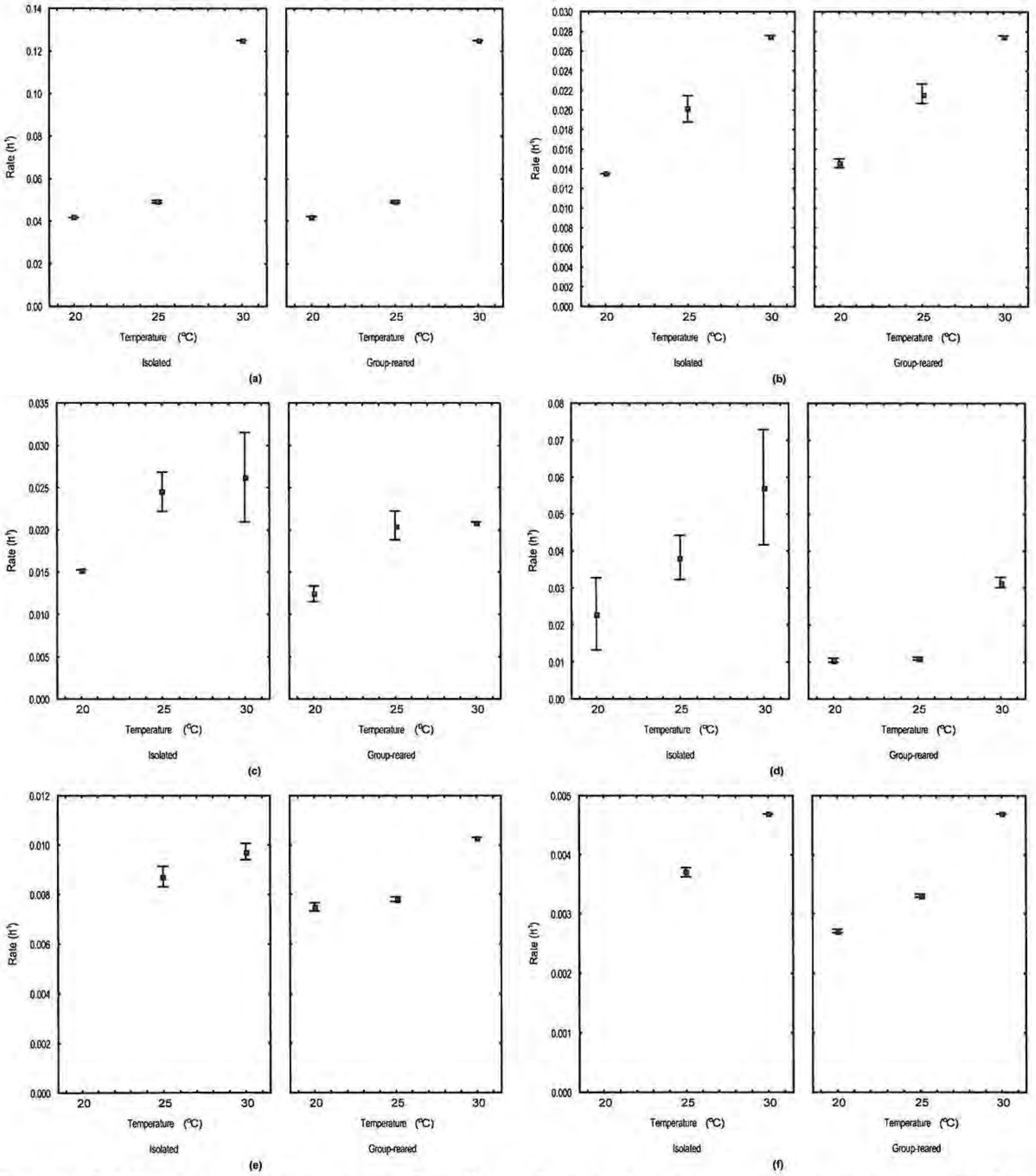

FIGURE 3.3.3 - Development rates of stadia of *C. putoria* categorized by treatment. (a) L1, (b) L2, (c) L3f, (d) L3w, (e) P, (f) total development.

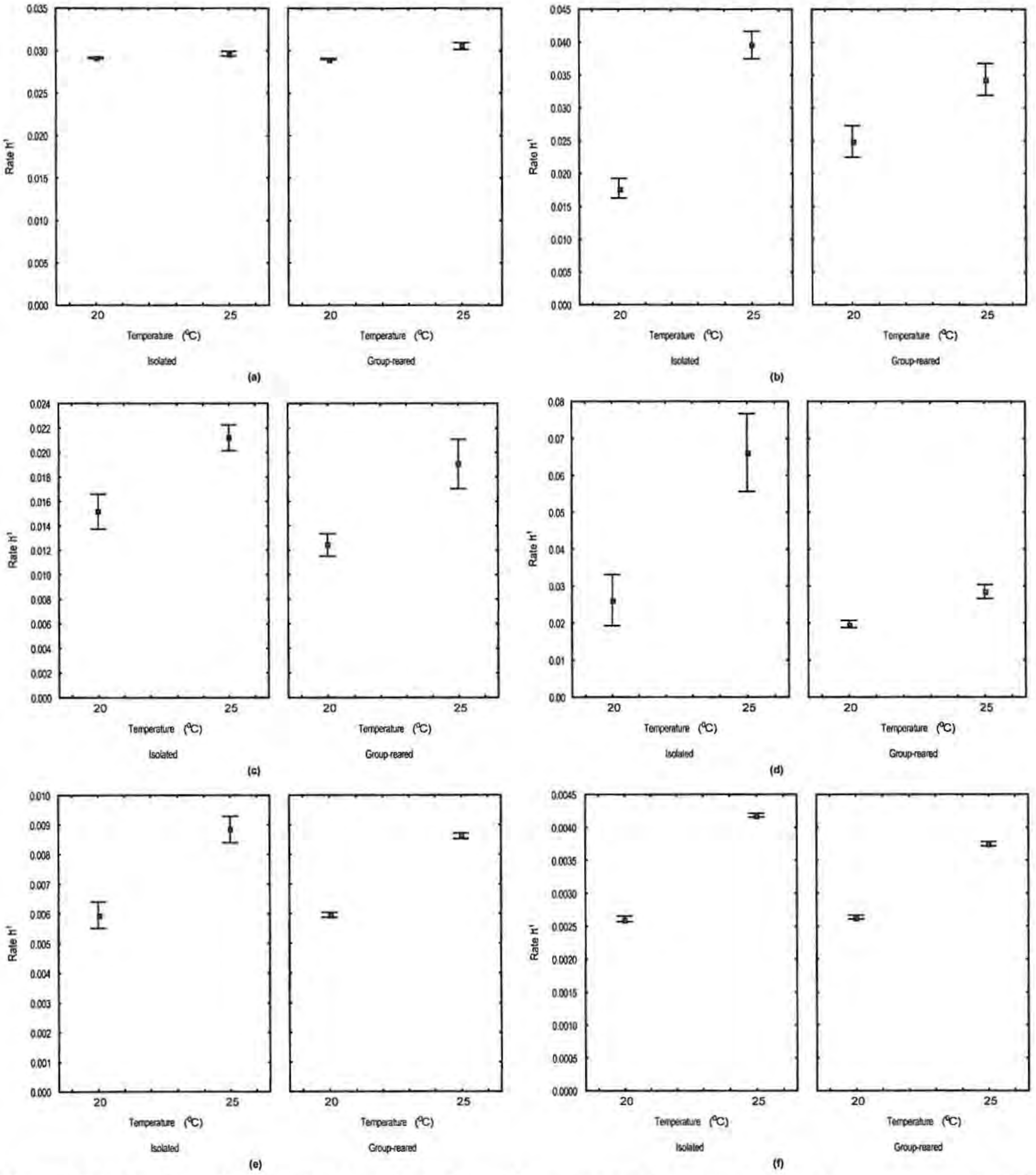

Figure 3.3.4 - Development rates of stadia of *C. megacephala* categorized by treatment. (a) L1; (b) L2; (c) L3f; (d) L3w; (e) P; (f) total development.

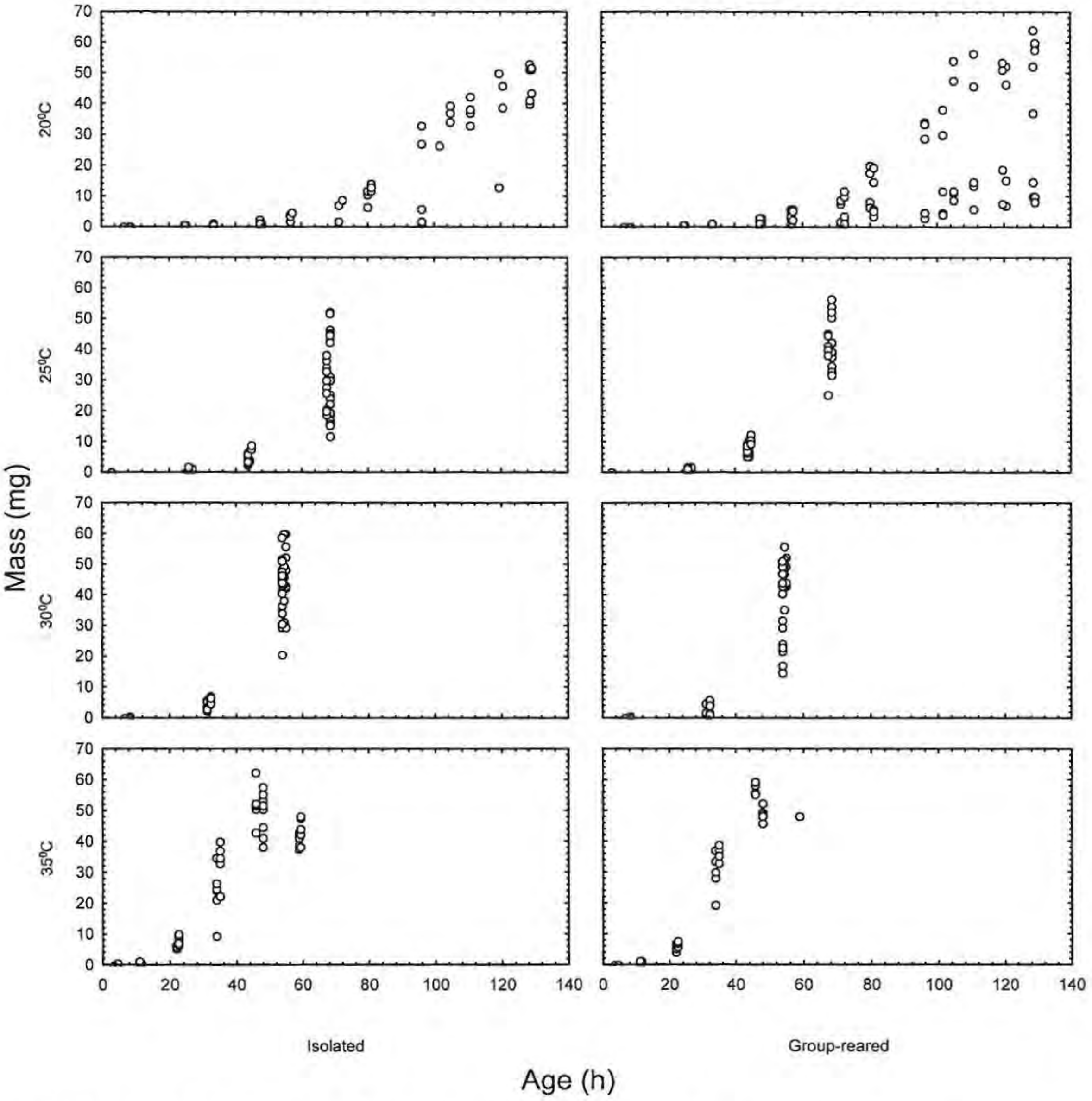

FIGURE 3.4.1 – Larval growth of *Lucilia sericata* categorized by temperature and treatment.

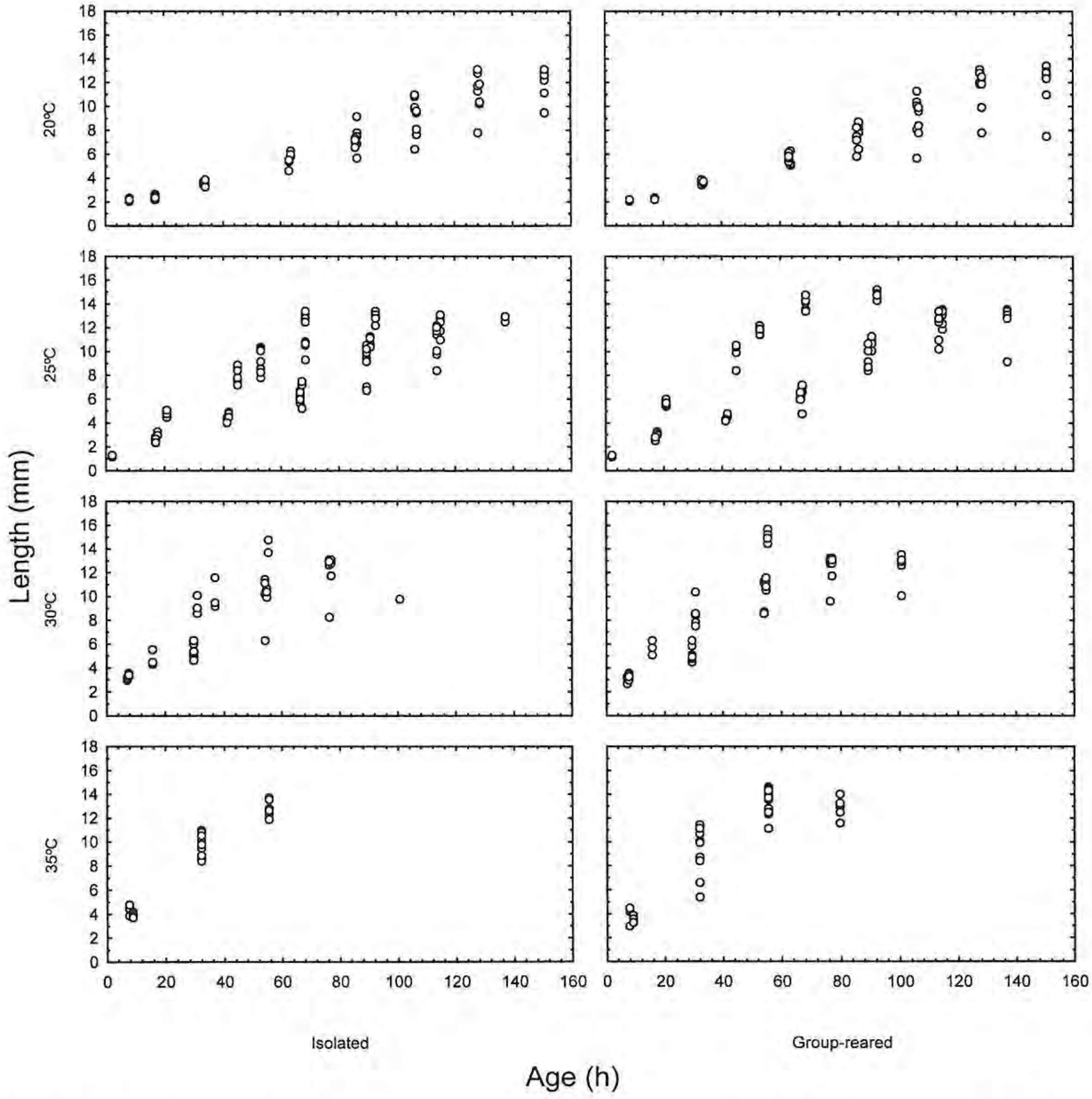

FIGURE 3.4.2 – Larval growth of *Chrysomya chloropyga*, categorized by temperature and treatment.

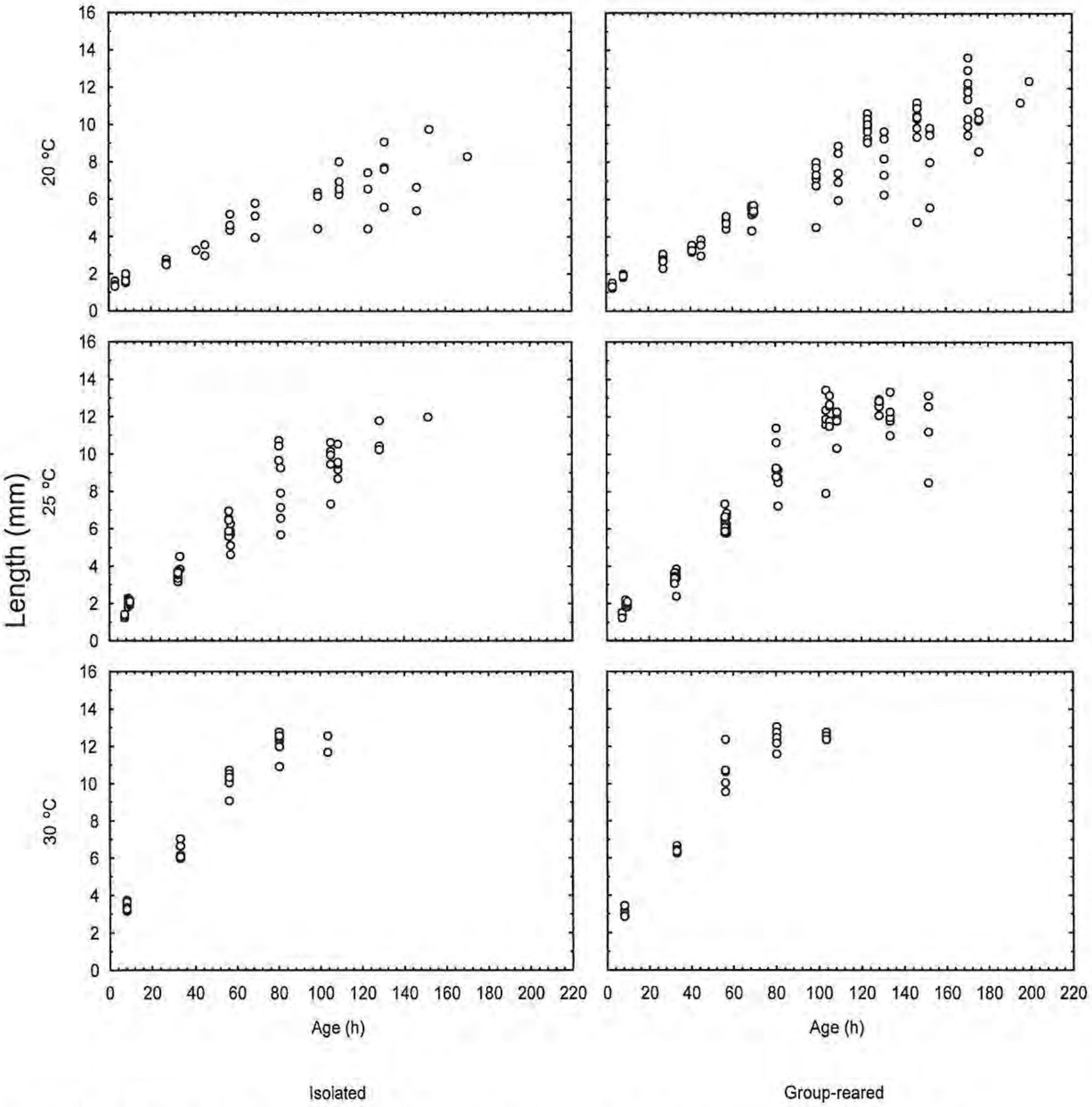

FIGURE 3.4.3 – Larval growth of *C. putoria* categorized by temperature and treatment.

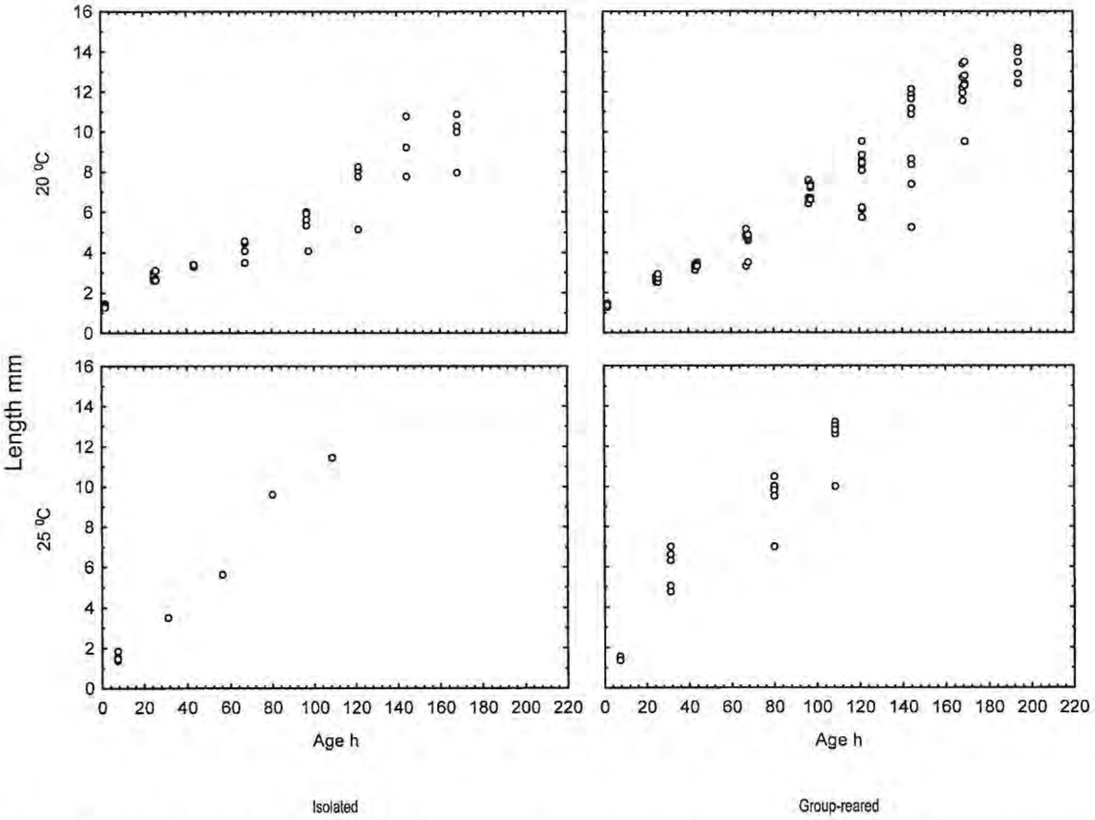

Figure 3.4.4 - Larval growth of *Chrysomya megacephala*, categorized by temperature and treatment.

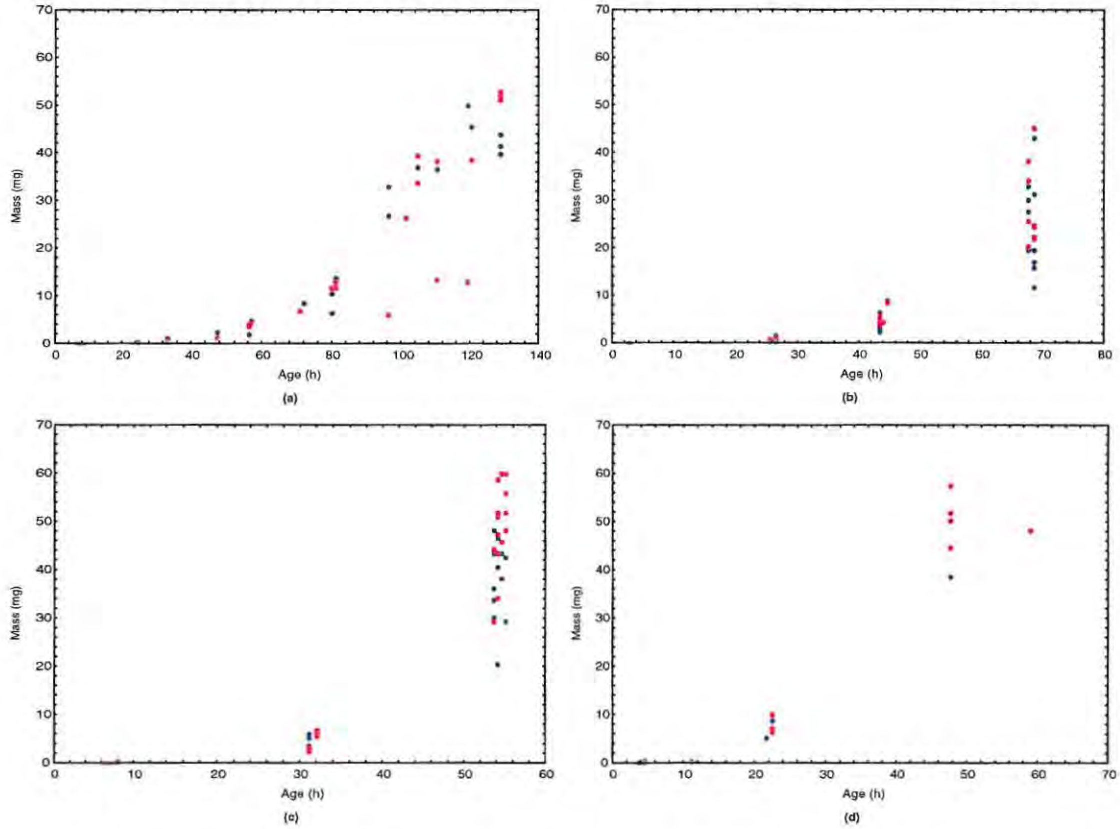

FIGURE 3.5.1 – Body masses of isolated *Lucilia sericata* larvae categorized by temperature and sex. (a) 20°C; (b) 25°C; (c) 30°C; (d) 35°C. ● Male; female. ○

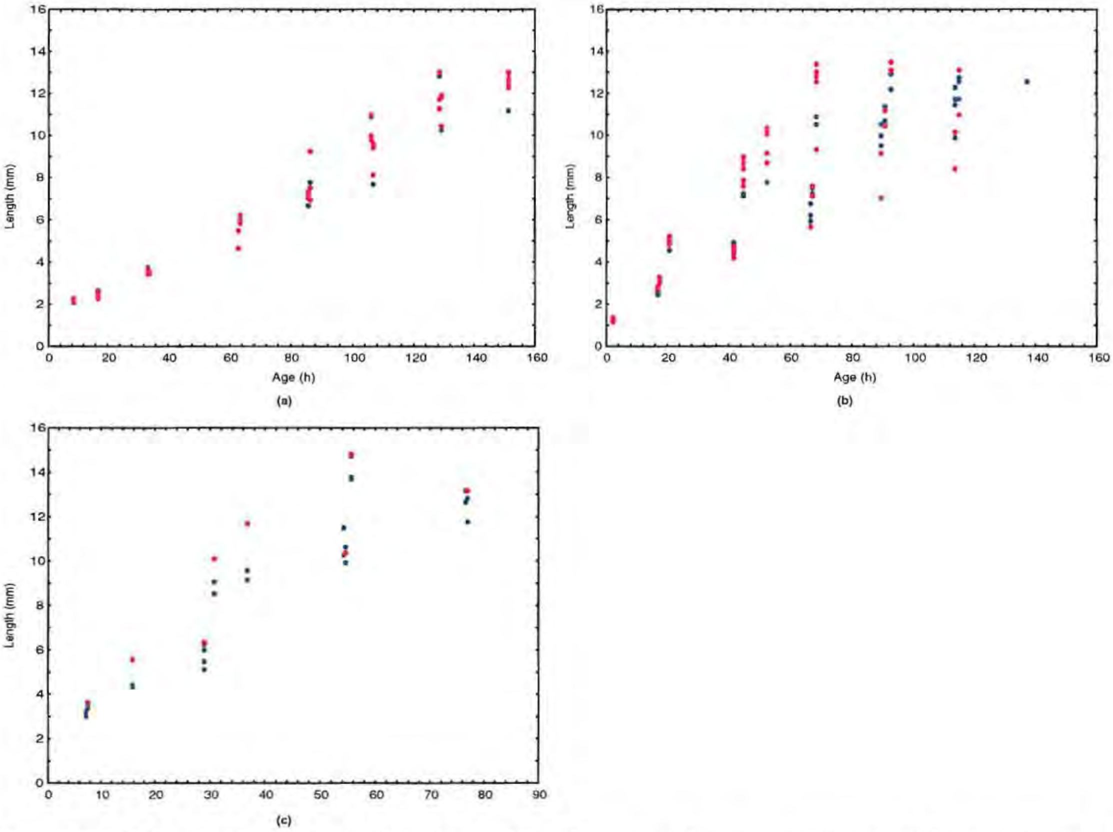

FIGURE 3.5.2 – Body masses of isolated *C. chloropyga* larvae, categorized by temperature and sex. (a) 20°C; (b) 25°C; (c) 30°C. • Male; ○Female. The sexes of larvae reared at 35°C were not determined, due to 100% mortality.

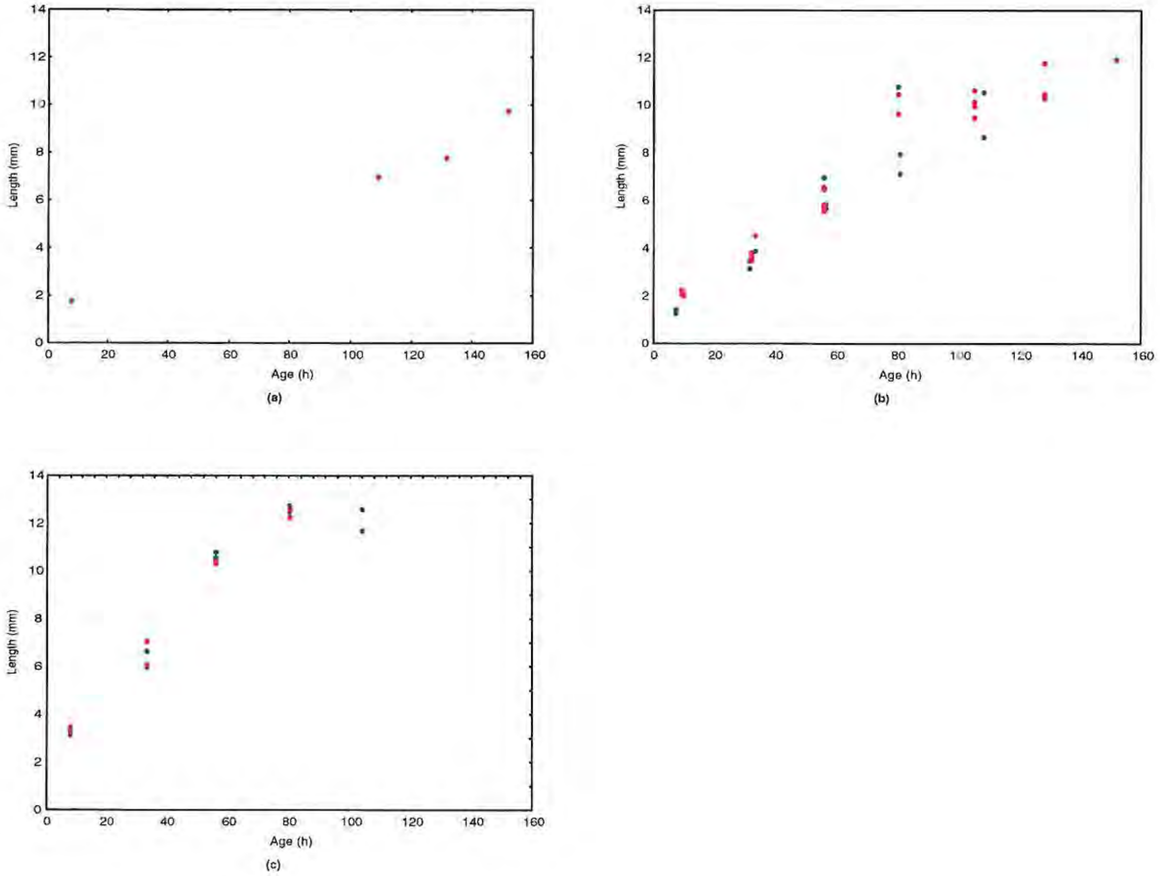

FIGURE 3.5.3 – Larval growth (isolated maggots only) of *C. putoria*, categorized by temperature and sex. (a) 20°C; (b) 25°C; (c) 30°C. • Male;○ female.

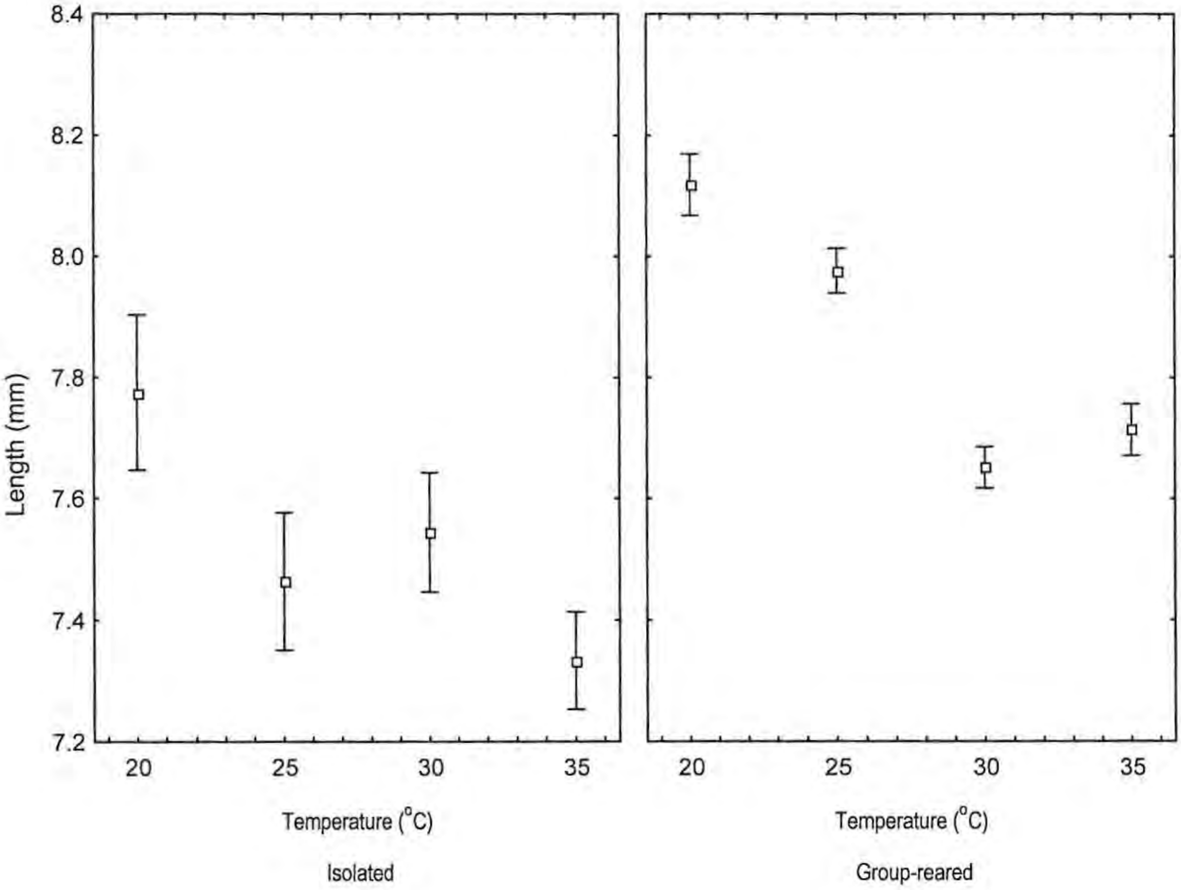

FIGURE 3.6.1 – Length (mean ± SE) of puparia of *L. sericata*, categorized by treatment.

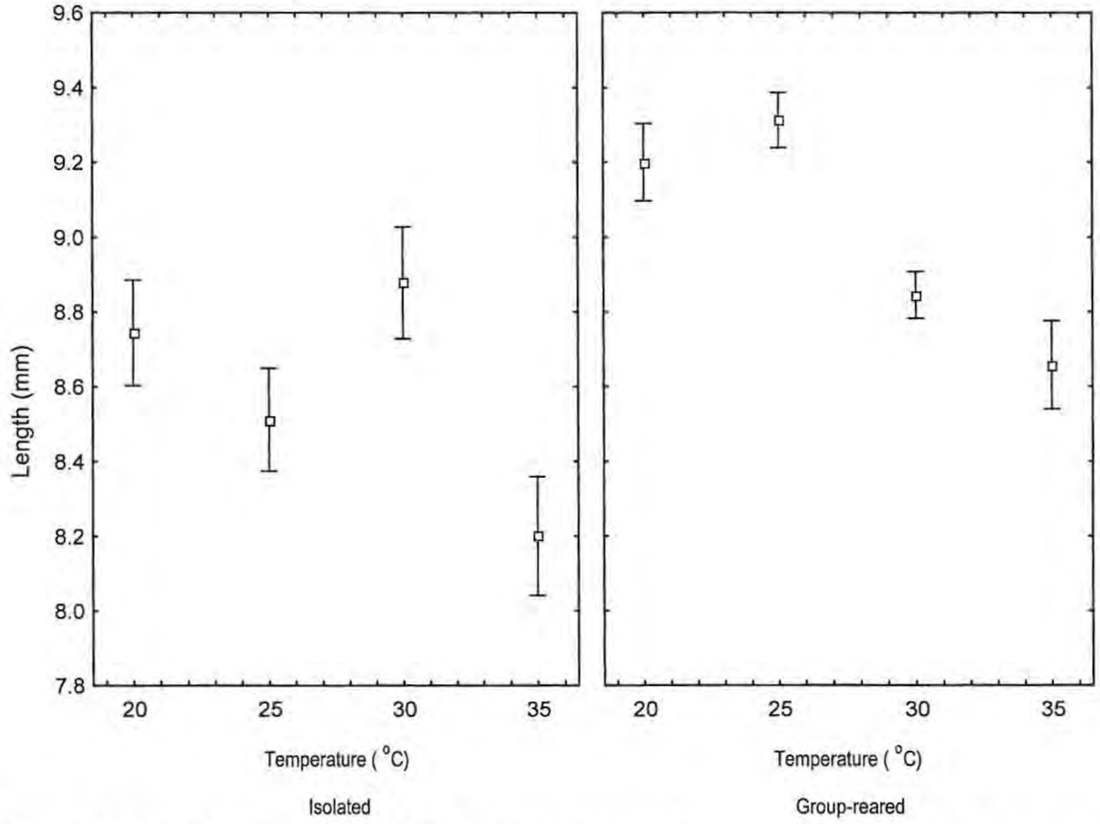

FIGURE 3.6.2 – Lengths (mean ± SE) of *C. chloropyga* puparia, categorized by treatment.

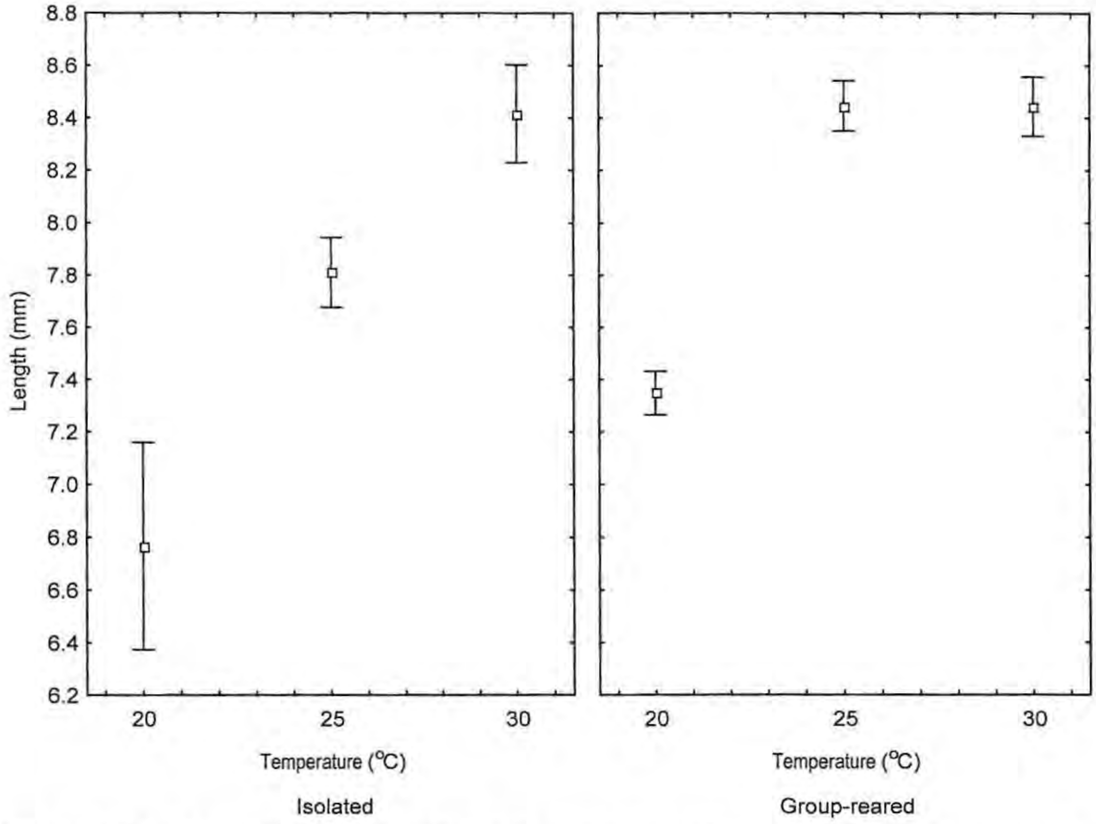

FIGURE 3.6.3 – Lengths (mean ± SE) of *C. putoria* puparia, categorized by treatment.

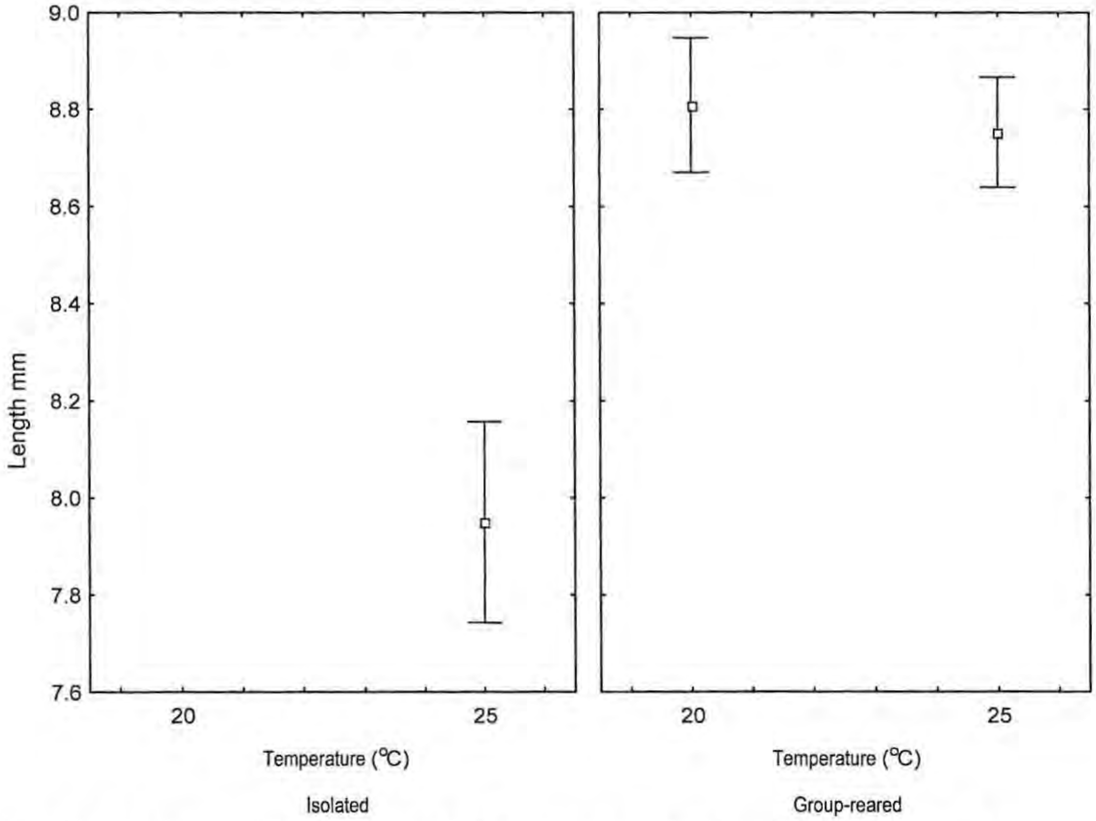

Figure 3.6.4 – Lengths (mean ± SE) of *C. megacephala* puparia, categorized by treatment.

Chapter 4

Combining Phylogeny And Development:

The Use Of Phylogeny For Predicting Developmental Parameters Of Forensically Important Flies

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4.0 – Introduction

The efficient use of fly larvae as indicators of the postmortem interval (PMI) of a corpse hinges on an investigator's ability to accurately determine the ages of the maggots or pupae present at a crime scene, and the lag between the time of death and oviposition by flies (Anderson, 2000). Cognisance of the factors that may affect maggot growth or corpse colonization (Catts, 1992; Goff & Lord, 1994; Christopherson & Gibo, 1997) has allowed investigators to utilise immature flies to estimate PMI with a high level of accuracy in a great number of cases worldwide (e.g. Smith, 1986; Kulshresha & Chandra, 1987; Lord, 1990; Anderson, 1997, 1999; Benecke, 1998; Benecke & Lessig, 2001; Byrd & Allen, 2001a; Hall, 2001).

The basic tenet underlying the use of maggots in PMI estimation is that their ages can be estimated if the environmental temperatures can be ascertained, since heterotherm development rates are largely dependent on temperature (Higley & Haskell, 2001). Provided that the environmental temperatures to which the maggots were exposed did not fluctuate excessively - especially at or near the thermal thresholds - the PMI estimate can be very accurate and fairly precise (Introna *et al.*, 1989; Worner, 1992; Davies & Ratcliffe, 1994; Higley & Haskell, 2001). However, any PMI estimate can only be as reliable as the data used to calculate it. Forensic investigators currently have two options available to them for acquiring the necessary developmental data. Firstly, they can identify the maggots (or resultant adults if larvae are difficult to identify) and refer to the literature or previous case studies to determine the age of the maggots at collection (e.g. Anderson, 1997; 1999). Alternatively, if the developmental data for the appropriate species are unavailable or unreliable, a second generation may be reared at appropriate temperatures and the necessary information gathered *ad hoc*. The second system is useful if one suspects that there is geographical variation in the estimation parameters, but it is also problematic for several reasons, not least of which is that it is time consuming. Additionally, in some cases, only dead maggots are available, making "rearing through" impossible (Smith, 1986; Wells *et al.*, 2001; Wells & Sperling, 2001), some species are difficult to rear in the laboratory (e.g. *Chrysomya albiceps, C. marginalis* and *Calliphora croceipalpis*, pers. obs.), and the accurate determination of the lower thermal threshold for development - which is essential for precise PMI determination - requires development rates to be investigated at several temperatures.

There are many publications of developmental data for a wide variety of forensically important taxa. Unfortunately, most of the earlier studies did not explicitly take into account the effects of maggot activity in dense aggregations. High maggot densities may raise the substrate temperature several degrees above ambient temperature, as observed by Payne (1965) and Goodbrod & Goff (1992). Furthermore, in several earlier cases, the ambient and substrate temperatures may not have been accurately measured or controlled (Hobson, 1932a,b; Cuthbertson, 1933; Hafez, 1940; Wijesundara, 1957b; Alwar & Seshiah, 1958; Kamal, 1958; Buri, 1959; Norris, 1965; Dasgupta & Roy, 1969; Abasa, 1970; Healey, 1970; Ash & Greenberg, 1975; Levot *et al.*, 1979; Subramanian & Raja-Mohan, 1980; Nawal *et al.*, 1982; Prins, 1985; Madubunyi, 1986). More recently, studies have been aimed at providing parameters exclusively for PMI estimation, with the added refinements of the maintenance of low larval densities and temperature control, and even incorporating the effects of drugs on larval development (O'Flynn, 1983; Reiter, 1984; Nishida *et al.*, 1986; Goff *et al.*, 1989, 1991, 1992, 1993, 1994, 1997; Goodbrod & Goff, 1990; Catts, 1992; Davies & Ratcliffe, 1994; Wells & Kurahashi, 1994; Byrd & Butler, 1996, 1997, 1998; Queiroz, 1996, 2001; Sadler *et al.*, 1997; Bourel *et al.*, 1999; Anderson, 2000; Grassberger & Reiter, 2001). However, the majority of these studies have focused on commonly encountered forensic flies or species that are easily reared in the laboratory. In view of these difficulties, an alternative and rapid means of determining growth parameters and maggot age would be useful. To date, phylogenetics and development have been viewed as separate fields, but the two potentially have strong links.

Sharpe & DeMichele (1977) illustrated that the kinetics of metabolic enzymes ultimately control development rates in heterotherms. Every enzyme has a lower (T_L) and upper (T_U) thermal threshold below and above which, respectively, its action will be inhibited. At some point between these two thresholds, the optimal temperature (T_{opt}) is reached, where the rate of activity of the enzyme reaches a maximum. Similarly, enzymes work optimally within a specific pH range. The overall metabolic - and further, growth - rate is limited by the slowest enzyme-mediated step of the process. Ignoring the effects of pH, the growth-rate limiting enzymes would theoretically be those with the highest T_L and the lowest T_U. There must also be an overall optimal temperature for development of the organism that is at, or close to, the optimal temperature for each metabolic enzyme. It follows, then, that for a heterotherm to be successful, all of its metabolic enzymes must have similar thermal tolerances and optima. Further, the organism must reside in a thermal regime that promotes enzymatic activity.

The tertiary, 3-dimensional (conformation) and quaternary (polymerisation) structures of an enzyme are dependent on the amino acid sequence and thus the pattern of folding of the peptide backbone and bonding between side-chains of the constituent amino-acids (McMurry, 1994). Each conformational arrangement of a metabolic enzyme has a unique temperature and pH optimum; beyond the thermal and pH thresholds and in reducing conditions, hydrogen bonds and disulphide bonds are broken, resulting in inactivation or denaturation of the enzyme. Since enzymes are proteins, their amino acid sequences are encoded in DNA in the form of triplet-base codons. Protein-coding genes evolve relatively slowly because nucleotide insertions or deletions (indels) and substitutions can alter the resultant amino acid sequence and affect the tertiary and quaternary structure of the protein. Such a change may result in the production of non-functional proteins or enzymes that would be selected against. However, there are synonymous codons (i.e. different codons that code for the same amino acid), so that "silent" nucleotide substitutions may occur that do not affect protein structure (Rodriguez *et al.,* 1990; Simon *et al.,* 1994).

Based on the knowledge that the development rates are enzyme-mediated, and that enzyme structure has a genetic basis, it is logical to assume then, that if related species have similar nucleotide sequences - including "silent substitutions" - they would have similar pH and thermal thresholds and optima. Thus, there is the potential that a robust phylogeny may be used to predict developmental parameters such as the developmental constant (K) and the lower developmental threshold temperature (D_0). This hypothesis relies heavily on two assumptions: firstly, that the species' phylogeny and the enzyme's (gene) phylogeny are congruent, and secondly that "noise" in the form of climatic or geographical adaptations by species does not override phylogeny. Over the past decade, the influence of shared ancestry in comparative physiology has been investigated and refined (Felsenstein, 1985; Harvey, 1996; Garland *et al.*, 1999; Garland & Ives, 2000), and statistical software to deal with phylogeny-based physiological trends has been developed (Garland & Ives, 2000).

The purpose of this paper is to (a) determine if predictions about developmental parameters (*viz.* K and D_0) can be made using phylogeny, (b) discover to what taxonomic level these predictions are accurate, and highlight any drawbacks to the method, and (c) determine if there are any phylogenetic trends in the proportion of the lifecycle spent in each stadium, as found in Anderson (2000) and Chapter 3. The overall aim is to provide forensic investigators with a further tool to make accurate predictions about the development of species that have not been reared under laboratory conditions, or for which insufficient data are available.

4.1 - Materials and Methods

4.1.1 – Acquisition of developmental data

Developmental data for a variety of forensically important Calliphoridae and Sarcophagidae were collated from the literature and the experimental results in Chapter 3 (Table 4.1). Where possible, more than one source of information was used for each species to allow inter-study comparisons to be made and to provide more data points for K and D_0 calculations.

If the published data included sufficient detail, the cumulative development times from hatching (H) to first ecdysis (E1), second ecdysis (E2), larval exodus (W), pupariation (P) and adult eclosion (AE) were recorded. The duration of egg incubation was not included in the cumulative times to allow for comparisons between oviparous and ovoviviparous species to be made, and also because egg retention by adult females has been shown to confound incubation estimates (Wells & King, 2001). Thus, any studies that did not explicitly separate oviposition from larval development were excluded from the analysis (Table 4.1).

4.1.2 – Determination of K and D_0 using the accumulated day degree (ADD) method

For each species, the development rates were calculated for each developmental stage for which data were available, and were plotted against temperature. Following Allen (1976) and Wagner *et al.* (1984), a Least Squares Regression (LSR) line with 95% confidence intervals was fitted through the linear portion of each graph. Points that lay either outside the 95% confidence limits or beyond the temperature at which development was most rapid, were then culled from the data set. Using the remaining data, K (in metric degree hours, °h) was calculated as the inverse of the gradient of the regression line, and D_0 (°C) was the ratio of the *y*-axis intercept to the gradient of the line. The fit (R^2) and degrees of freedom (n-1) were calculated to give an indication of the goodness-of-fit of the regression line.

4.1.3 – Determination of K and D_0 using the Ikemoto & Takai (2000) linearised formula (LF) method

In order to calculate K and D_0 with this method, DT values (D = duration of stadium, T = temperature) were plotted against D. The points were joined in temperature order, and *z*- or *N*-shaped graphs were produced. Points that lay at an acute angle to the major linear portion of the graph were discarded from the data set prior to the calculation of regression lines. If fewer than three linearly-arranged points were retained, this method was not used, because the resultant regression would be meaningless. Since D and DT do not have a causal relationship, Reduced Major Axis Regression (RMAR) (Sokal & Rohlf, 1981) was applied in preference to LSR. The slope of the regression line (s_y/s_x) is equivalent to the developmental zero (D_0), and the *y*-axis intercept describes the developmental constant, K. R^2 values and degrees of freedom were calculated as for the ADD method.

4.1.4 - Elucidation of phylogenetic trends in K and D_0 using PDTree v. 5.0 (Garland et al., 2002)

When K was plotted against D_0, a negative correlation was evident (Fig. 4.4). To determine the influence of species' relatedness on physiological parameters, PDTree software (Garland *et al.*, 2002) was employed, and Felsenstein's independent contrasts (IC) method was applied to the data set. Due to its high bootstrap support, the neighbor-joining tree derived from the conservative data set (Fig. 2.2.1) was used as a baseline phylogeny. After importation into PDTree, species for which no physiological data were available were trimmed from the tree.

To determine the strength of prediction, taxa with known physiological characteristics were trimmed from the tree (Table 4.4), the tree was rerooted on the trimmed node and the IC method was applied to the remainder of the data set. Ninety-five percent prediction intervals for K and D_0 were calculated, for the "hypothetical" descendent, with the branch length adjusted from the mean branch length calculated by PDTree to the actual branch length derived from phylogenetic analysis. Thereafter, the calculated K and D_0 values of the trimmed branches or taxa were compared to the values predicted by PDTree. Additionally, prediction intervals were calculated for taxa for which no physiological data were available.

4.1.5 - Comparison within and among species and genera, of the proportion of total development assigned to each developmental stadium

Due to differences in terminology and methods of various authors, two separate analyses were run to maximise the number of species included in stadial proportion comparisons.

(i) Each stadium was treated separately (i.e. L1, L2, L3f, L3w, P).

(ii) The larval stadia (L1 to L3w) were combined, with pupariation (P) treated separately.

The proportions of total development assigned to all stages were arcsine-transformed (Zar, 1996) in order that the distributions approached normality prior to statistical analysis. ANOVAs, with species and temperature as factors and stadial proportions as dependent variables, were undertaken to determine if the time apportionments of each stage of the lifecycle were comparable within and among temperatures and congeneric species. Incubation was excluded because several species (e.g. *Sarcophaga* spp.) are larviparous.

4.2 - Results

4.2.1 - Comparison of K and D_0 values derived from the Accumulated Day Degree (ADD) model and the Ikemoto & Takai (2000) (LF) model

Tables 4.2.1 - 4.2.6 detail the K and D_0 values to each developmental landmark (H, E1, E2, W, P, AE) calculated for each species in the analysis. The results from the ADD and LF methods were comparable in the majority of cases where the fit of the regression line (R^2) exceeded 0.98. However, when R^2 values were mediocre to poor in either method, and when the data from different studies were widely divergent, the calculated parameters differed markedly (Fig. 4.1.1 - 4.1.24; 4.2.1 - 4.2.24; Tables 4.2.1 - 4.2.6). When data from different studies were obviously divergent (e.g. Fig. 4.1.19; 4.2.19), the determination of outliers was easier with the ADD method, so the R^2 values tended to be greater than the corresponding LF values. Large differences in developmental durations at similar temperatures, or similar durations at quite different temperatures, resulted in a "zig-zag" pattern being produced by the LF method; the differences were exaggerated on the *y*-axis and the determination of which points were displaced from the linear section of the relationship became subjective (e.g. *C. vicina* - Fig. 4.2.9).

4.2.2 - Changes in D_0, and the pattern of increase in K with the progression of development

During larval development, the developmental zero fluctuated slightly for all species, although the amplitudes of the changes were relatively small, rarely exceeding 2°C between stadia (Tables 4.2.2 – 4.2.4). Although the threshold temperature fluctuated by up to 5°C from egg incubation to adult eclosion (*C. rufifacies* - Tables 4.2.1 – 4.2.6), there was no clear pattern of fluctuations with development, nor were there similarities in fluctuations between congeneric species. Between larval exodus and adult eclosion, 11 species exhibited a change in D_0 of less than 1°C, the thresholds of eight species differed by between 1°C and 3°C, and only three species showed a change of more than 4°C in either direction (Tables 4.2.5 – 4.2.6).

In all species and at all temperatures, the puparial period comprised more than 40% of the total development time (Fig. 4.3.1). In general, the puparial proportion was greatest in the Sarcophagidae and Calliphorinae (Fig. 4.3.1). Arcsine-transformed stadial proportions, when treated separately, were significantly different among species (Wilk's test; $F = 11.1899$, $df_{effect} = 25$; $df_{error} = 86.9431$; $p = 0.0000$) but not among temperatures (Wilk's test; $F = 1.0122$, $df_{effect} = 75$; $df_{error} = 114.3809$; $p = 0.4712$). The interaction between species and temperature was also highly significant (Wilk's test; $F = 269.1442$; $df_{effect} = 5$; $df_{error} = 23$; $p = 0.0000$). Unequal N HSD tests indicated that all species formed homogenous groups (Table 4.3.1). Combined stadia (L1 to L3w, P separate) produced similar results. Again there was no significant difference between temperatures (Wilk's test; $F = 1$; $df_{effect} = 40$; $df_{error} = 144$; $p = 0.0522$) but species had a significant effect (Wilk's test; $F = 194$; $df_{effect} = 40$; $df_{error} = 144$; $p = 0.0000$), as did the interaction of species and temperature (Wilk's test; $F = 309689895$; $df_{effect} = 2$; $df_{error} = 72$; $p = 0.0000$). Closely related (congeneric) species tended to form homogenous groups (Table 4.3.2).

4.2.3 - Phylogenetic trends in developmental parameters, and prediction using phylogeny

Trends in K and D_0 are evident at familial, subfamilial, generic and specific levels (Fig. 4.4). The Sarcophagidae generally had high K values (>5000°h) and their lower thermal thresholds for complete development ranged from 4°C to 9°C (Table 4.2.6). The calliphorines had similarly high K and low D_0 values ($7169 < K < 9879$; $2.57 < D_0 < 6.14$). By contrast, although the ranges of K and D_0 were large in the Luciliinae and Chrysomyinae, K values were generally lower than those calculated for the Sarcophagidae and Calliphorinae, and D_0 values exceeded 6.5°C (Table 4.2.6).

There was a negative trend evident in a plot of K against D_0 for the Calliphoridae, but not for the Sarcophagidae (Fig. 4.4). A generalised least squares regression (LSR) described the relationship as $y = 9188.089 - 450.623x$ ($R^2 = 0.826$). However, the data were partitioned phylogenetically; the Calliphorinae had high K and low D_0 values, the Luciliinae, *Phormia, Protophormia* and *Cochliomyia* were intermediate and *Chrysomya* had low K and high D_0 values.

Using Felsenstein's Independent Contrasts approach, and rooting the tree (Fig. 4.5) on different nodes (Table 4.4) resulted in more precise prediction intervals for taxa within a clade (Table 4.4). When rooted on the calliphorid root (node C6 – Fig. 4.5), the prediction intervals were wide for both K and D_0 (Table 4.4), but when rooted on subfamily nodes, the confidence and prediction intervals for taxa within those subfamilies were smaller. The known D_0 values fell well within the prediction intervals, but K was consistently underestimated for the Calliphorinae and overestimated for the Chrysomyinae (Table 4.4). The long branch lengths within the Calliphorinae (Fig. 4.5) and random taxon sampling may account for this disparity; the branch lengths leading to predicted tips are calculated as the average length of all branches in the phylogeny (Garland & Ives, 2000), and adjusting the branch lengths to "known" lengths, or mean lengths for the appropriate clade, generally improved the accuracy of the prediction intervals.

To assess the magnitude of the disparity between values predicted by PDTree and calculated values, species within the Chrysomyinae were trimmed from the tree (Table 4.4) and their K and D_0 values predicted using the remainder of the data set. Chrysomyines were used in preference to luciliines and calliphorines because the group was better sampled. Branch lengths (Vh) leading to the hypothetical tips were adjusted to the known branch length of the trimmed species. *Chrysomya rufifacies* has a low calculated K (2427°h) and relatively low D_0 (12.4°C). When the phylogeny included the Calliphorinae and the Luciliinae (but not the Sarcophagidae), the predicted D_0 fell between 9.14°C and 20.17°C, and the predicted K ranged between 3094°h and 3103°h. Using a mean of the K and D_0 values (14.655°C and 3098.5°h, respectively) would result in an overestimate of the development time of 107 hours (or 4.5 days) at 25°C.

By contrast, the predictions for *Chrysomya chloropyga* were much more accurate. When a tip with a branch length equal to the branch length for *C. chloropyga* was predicted using the entire data set minus the Sarcophagidae, the D_0 fell between 3.25 and 13.52°C (calculated value 10.5°C). K was slightly overestimated (predicted range 4649 to 4657°h *cf.* calculated value of 4114°h). However, taking the mean of both predicted ranges (D_0 = 8.38°C; K = 4653.5°h) resulted in only a slight underestimate of the developmental duration (3.76 hours) at 25°C compared with the calculated values.

In the predictions of both *Chrysomya chloropyga* and *C. rufifacies*, trimming the calliphorines and luciliines from the tree prior to analysis obviously resulted in wider prediction intervals because of the reduction in sample size. Although trimming the other subfamilies from the tree would improve a PMI estimate by 3.5 hours at 25°C for *C. rufifacies*, the accuracy of the estimate for *C. chloropyga* would be reduced by a similar magnitude (3 hours).

4.3 - Discussion

4.3.1 - Changes in D_0 through development, fly distribution, and predictions about carcass size preference and behaviour

The temperature tolerances of *Calliphora vicina* have been shown to change with fly age, with eggs being most susceptible, and puparia the most resistant, to high temperatures (Davison, 1969). It would similarly be expected that the exposed or immobile stages of the immature fly (i.e. egg, wandering larva and puparium) would necessarily be more tolerant of low temperatures than would the feeding larva. Feeding larvae effectively live in a temperature-buffered habitat, because heat produced by maggot metabolism, bacterial breakdown of the substrate and maggot activity may increase the temperature of the carcass by several degrees above air temperature (Payne, 1965; Goodbrod & Goff, 1990), and the carcass itself possesses a degree of thermal inertia. By contrast, post-feeding larvae are exposed to ambient temperature for some time before they bury themselves, so a wider temperature tolerance is imperative for continued development. Wandering larvae and puparia are the stages that tend to enter diapause when conditions are unsatisfactory (Fraser & Smith, 1963; Davies & Ratcliffe, 1994), but this prolonged period of suspended animation make the flies prone to predation and parasitism. Thus, a low D_0 would reduce the likelihood of entering diapause during brief bouts of inclement weather. Although D_0s fluctuated from hatching to adult eclosion, there was no clear trend of a decrease in D_0 at the onset of wandering or pupariation (Tables 4.2.4 – 4.2.6). Some species (e.g. *C. rufifacies*) exhibited a decreasing trend in D_0 with development, but the remainder of the species showed either an insignificant change of less than 1°C, or a slight increase in the D_0 with maturation. It is highly likely, then, that the choice of pupariation sites by wandering larvae is critical for continued development.

Trends in K and D_0 apparently have a geographical as well as a phylogenetic bias. *Calliphora* and *Sarcophaga* are most speciose in temperate latitudes (Hall, 1948; Rognes, 1991) where low ambient temperatures are commonly encountered. Both taxa have relatively low D_0s (<9°C) and are the dominant forensic flies in Europe, North America and its islands, and parts of Australia (Introna *et al.,* 1990; Goff *et al.*, 1991, 1992; Wallman & Adams, 1997; Stevens & Wall, 2001; Wallman & Donnellan, 2001; Wells *et al.,* 2001). Similarly, the temperate chrysomyine genera *Phormia, Protophormia* and *Cochliomyia* have lower D_0s than the majority of the tropical *Chrysomya* spp. Recent introductions of four species of *Chrysomya* into the Americas has resulted in the rapid spread of these species, but at present, their distributions are restricted to heavily (human) populated or warmer areas. *Chrysomya chloropyga* has not yet been found north of Panama (Wells & Sperling, 2001), but *C. rufifacies* and *C. megacephala* are present on the Hawaiian islands (Goodbrod & Goff, 1990) and the warmer southern states of North America (Byrd & Butler, 1997). Baumgartner and Greenberg (1984) reported on the altitudinal distributions of *C. putoria* and *C. albiceps* in the Peruvian Andes. Both species were eusynanthropic at higher altitudes where median annual temperatures were below 17°C (Baumgartner & Greenberg, 1984), which indicates that in the absence of human interactions, these flies cannot survive low temperatures.

Small carcasses (e.g. rodents, birds), due to their high surface area to volume ratios, are likely to track ambient temperatures more closely than are larger carcasses (Hewardikaram & Goff, 1991). Additionally, small carcasses are unlikely to generate the same amount of localised heat as large carcasses, partly due to the limited resource available for bacterial and/or maggot action, and partly because of the loss of heat to the atmosphere. Thus, it can be expected that temperatures within a small carcass will drop dramatically at night (or during inclement weather), and species with lower D_0s will gain an advantage over species with higher thermal thresholds. No direct tests of this possibility have been undertaken, but it is notable that *Sarcophaga* spp. and *Calliphora croceipalpis* - which have larval D_0s<10°C (Tables 4.2.2 – 4.2.6) - are frequently found in small carcasses even in the cooler months of the year in the Grahamstown region, but larval *Chrysomya* spp. (D_0>10°C) are virtually absent from small carcasses year-round (pers. obs.). The almost total absence of *Sarcophaga* and *Calliphora* from larger carcasses (pers. obs.) - which are presumably more suitable for larval growth - is more difficult to explain, but biological (e.g. interspecific competition) rather than physical (e.g. temperature) factors may be responsible.

4.3.2 - Use of phylogeny to predict developmental parameters

Anderson (2000) illustrated that the proportions of total development spent in each stadium (developmental patterns) were similar between congeneric species and across temperatures, but she did not statistically test for differences between species or temperatures. Results from chapter 3 (this study) indicated that both temperature and species had significant effects on developmental patterns. From a collection of data in the literature, the developmental patterns were found to be statistically similar between temperatures but not between species (Table 4.3). For any species, then, the total development time at a given temperature can be estimated by simple ratios if the duration of a single stadium is known. However, as shown in chapter 3, the relative duration of, for example, the L3w stage, may be dramatically altered at temperature extremes, so extrapolation should be restricted to a median range of environmental temperatures.

The influence of common ancestry on the physiological phenotypes of organisms has been recognised for some time (Harvey, 1996). If, in a given taxon, two characters are strongly correlated, prediction and confidence limits can be calculated either for unsampled species, or for the parental (ancestral) node. Traditionally, generalised least squares regression (GLSR) has been used to determine the relationships between two physiological variables and to determine the limits of the correlation (Harvey, 1995; Schmidt-Nielsen, 1995). However, the major drawbacks to using GLSR for predictive purposes are, firstly, that one character state has to be known to predict the other, and secondly, the influence of shared ancestry is often strong and may confound a prediction (Felsenstein, 1985; Harvey, 1996; Garland et al., 1999; Garland & Ives, 2000). By contrast, Felsenstein's independent contrasts (FIC) method takes into account the effects of shared ancestry, by determining the rate of evolution of a clade and applying directional changes to the characters to be predicted. Thus, the characteristics of both ancestral (parental) and descendant (daughter) nodes can be determined with this method (Felsenstein, 1985; Garland et al., 1999; Garland & Ives, 2000). Additionally, PDTree (Garland et al., 2002) allows the characteristics of hypothetical tip species to be determined (Garland & Ives, 2000).

Although the determination of ancestral states using a GLSR approach may give similar results to FIC, it has been determined that the FIC method is more robust and conservative (Garland & Ives, 2000). FIC also has the advantage of being able to predict both the independent and dependent variables if the phylogeny is known, whereas with GLSR, one the parameters must be known in order for the other to be determined.

For the calliphorids (including *Calliphora augur, C. vomitoria* and *C. stygia* for which no phylogenetic data were available), a plot of K against D_0 produced a negative relationship (Figure 4.4). It is possible that the relationship is hyperbolic rather than linear, but the small data set (n = 16) makes the determination of the type of relationship difficult. Log or inverse transformation of either or both parameters did not improve the linearity of the relationship, so the non-transformed data were used for analysis. A GLS regression fitted to the non-transformed data had a reasonable fit ($R^2 = 0.825518$). Although the linear regression described the K/D_0 relationship adequately, close scrutiny indicated that there was a phylogenetic pattern in both K and D_0. For example, the Calliphorinae and Chrysomyinae were restricted to the upper and lower K range, respectively. Thus, using the GLSR equation to calculate K and D_0 for species for which no developmental data are available may over- or underestimate values for taxa at the extremes of the K/ D_0 range. Obviously, if either K or D_0 of a species is known, the magnitude of the error will be smaller.

The prediction intervals produced by PDTree and FIC ranged widely in their accuracy. The clade on which the tree was rooted was found to be important for accurate predictions of individual species. When rooted on the Calliphorid node (C6, Figure 4.5), the confidence intervals (CI) and prediction intervals (PI) for both parameters were wide (Table 4.4), and determining the K and D_0 values for tip species with any accuracy would be tenuous. However, when the tree was rooted on subfamily nodes (e.g. B9, C2, C8, Fig. 4.5), the prediction intervals for both K and D_0 were reduced (Table 4.4). Rooting the tree on clades for which estimates are to be made results in a refinement of the branch lengths of hypothetical taxa, because the rates of character evolution may differ between, for example, subfamilies.

When testing the predictive accuracy of the FIC method, it was found that tree rooting, the clade size and number of close relatives, and branch lengths all affected the prediction intervals. For example, when the tree was rooted on the *C. albiceps*-*C. rufifacies* node (node D9, Fig. 4.5) and the latter species was trimmed from the data set, the "predicted" K and D_0 were greatly overestimated. *Chrysomya rufifacies* and its sister species *C. albiceps*, are positioned on a long branch within the Chrysomyinae. Furthermore, the calculated K and D_0 for the two species are quite different (*C. albiceps* K = 2709°h, D_0 = 16.2°C; *C. rufifacies* K = 2427°h, D_0 = 12.4°C). Thus, the predicted values for *C. rufifacies* more closely approximated *C. albiceps* than was apparent from ADD calculations. However, as is evident in the scatterplot of K against D_0 (Fig. 4.4), *C. rufifacies* had a markedly lower K than would be expected for its D_0, and such anomalies may also complicate the prediction.

In contrast to the *C. rufifacies* example, rooting the tree on the *C. chloropyga*-*C. putoria* node (node E4, Fig. 4.5), and trimming *C. chloropyga* from the data set resulted in a close approximation of K and D_0 to the calculated values (Table 4.4). Although the mean K was slightly overestimated (4653°h *cf.* 4114°h) and D_0 was slightly underestimated (8.38°C *cf.* 10.5°C), the net effect would be a minor difference in the estimated total development time; however, the error would increase at lower temperatures. *Chrysomya chloropyga* and *C. putoria* are positioned on a relatively short branch, and their nearest relative, *C. varipes*, is also positioned on a short branch. Additionally, the K/D_0 co-ordinates for *C. chloropyga* fall close to the GLRS regression line (Fig. 4.4).

It is evident, therefore, that phylogenetic and physiological data are equally important when using FIC to predict the physiological parameters for untested species. Long branches in the phylogeny affect the calculation of the evolution rate (Garland *et al.*, 1999). Since the prediction of a continuous variable using FIC is dependent on the branch length, the most reliable results will probably be acquired when the branch leading to the untested species is adjusted from PDTree's default "mean length" branch to the actual branch length derived from phylogenetic analysis.

The Calliphorinae generally have high Ks and low D_0s. Appropriate COI data were not available for *Calliphora augur, C. vomitoria* and *C. stygia,* all three of which have higher K values than either *C. vicina* or *Eucalliphora latifrons*. The ancestral calliphorine character confidence limits for K were calculated by FIC as 5967°h to 5981°h (root node B9, Table4.4). When the tree was rooted midway along the *C. vicina* branch, the prediction intervals rose to

7285°h to 7302°h. This indicates that *C. augur*, *C. vomitoria* and *C. stygia* are more derived than *C. vicina*. Wallman *et al.* (2001) produced a phylogeny that indicates that this hypothesis is correct; *C. vicina* was basal to the rest of the Australian *Calliphora* clade. Thus, phylogeny and physiology can be of reciprocal use. If the direction of character change within a clade is constant and known, the relative derivation of species can be determined using physiological parameters.

4.3.3 – Utility of the ADD and LF methods, comparison of ease of use and potential drawbacks

The general accumulated day-degrees (ADD) method, or variations of it, has been refined and modelled by a variety of authors (e.g. Allen, 1976; Wagner et al., 1984; Higley et al., 1986; Worner, 1992; Byrd & Allen, 2001a), for use in either predicting pest outbreaks or estimating the time of insect infestation (e.g. of a corpse). The underlying theory to this method is that the relationship between temperature and development rates of poikilotherms is sigmoidal. Fitting a linear regression to the portion of greatest (linear) acceleration in rate allows the K and D_0 to be estimated. Confidence intervals can be fitted to the linear portion of the graph and error terms thus incorporated into estimates. However, the determination of the linear portion of the graph is necessarily subjective.

Ikemoto & Takai (2000) devised a fully linearised method for determining K and D_0. A plot of developmental duration multiplied by temperature against developmental duration results in a Z-shaped graph. Regression analysis is applied to the major linear portion of the graph. The formula was specifically designed to reduce the magnitude of the error terms of the estimates and thus to increase the precision of estimates of K and D_0. However, some degree of subjectivity is still present, as the points that depart from the major linear portion of the graph are culled prior to regression analysis.

Provided that data are accurate and cover a wide range of temperatures, both the ADD and LF methods will provide similar results (Ikemoto & Takai, 2000); the latter method is simply easier to use. However, as was found in this study, the range of rearing temperatures and the quality of the data available have marked effects on the calculations of K and D_0. In all examples illustrated in Ikemoto & Takai (2000), a wide range of temperatures (and thus durations) was used in their calculations. In this study, only a narrow range of (or few) temperatures were available for analysis for the majority of species (Table 4.1), so use of the

LF method increased the danger of introducing Type II errors. In such cases, provided that the temperatures investigated did not lie beyond the linear portion of the rate/temperature curves, the ADD method would give more reliable results.

Logically, an estimate of K and D_0 can only be as accurate as the data used to derive them. In many cases, data produced by different authors were widely divergent and determining which were more reliable was difficult (see below). The ADD method was apparently less affected by disparities and outliers were relatively easy to identify, but when the LF method was applied, it was virtually impossible to distinguish the linear portion of the graph from the outliers (e.g. *C. vicina*, Fig. 4.2.9). Therefore, if the reliability of the data is in question, or the spread of rates at a given temperature is wide, the ADD method should be used in preference to the LF method.

4.3.4 - Inter-study comparisons and recommendations for further research

The degree of congruence between studies of the same species differed substantially. In most cases where major developmental variations occurred (e.g. *Chrysomya megacephala*), differences were evident between studies undertaken by different authors on different populations of the fly (Bohart & Gressit, 1951; Wijesundara, 1957a; Levot *et al.*, 1979; Subramanian & Raja Mohan, 1980; Prins, 1982; O'Flynn, 1983; Nishida *et al.,* 1984; Goodbrod & Goff, 1990; Wells & Kurahashi, 1994; Lunt, unpub.). However, despite similar methods, researchers, temperatures and sympatric fly populations being used, widely divergent results were also published for *Sarcophaga ruficornis* (Goff *et al.,* 1992, 1993, 1994, 1997). The three earlier studies, which used flies from a single colony established in 1990, were comparable with each other, but development to adult eclosion was some 80 hours faster at 25°C than for flies from a colony established in 1994.

There are several possibilities for developmental disparities. Firstly, and most likely in the majority of cases, the temperature recordings of the substrate may have differed from the measured ambient temperature (Catts, 1992). In the earlier studies especially, authors did not control maggot density (e.g. Hobson, 1932; Smith, 1933; Wijesundara, 1957a; Kamal, 1958; Buri, 1959; Norris, 1965; Abasa, 1970; Subramanian & Raja Mohan, 1980), so the temperature in the maggot mass might have been several degrees higher than the air temperature (Payne, 1965). Measurement of the substrate temperature in addition to the air temperature will circumvent this problem, as indicated by Byrd & Butler (1996, 1997,1998).

Secondly, the precision of measurements in source data and the conversion to hours in this study may have introduced a degree of error. For example, Nishida *et al.* (1984) made observations at 24-hour intervals from hatching, which would result in an overestimate of some of the stadial durations. More frequent observations (e.g. Reiter, 1984; Davies & Ratcliffe, 1994; Queiroz, 1996; Byrd & Butler, 1996, 1997, 1998; Byrd & Allen, 2001b; Grassberger & Reiter, 2001) or staggered rearing of cohorts (e.g. chapter 3, this study) will improve the resolution of stadial duration estimates.

Thirdly, it is possible that there is a degree of geographical or population variation. Populations may become adapted to local conditions, as found in the hymenopteran, *Lysiphlebus testaceipes* (Royer *et al.*, 2001) or not, as in *Plutella xylostella* (Lepidoptera: Plutellidae) (Shirai, 2000). Laurence (1988) found that *Chrysomya putoria* from different geographical regions exhibited substantially different development rates. Development rates of a South African population (chapter 3, this study) were coincident with Laurence's (1988) Liberian and Brazilian colonies, but his inbred Tanzanian colony had a greatly increased hatch to pupariation period. Unfortunately, it is impossible to determine whether the variation in development rate of *C. putoria* was a result of local population adaptations or inbreeding depression; the Tanzanian colony had been inbred for some 10 years (Laurence, 1988).

Finally, the form of the data published by authors makes inter-study comparisons difficult. Marchenko (1985, 1986, 2001) published secondary data for a wide range of species, but unfortunately he did not separate development into stadia, and the egg stage (if present) was incorporated into the overall development rate. The data were also all processed, and development rates were derived from linear regression equations and applied across a wide range of temperatures. Incorporation of his data into this study was, therefore, impossible.

The lack of standardisation of methods in the literature to date has resulted in a large amount of potentially useful data being excluded from this analysis, and it was often impossible to separate the effects of differing terminology or data types from true variations in developmental patterns. There is a need to standardise the methods and terminology used in forensic entomology so that meaningful comparisons can be made and parameter estimates or predictions can be accurate. The higher the resolution of stadial duration records, the more flexible data will become for comparative purposes.

Although constant-temperature rearings are useful for baseline studies (especially for the determination of K and D_0), the Kauffman effect (Worner, 1992) needs to be investigated further (e.g. Introna *et al.*, 1989; Davies & Ratcliffe, 1994). Several authors separate the wandering stage from larval feeding, some incorporate wandering into the pupariation stage, and some combine wandering with the third (feeding) larval instar. Due to the variation in the duration of wandering at high temperatures (chapter 3, this study) or when wandering larvae are continually disturbed (Denlinger & Zdarek, 1994), it is recommended that wandering be treated as a separate stage. Additionally, precocious egg development (Wells & King, 2001) may result in an overestimate of a PMI, so the separation of egg and larval development is also necessary. Appendix C provides guidelines for rearing forensically important flies and for reporting developmental data.

In summary, the potential for using phylogeny for predicting development patterns of untested species is great, but the accuracy and precision of predictions relies heavily on the quality of the source data available. A robust phylogeny that is not subject to random taxon sampling error is required, and developmental data for related species must be of a high quality.

TABLE 4.1 – Source data for species included in the analysis. * denotes data unsuitable for inclusion (processed data or insufficient resolution). See text for stadia codes.

Species	Author	Stadia
Wohlfahrtia trina	Tawfik (1962) cited in Zohdy & Morsy (1982)	P; AE
Sarcophaga (Bercaea) africa	Byrd & Butler (1998)	H; E1; E2; P; AE
Sarcophaga (Boettcherisca) peregrina	Utsumi (1958) Nishida *et al.* (1986)	* H; E1; E2; P; AE
Sarcophaga (B.) septentrionalis	Marchenko (1988)	P
Sarcophaga (Liopygia) aegyptiaca	Kamal (1979)	AE
Sarcophaga (L.) argyrostoma	Kamal (1979) Marchenko (1980) Zhody & Morsy (1982)	P AE * E1; E2; P; AE
S. (L.) ruficornis	Bohart & Gressit (1951) Amoudi *et al.* (1994)	P; AE W; P; AE
S. (L.) crassipalpis	Levot *et al.* (1979) Nishida *et al.* (1986)	W E1; E2; P; AE
Calliphora vicina	Anderson (2000) Greenberg (1991) Kamal (1958) Marchenko (1980) Marchenko (1988) Reiter (1984) Williams & Richardson (1984)	E1, E2, W, P, AE H; E1; E2; W; P AE E1, E2, W, P, AE E1, E2, W, P, AE P; AE * H H
Calliphora augur	Levot *et al.* (1979) O'Flynn (1983) Kamal (1958) Greenberg & Tantawi (1993)	W E1, E2, W, P, AE E1, E2, W, P, AE E1, E2, P, AE

TABLE 4.1 continued - Source data

Species	**Author**	**Stadia**
Calliphora stygia	O'Flynn (1983)	E1, E2, W, P, AE
	Levot *et al.* (1979)	L3w
	Williams & Richardson (1984)	P
Calliphora vomitoria	Kamal (1958)	H; E1; E2; W; P; AE
	Marchenko (1988)	P; AE *
	Greenberg & Tantawi (1993)	H; E1; E2; P; AE
Eucalliphora latifrons	Anderson (2000)	H, E1, E2, W, P, AE
Lucilia cuprina	Bohart & Gressit (1951)	H
	Buei (1959)	*
	Subramanian & Raja Mohan	H; E1; E2; W; P; AE
	(1980)	H; E1; E2; W; P; AE
	O'Flynn (1983)	*
	Dallwitz (1984)	H; E1; E2; W
	Nishida (1984)	
Lucilia sericata	Melvin (1934)	H
	Kamal (1958)	H; E1; E2; W; P; AE
	Prins (1982)	H; E1; E2; P; AE
	Ash & Greenberg (1975)	H; P; AE
	Marchenko (1988)	P; AE *
	Greenberg (1991)	H; E1; E2; P; AE
	Grassberger & Reiter (1991)	H; E1; E2; W; P; AE
	Anderson (2000)	H; E1; E2; P; AE
Cochliomyia macellaria	Byrd & Butler (1996)	H; E1; E2; P; AE
Protophormia terraenovae	Kamal (1958)	H; E1; E2; P; AE
	Marchenko (1988)	AE *
	Greenberg & Tantawi (1993)	H; E1; E2; P; AE
Phormia regina	Melvin (1934)	H
	Kamal (1958)	H; E1; E2; P; AE
	Marchenko (1980, 1988)	P; AE *
	Nishida (1984)	H; E1; E2; W
	Greenberg (1991)	H; E1; E2; P; AE
	Anderson (2000)	H
	Byrd & Allen (2001b)	H; E1; E2; W; P; AE

TABLE 4.1 continued - Source data

Species	**Author**	**Stadia**
Chrysomya megacephala	Bohart & Gressit (1951)	H; &
	Levot *et al.* (1979)	&
	Prins (1982)	E1; E2; P
	Nishida (1984)	&
	Goodbrod & Goff (1990)	E1; E2; P; AE
	Wells & Kurahashi (1994)	H; E1; E2; P; AE
	Lunt (unpub.)	E1; E2; W; P; AE
Chrysomya albiceps	Prins (1982)	H; E1; E2; P; AE
	Marchenko (1988)	P; AE *
	Queiroz (1996)	W; P; AE
	Lunt (unpub.)	E1; E2; W
Chrysomya rufifacies	Bohart & Gressit (1951)	H
	Subramanian & Raja Mohan	H; E1; E2; W; P; AE
	(1980)	H; E1; E2; AE
	O'Flynn (1983)	E1; E2; P
	Goodbrod & Goff (1990)	H; E1; E2; P; AE
	Byrd & Butler (1997)	
Chrysomya varipes	Levot *et al.* (1979)	W; AE
	O'Flynn (1983)	AE
Chrysomya chloropyga	Prins (1982)	E1; E2; P; AE
	Lunt (unpub.)	E1; E2; W; P; AE
Chrysomya putoria	Laurence (1988)	H; P; AE
	Lunt (unpub.)	E1; E2; W; P; AE

TABLE 4.2.1 – Developmental parameters for the incubation period.

Species	**Accumulate Day Degree (ADD) method**					**Ikemoto & Takai (2000) (LF) method**				
	R^2	**d.f.**	**K**	**D_0**	**Fig. #**	**R^2**	**d.f.**	**K**	**D_0**	**Fig. #**
Calliphora vicina	0.6908	11	409	3.4	4.1.9	0.8176	6	304	6.7	4.2.9
C. vomitoria	0.8899	4	352	8.1	4.1.11	0.9936	3	348	7.1	4.2.11
C. stygia	0.8673	2	225	7.0	4.1.12	0.8644	2	275	5.4	4.2.12
Eucalliphora latifrons	0.9851	2	253	10.3	4.1.13	0.9933	2	261	10.1	4.2.13
Lucilia cuprina	0.8314	4	178	9.7	4.1.14	0.9999	7	8*	24.6*	4.2.14
L. sericata	0.8131	21	234	9.8	4.1.15	0.7937	21	232	10.2	4.2.15
Cochliomyia macellaria	0.8295	2	172	9.2	4.1.16	1.0*	1*	106*	12.3*	4.2.16
Protophormia terraenovae	0.9459	4	310	7.2	4.1.17	0.9986	2	236	11.6	4.2.17
Phormia regina	0.7986	11	344	7.2	4.1.18	0.9473	10	243	11.0	4.2.18
Chrysomya rufifacies	0.8337	8	178	12.2	4.1.21	0.9276	3	147	14.2	4.2.21

* insufficient points for meaningful regression calculation, or unreliable result

TABLE 4.2.2 – Developmental parameters for the cumulative development from hatching (H) to first ecdysis (E1).

Species	Accumulate Day Degree (ADD) method					Ikemoto & Takai (2000) (LF) method				
	R^2	d.f.	K	D_0	Fig. #	R^2	d.f.	K	D_0	Fig. #
Sarcophaga (Bercaea) africa	0.84588	2	116	8.67	4.1.2	-	-	*	*	4.2.2
S. (Boettcherisca) peregrina	0.782261	5	680	4.15	4.1.3	0.9122	2	436	9.5	4.2.3
S. (Liopygia) argyrostoma	0.951706	4	437	4.08	4.1.6	0.9254	4	362	8.2	4.2.6
S. (L.) crassipalpis	0.882353	3	480	8.33	4.1.8	0.9091	3	440	9.5	4.2.8
Calliphora vicina	0.703534	4	458	2.95	4.1.9	*	*	*	*	4.2.9
C. augur	0.988087	2	395	4.57	4.1.10	0.9914	2	379	5.1	4.2.10
C. vomitoria	0.852253	2	236	9.67	4.1.11	0.8226	2	268	8.6	4.2.11
C. stygia	0.818439	2	225	6.99	4.1.12	-	-	*	*	4.2.12
Eucalliphora latifrons	-	-	*	*	4.1.13	-	-	*	*	4.2.13
Lucilia cuprina	0.987433	3	197	11.6	4.1.14	0.9670	3	224	12.1	4.2.14
L. sericata	0.778486	24	286	8.0	4.1.15	-	-	*	*	4.2.15
Cochliomyia macellaria	0.989539	2	143	11.7	4.1.16	0.9909	3	102	18.4	4.2.16
Protophormia terraenovae	0.993305	3	180	15.96	4.1.17	0.9996	4	251	11.7	4.2.17
Phormia regina	0.798603	11	344	7.2	4.1.18	0.9384	11	311	13.6	4.2.18
Chrysomya megacephala	0.971993	3	133	16.31	4.1.19	-	-	*	*	4.2.19
C. albiceps	0.999999	2	158	17.5	4.1.20	1.00	2	158	17.5	4.2.20
C. rufifacies	0.705842	7	186	16.5	4.1.21	0.9544	3	152	18.2	4.2.21
C. chloropyga	0.853856	6	125	16.0	4.1.23	0.9253	8	139	15.6	4.2.23
C. putoria	0.814216	5	126	16.3	4.1.24	0.9008	5	102	17.7	4.2.24

* insufficient points for regression calculation, or unreliable results

TABLE 4.2.3 – Developmental parameters for the cumulative development from hatching (H) to second ecdysis (E2).

Species	**Thermal summation method**					**Ikemoto & Takai (2000)**				
	R^2	**d.f.**	**K**	**D_0**	**Fig. #**	**R^2**	**d.f.**	**K**	**D_0**	**Fig. #**
Sarcophaga (Bercaea) africa	0.967497	2	369	11.89	4.1.2	-	-	*	*	4.2.2
S. (Boettcherisca) peregrina	0.78125	4	1440	4.0	4.1.3	0.9423	2	948	8.3	4.2.3
S. (Liopygia) argyrostoma	0.959885	4	842	8.76	4.1.6	-	-	*	*	4.2.6
S. (L.) crassipalpis	0.882353	3	960	8.3	4.1.8	0.9231	2	761	10.4	4.2.8
Calliphora vicina	0.71403	5	821	5.15	4.1.9	-	-	*	*	4.2.9
C. augur	0.995337	5	729	3.59	4.1.10	0.9936	5	745	3.4	4.2.10
C. vomitoria	0.98572	2	646	7.3	4.1.11	0.9820	2	676	6.7	4.2.11
C. stygia	1.00	1	1188*	3.5*	4.1.12	-	-	*	*	4.2.12
Eucalliphora latifrons	1.0	1	1547*	4.83*	4.1.13	-	-	*	*	4.2.13
Lucilia cuprina	0.967997	3	454	11.1	4.1.14	0.9826	3	461	12.4	4.2.14
L. sericata	0.787875	25	585	10.96	4.1.15	-	-	*	*	4.2.15
Cochliomyia macellaria	0.901228	4	502	11.9	4.1.16	0.8886	4	580	10.2	4.2.16
Protophormia terraenovae	0.988094	3	600	11.29	4.1.17	0.9991	2	518	11.5	4.2.17
Phormia regina	0.939206	10	1130	10.2	4.1.18	0.9338	11	769	13.4	4.2.18
Chrysomya megacephala	0.736684	6	834	10.34	4.1.19	-	-	*	*	4.2.19
C. albiceps	0.997282	2	450	16.5	4.1.20	0.9999	2	445	16.5	4.2.20
C. rufifacies	0.884323	7	490	13.8	4.1.21	0.9579	2	574	12.5	4.2.21
C. chloropyga	0.880153	8	406	16.5	4.1.23	0.9444	8	422	16.3	4.2.23
C. putoria	0.944863	5	836	11.8	4.1.24	0.9156	5	829	11.9	4.2.24

* insufficient linearly-arranged points for regression calculation, or unreliable data.

TABLE 4.2.4 – Developmental parameters for the cumulative development from hatching (H) to larval exodus (W).

Species	**Thermal summation**					**Ikemoto & Takai (2000)**				
	R^2	**d.f.**	**K**	**D_0**	**Fig. #**	**R^2**	**d.f.**	**K**	**D_0**	**Fig. #**
S. (Liopygia) ruficornis	0.9814	7	1675	9.3	4.1.7	0.9762	7	1501	10.8	4.2.7
Calliphora vicina	0.7993	15	2114	3.7	4.1.9	0.8453	10	1917	6.9	4.2.9
C. augur	0.9811	7	1776	3.55	4.1.10	0.9755	8	1658	3.8	4.2.10
C. stygia	0.8215	4	2259	3.1	4.1.12	0.9404	3	1217	13.4	4.2.12
Eucalliphora latifrons	0.6087	2	3251	3.58	4.1.13	1.0	1	914*	15.1*	4.2.13
Lucilia cuprina	0.9666	2	842	13.2	4.1.14	0.9133	3	2525	14.3	4.2.14
L. sericata	0.8699	19	1260	9.8	4.1.15	-	-	*	*	4.2.15
Phormia regina	0.7527	10	3550	7.1	4.1.18	0.8924	9	2291	13.2	4.2.18
Chrysomya megacephala	0.9506	4	1899	8.51	4.1.19	1.0	3	-302*	28.7*	4.2.19
C. albiceps	0.8815	5	1199	14.3	4.1.20	0.9910	5	993	15.9	4.2.20
C. varipes	0.9437	2	1511	14.6	4.1.22	0.9942	2	1395	15.3	4.2.22
C. chloropyga	0.9361	7	1894	8.4	4.1.23	0.8636	5	1626	10.9	4.2.23
C. putoria	0.9838	5	1874	9.0	4.1.24	0.9882	3	1722	10.3	4.2.24

* insufficient linearly-arranged points for regression calculation, or unreliable data.

TABLE 4.2.5 – Developmental parameters for the cumulative development from hatching (H) to pupariation (P).

Species	**Thermal summation**					**Ikemoto & Takai (2000)**				
	R^2	**d.f.**	**K**	**D_0**	**Fig. #**	**R^2**	**d.f.**	**K**	**D_0**	**Fig. #**
Wohlfahrtia trina	0.996577	5	2272	12.59	4.1.1	0.9996	5	2303	12.4	4.2.1
Sarcophaga (Bercaea) africa	0.924413	4	2449	6.57	4.1.2	0.7542	4	2348	7.3	4.2.2
S. (Boettcherisca) peregrina	0.988698	2	1867	10.9	4.1.3	0.9968	2	1937	10.6	4.2.3
S. (B.) septentrionalis	0.999935	19	2808	7.80	4.1.4	1.0	19	2806	7.8	4.2.4
S. (Liopygia) argyrostoma	0.861367	6	2115	13.12	4.1.6	0.9849	7	1785	15.1	4.2.6
S. (L.) ruficornis	0.977662	8	3635	6.7	4.1.7	0.9634	6	2574	9.9	4.2.7
S. (L.) crassipalpis	0.938939	4	4851	5.96	4.1.8	0.8981	2	3647	9.0	4.2.8
Calliphora vicina	0.887859	6	5074	2.6	4.1.9	-	-	*	*	4.2.9
C. augur	0.982050	4	3169	4.86	4.1.10	0.9839	3	2720	6.4	4.2.10
C. vomitoria	0.998444	2	4899	3.72	4.1.11	0.9853	2	4961	3.5	4.2.11
C. stygia	0.844604	10	3311	3.74	4.1.12	-	-	*	*	4.2.12
Eucalliphora latifrons	0.449526	2	4182	1.07	4.1.13	1.0	1	924*	17.2*	4.2.13
Lucilia cuprina	1.00	1	1439	12.5	4.1.14	1.0	2	3029	12.4	4.2.14
L. sericata	0.7421	26	3242	7.3	4.1.15	-	-	*	*	4.2.15
Cochliomyia macellaria	0.9734	4	1871	8.1	4.1.16	0.9489	4	1812	8.6	4.2.16
Protophormia terraenovae	0.9752	4	2629	9.36	4.1.17	0.9999	2	2166	10.9	4.2.17
Phormia regina	0.7290	5	2787	11.3	4.1.18	-	-	*	*	4.2.18
Chrysomya megacephala	0.9846	5	2140	10.63	4.1.19	-	-	*	*	4.2.19
C. albiceps	0.8658	5	1697	15.4	4.1.20	0.9839	5	2189	15.0	4.2.20
C. rufifacies	0.6666	5	894	17.2	4.1.21	0.9993	6	548	20.2	4.2.21
C. varipes	1.00	1	1938*	14.2*	4.1.22	0.8854	2	2970	10.9	4.2.22
C. chloropyga	0.735109	6	2141	10.9	4.1.23	0.9503	4	1499	14.6	4.2.23
C. putoria	0.668353	8	2538	8.7	4.1.24	0.8204	5	1511	15.2	4.2.24

* insufficient linearly-arranged points for regression calculation, or unreliable data.

TABLE 4.2.6 – Developmental parameters for the cumulative development from hatching (H) to adult eclosion (AE).

Species	**Thermal summation**					**Ikemoto & Takai (2000)**				
	R^2	**d.f.**	**K**	**D_0**	**Fig. #**	**R^2**	**d.f.**	**K**	**D_0**	**Fig. #**
Wohlfahrtia trina	0.998103	5	7509	11.83	4.1.1	0.9996	5	7602	8.3	4.2.1
Sarcophaga (Bercaea) africa	0.729145	3	4979	10.42	4.1.2					4.2.2
S. (Boettcherisca) peregrina	0.952234	4	6261	6.7	4.1.3	0.9212	2	5075	8.9	4.2.3
S. (B.) septentrionalis	0.998830	23	6732	8.25	4.1.4	1.0	23	6704	8.3	4.2.4
S. (Liopygia) aegyptiaca	0.997829	2	11269	0.63	4.1.5	-	-	*	*	4.2.5
S. L. argyrostoma	0.954225	4	6458	13.05	4.1.6	0.9849	9	6006	13.7	4.2.6
S. L. ruficornis	0.974874	6	9098	7.9	4.1.7	0.8764	5	6200	11.6	4.2.7
S. L. crassipalpis	0.960259	4	8866	6.1	4.1.8	0.9256	2	7016	8.7	4.2.8
Calliphora vicina	0.996628	5	8613	3.9	4.1.9	-	-	*	*	4.2.9
C. augur	0.99062	4	7190	6.14	4.1.10	0.9910	4	7512	5.7	4.2.10
C. vomitoria	1.0	1	8856*	5.51*	4.1.11	1.0000	1	8856	5.5	4.2.11
C. stygia	0.987214	3	9879	2.57	4.1.12	0.9501	3	9273	3.4	4.2.12
Eucalliphora latifrons	0.827093	2	7169	4.48	4.1.13	1.0	1	2917*	15.1*	4.2.13
Lucilia cuprina	1.00	2	3028	12.4	4.1.14	1.0000	2	3029	12.4	4.2.14
L. sericata	0.70894	24	6166	7.8	4.1.15	-	-	*	*	4.2.15
Cochliomyia macellaria	0.92343	3	2791	11.0	4.1.16	0.9740	3	2929	10.5	4.2.16
Protophormia terraenovae	0.969723	4	5168	8.71	4.1.17	0.9990	3	4416	10.4	4.2.17
Phormia regina	0.732566	5	4996	10.7	4.1.18	0.7034		5553	13.2	4.2.18
Chrysomya megacephala	0.651364	10	2998	12.26	4.1.19	0.9802	5	710*	22.4*	4.2.19
C. albiceps	0.923624	4	2709	16.2	4.1.20	0.9477	4	1113	21.1	4.2.20
C. rufifacies	0.704577	6	2427	12.4	4.1.21	0.8023	8	1642	17.0	4.2.21
C. varipes	0.976878	2	3177	14.7	4.1.22	0.9603	3	4029	12.9	4.2.22
C. chloropyga	0.858528	6	4114	10.5	4.1.23	0.9857	3	2127	17.8	4.2.23
C. putoria	0.894591	8	5125	6.6	4.1.24	0.8268	5	3889	10.8	4.2.24

* insufficient linearly-arranged points for regression calculation, or unreliable data.

TABLE 4.3.1 - Unequal N HSD tests of homogeneity, of the arcsine-transformed stadial durations of Calliphoridae and Sarcophagidae. Stadia were treated separately (i.e. L1, L2, L3f, L3w and P). Homogenous groups are depicted by "XX"

Species	Mean	1	2	3	4
S. ruficornis	0.449551	XX			
S. tibialis	0.577316	XX	XX	XX	XX
W. trina	0.607043	XX	XX		
S. africa	0.620977	XX	XX		
C. croceipalpis	0.650523	XX	XX	XX	XX
C. vicina	0.680271		XX	XX	
C. saffranea	0.684719	XX	XX	XX	XX
E. latifrons	0.716768		XX	XX	XX
C. albiceps	0.765382		XX	XX	XX
C. macellaria	0.798606			XX	XX
C. vomitoria	0.798777		XX	XX	XX
C. megacephala	0.799216			XX	XX
L. sericata	0.807722			XX	XX
C. chloropyga	0.826391				XX
S. crassipalpis	0.828862			XX	XX
S. peregrina	0.833081			XX	XX
L. illustris	0.854220		XX	XX	XX
C. rufifacies	0.856477			XX	XX
C. putoria	0.869808				XX
P. regina	0.913091			XX	XX

TABLE 4.3.2 – Tukey's unequal N HSD tests of homogeneity, of the arcsine-transformed stadial durations of Calliphoridae and Sarcophagidae. Larval stadia (i.e. L1 to L3w) were combined, pupariation was treated separately. Homogenous groups are depicted by "XX"

Species	Mean	1	2	3	4	5
C. vomitoria	0.690629	XX	XX	XX		
L. sericata	0.707043	XX				
C. megacephala	0.733823	XX			XX	
C. chloropyga	0.736822	XX	XX		XX	
C. putoria	0.770963	XX	XX		XX	
E. latifrons	0.857028		XX	XX		XX
C. vicina	0.870743			XX		XX
C. saffranea	0.886077	XX	XX	XX	XX	XX
C. croceipalpis	0.920273				XX	XX
S. tibialis	0.993480					XX

TABLE 4.4 – Prediction intervals and regression equations derived from Felsenstein's Independent Contrasts, with the tree rooted on various nodes.

Root	**Taxa trimmed**	**Taxon predicted**	**Regression equation**	**D_0 (°C) [95% PI]**	**K (°h) [95% PI]**
C6	Sarcophagidae	Calliphoridae	y = 8473.73 – 399.37x	1.56 – 17.42	4675 – 4688
B9	Sarcophagidae	Calliphorinae	y = 8914.72 – 399.37x	-0.91 – 15.63	5967 – 5981
C2	Sarcophagidae	Luciliinae	y = 8945 – 399.37x	0.47 – 17.13	5423 – 5436
B9	Sarcophagidae; Chrysomyinae	Calliphorinae	y = 10576.47 – 624.37x	-1.59 – 13.97	6705 – 6717
C2	Sarcophagidae; Chrysomyinae	Luciliinae	y = 10926.32 – 624.375x	2.64 – 14.55	5551 – 5561
C8	Sarcophagidae	Chrysomyinae	y = 8320.87 – 399.37x	2.47 – 17.84	4258 – 4271
E1	Sarcophagidae	*Chrysomya*	y = 8372.8 – 399.37x	3.62 – 18.5	3947 – 3960
D9	Sarcophagidae; *Chrysomya rufifacies*	*Chrysomya rufifacies*	y = 9356.10 – 426.76x	9.14 – 20.17	3095 - 3104
D9	Sarcophagidae; Calliphorinae; Luciliinae; *Chrysomya rufifacies*	*Chrysomya rufifacies*	y = 7082.11 – 274.30x	9.06 – 20.48	3025 - 3034
E4	Sarcophagidae *Chrysomya chloropyga*	*Chrysomya chloropyga*	y = 8049.59 – 404.75x	3.25 – 13.52	4649 – 4657
E4	Sarcophagidae; Calliphorinae; Luciliinae; *Chrysomya chloropyga*	*Chrysomya chloropyga*	y = 6584.13 – 236.80x	3.16 – 13.89	4559 – 4568

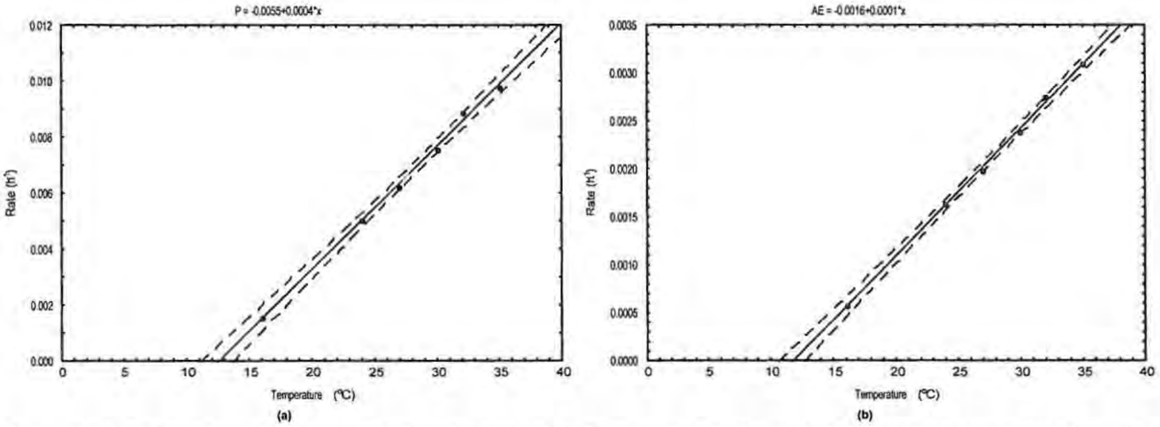


FIGURE 4.1.1 – Development to landmarks (ADD method) for *Wohlfahrtia trina*. (a) P; (b) AE.

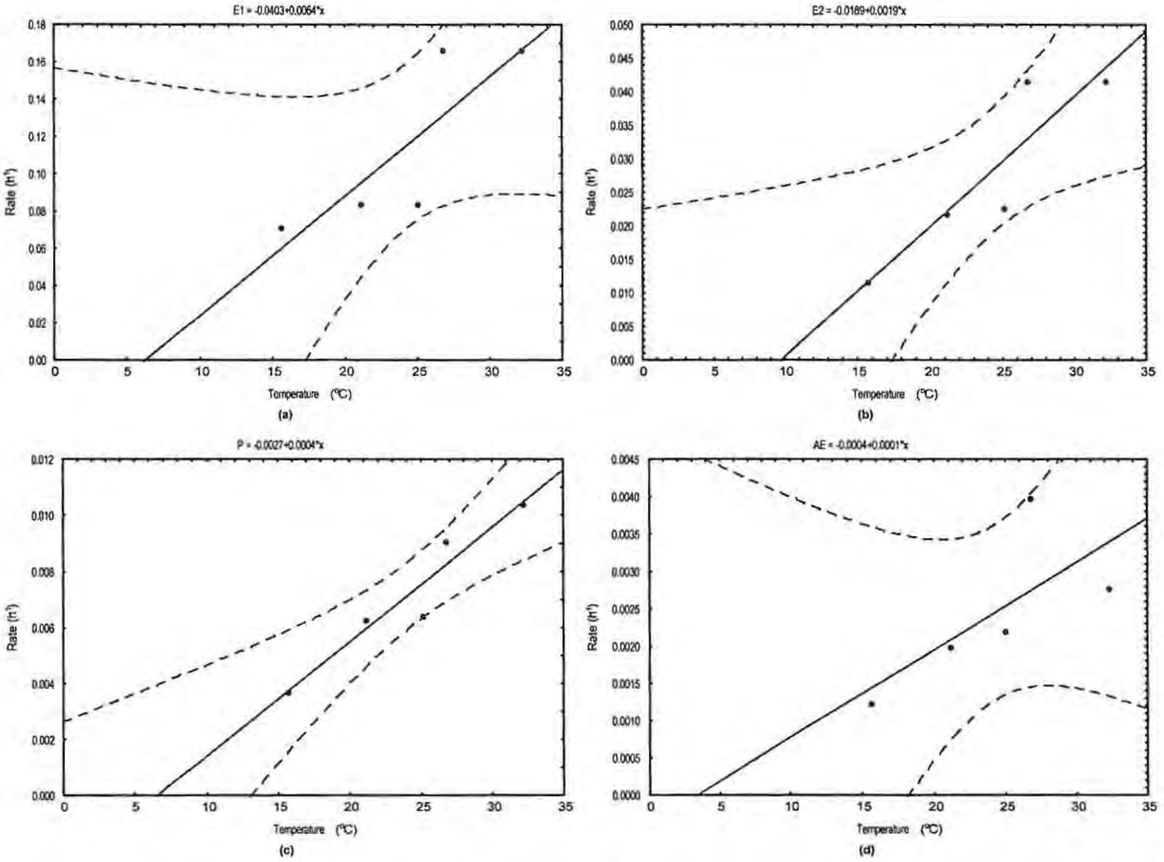


FIGURE 4.1.2 – Development to landmarks (ADD method) for *Sarcophaga africa.* (a) E1; (b) E2; (c) P; (d) AE.

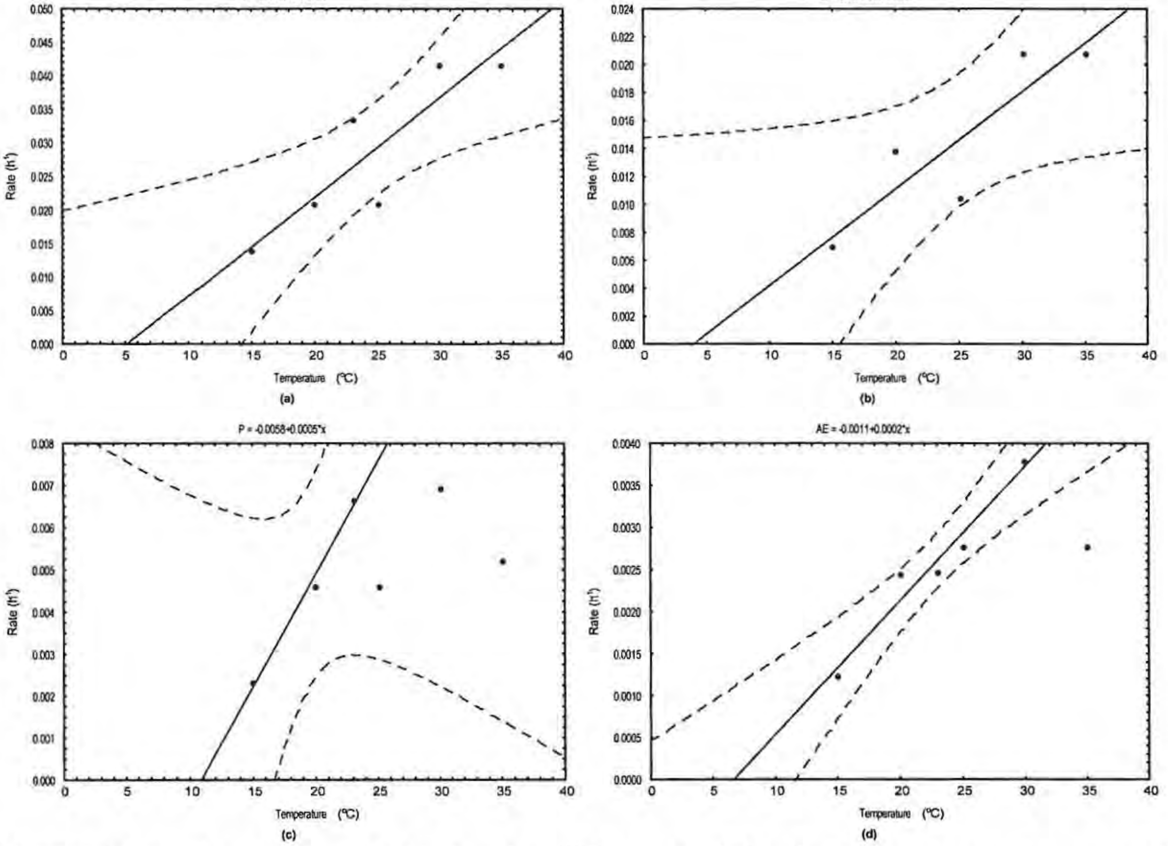


FIGURE 4.1.3 – Development to landmarks (ADD method) for *Sarcophaga peregrina*. (a) E1; (b) E2; (c) P; (d) AE.

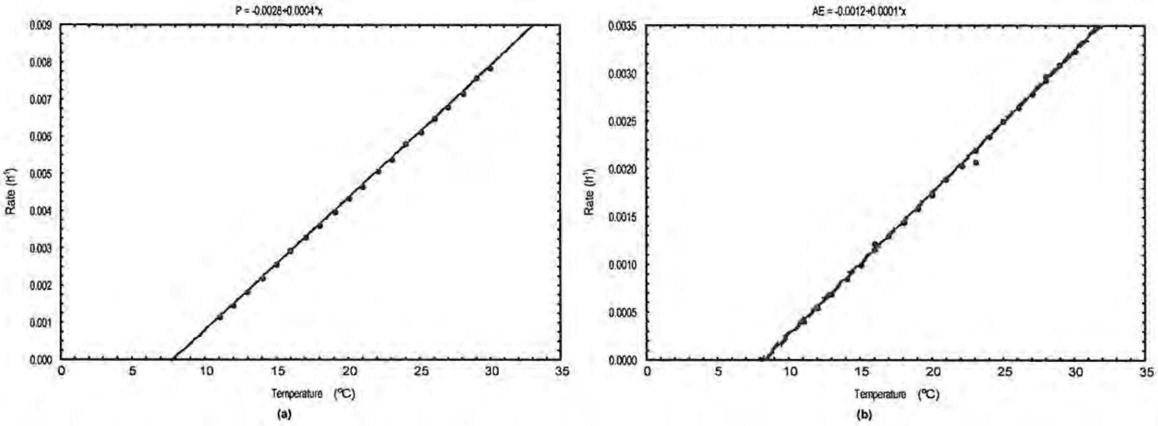
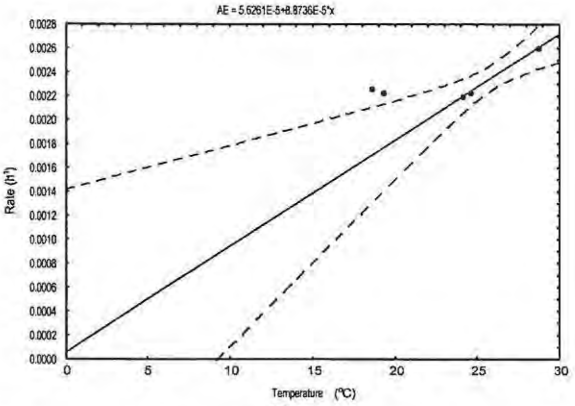


FIGURE 4.1.4 – Development to landmarks (ADD method) for *Sarcophaga septentrionalis*. (a) P; (b) AE.



(a)

FIGURE 4.1.5 - Development to eclosion (ADD method) for *Sarcophaga aegyptiaca*..

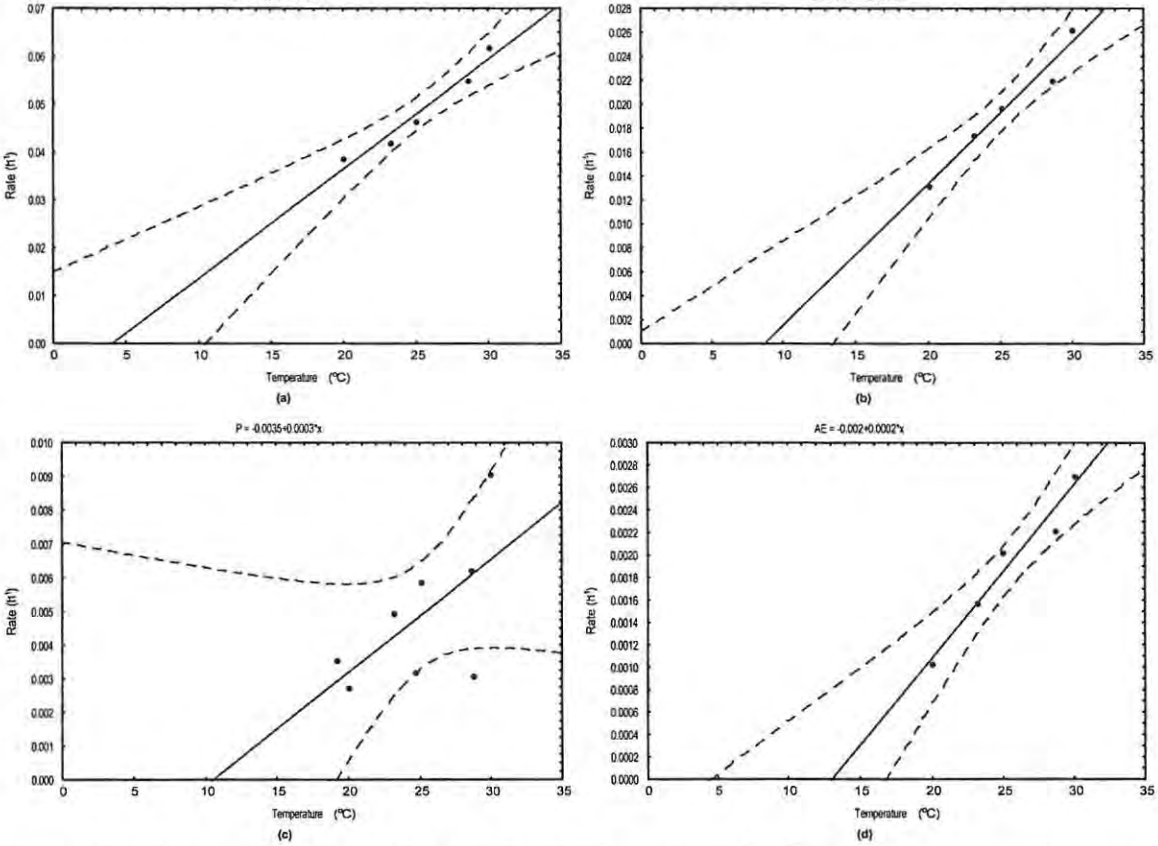


FIGURE 4.1.6 – Development to landmarks (ADD method) for *Sarcophaga argyrostoma*. (a) E1; (b) E2; (c) P; (d) AE.

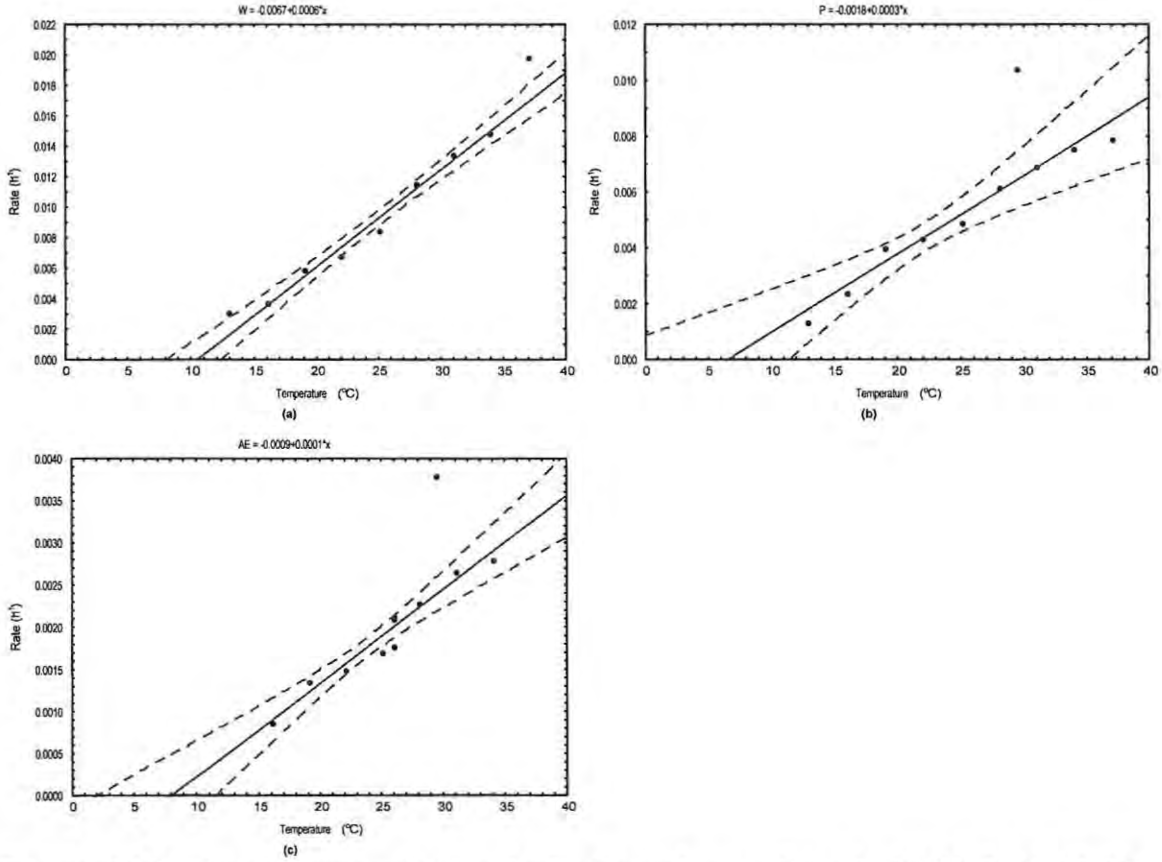


FIGURE 4.1.7 – Development to landmarks for *Sarcophaga ruficornis.* (a) W; (b) P; (c) AE.

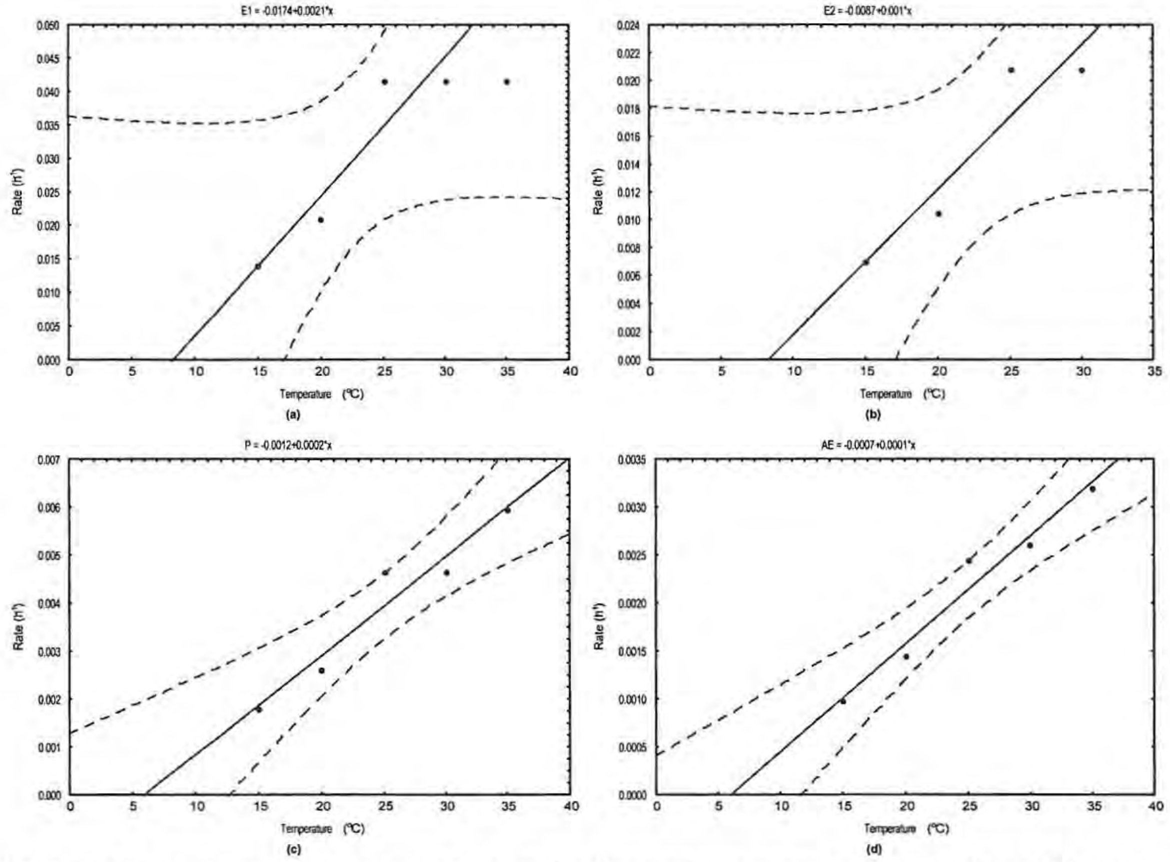


FIGURE 4.1.8 – Development to landmarks for *Sarcophaga crassipalpis*. (a) E1; (b) E2; (c) P; (d) AE.

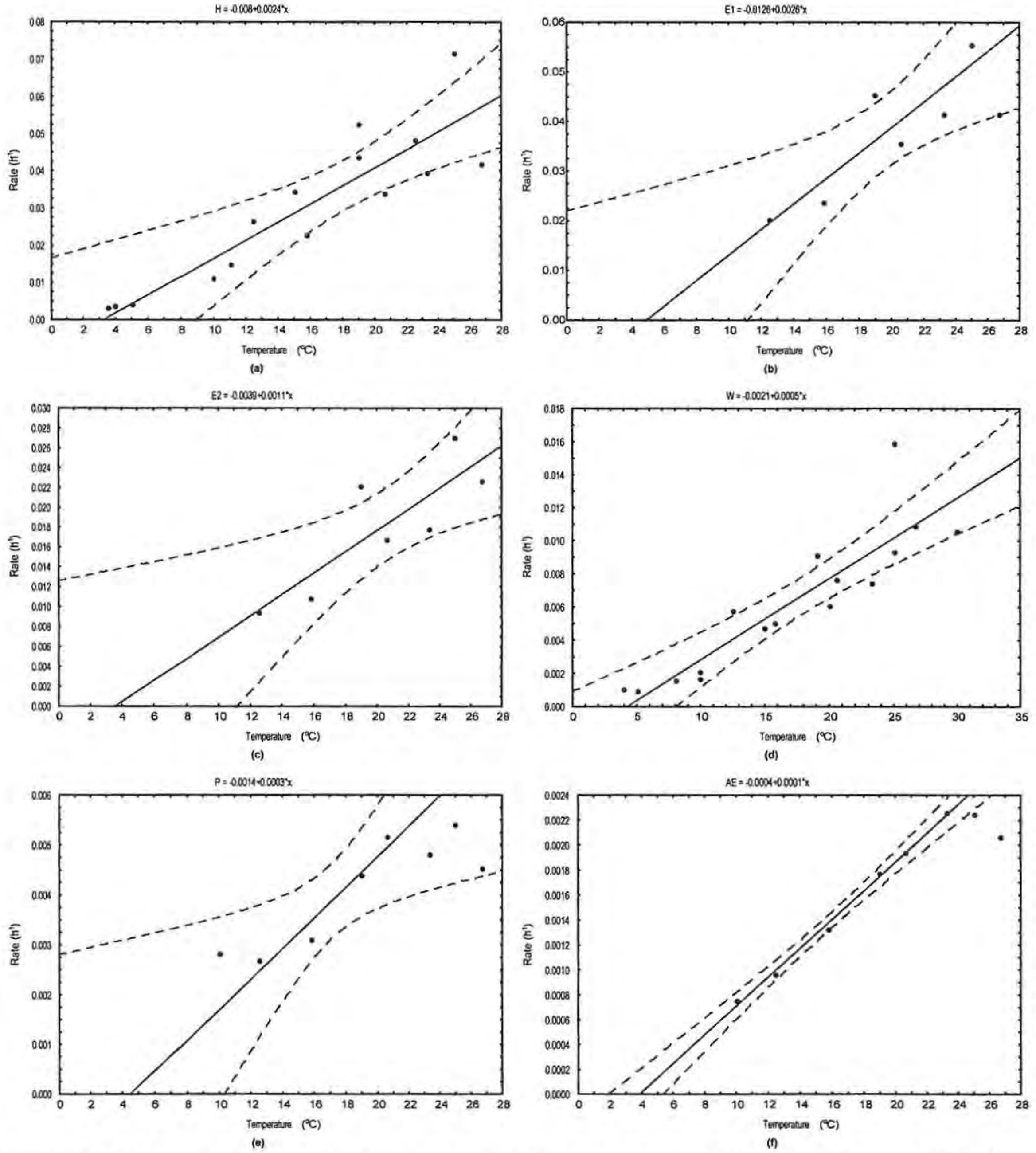


FIGURE 4.1.9 – Development to landmarks (ADD method) for *Calliphora vicina*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

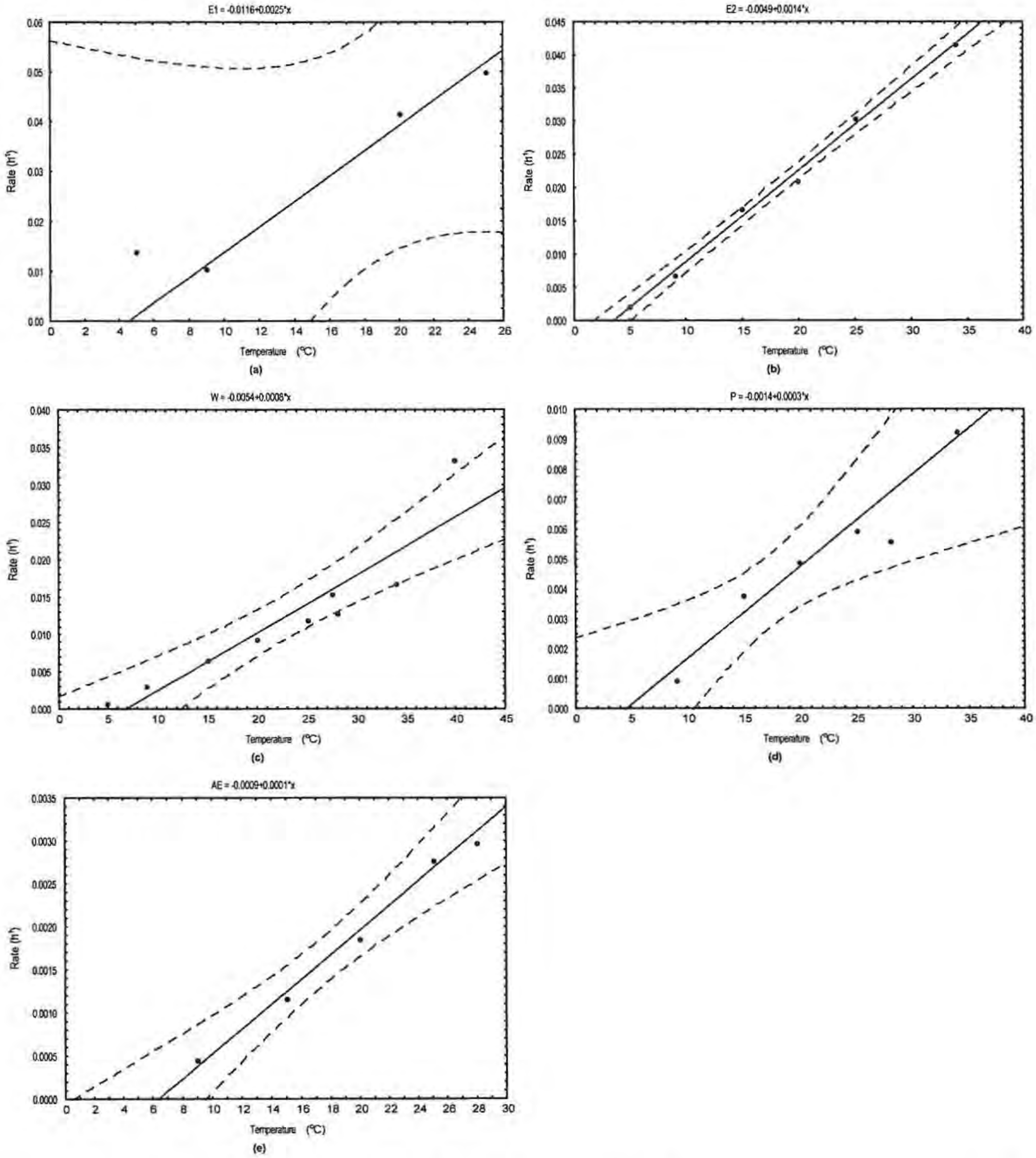


FIGURE 4.1.10 – Development to landmarks (ADD method) for *Calliphora augur*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

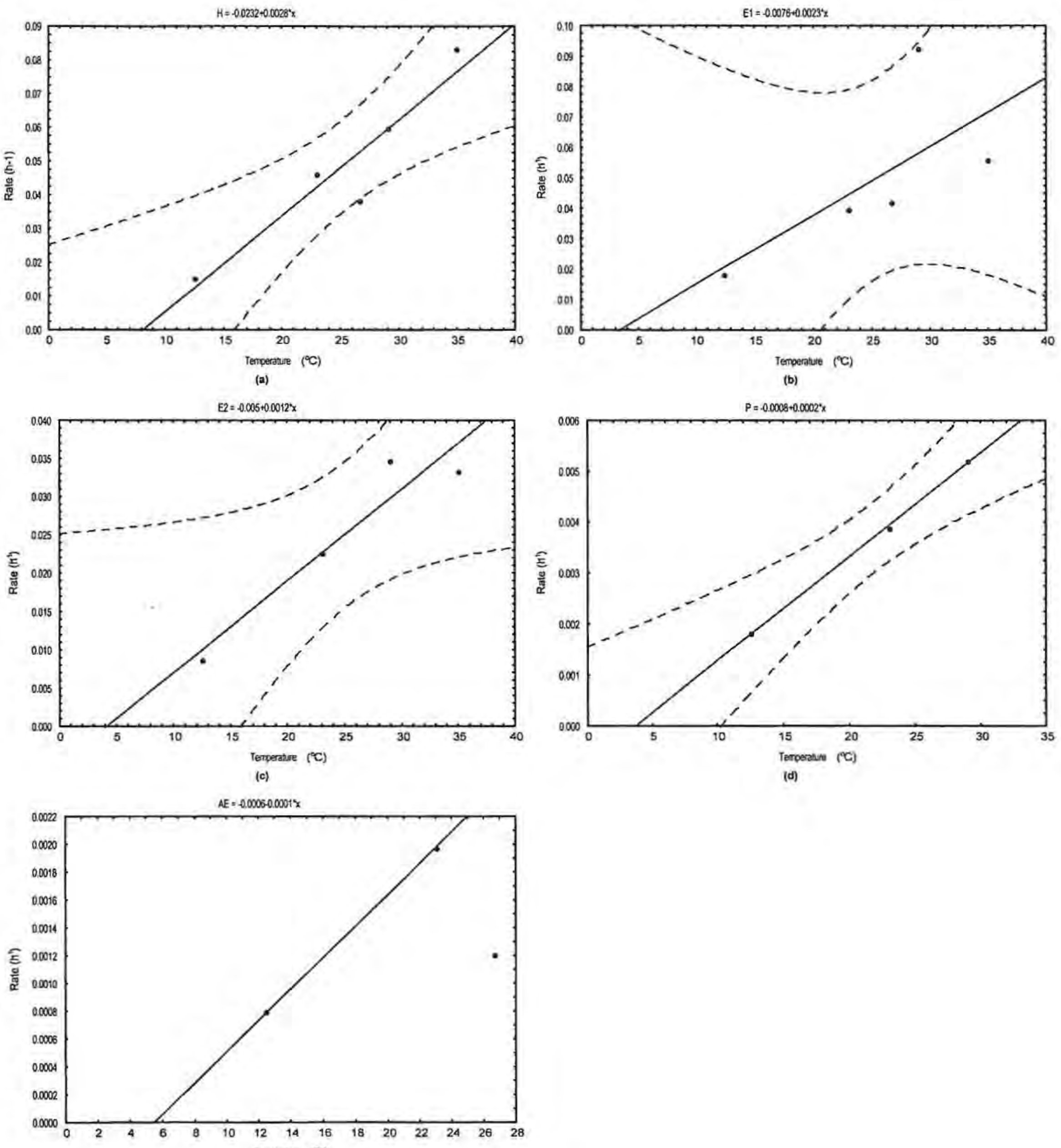


FIGURE 4.1.11 – Development to landmarks (ADD method) for *Calliphora vomitoria*. (a) hatch, (b) first ecdysis, (c) second ecdysis, (d) pupariation (e) eclosion.

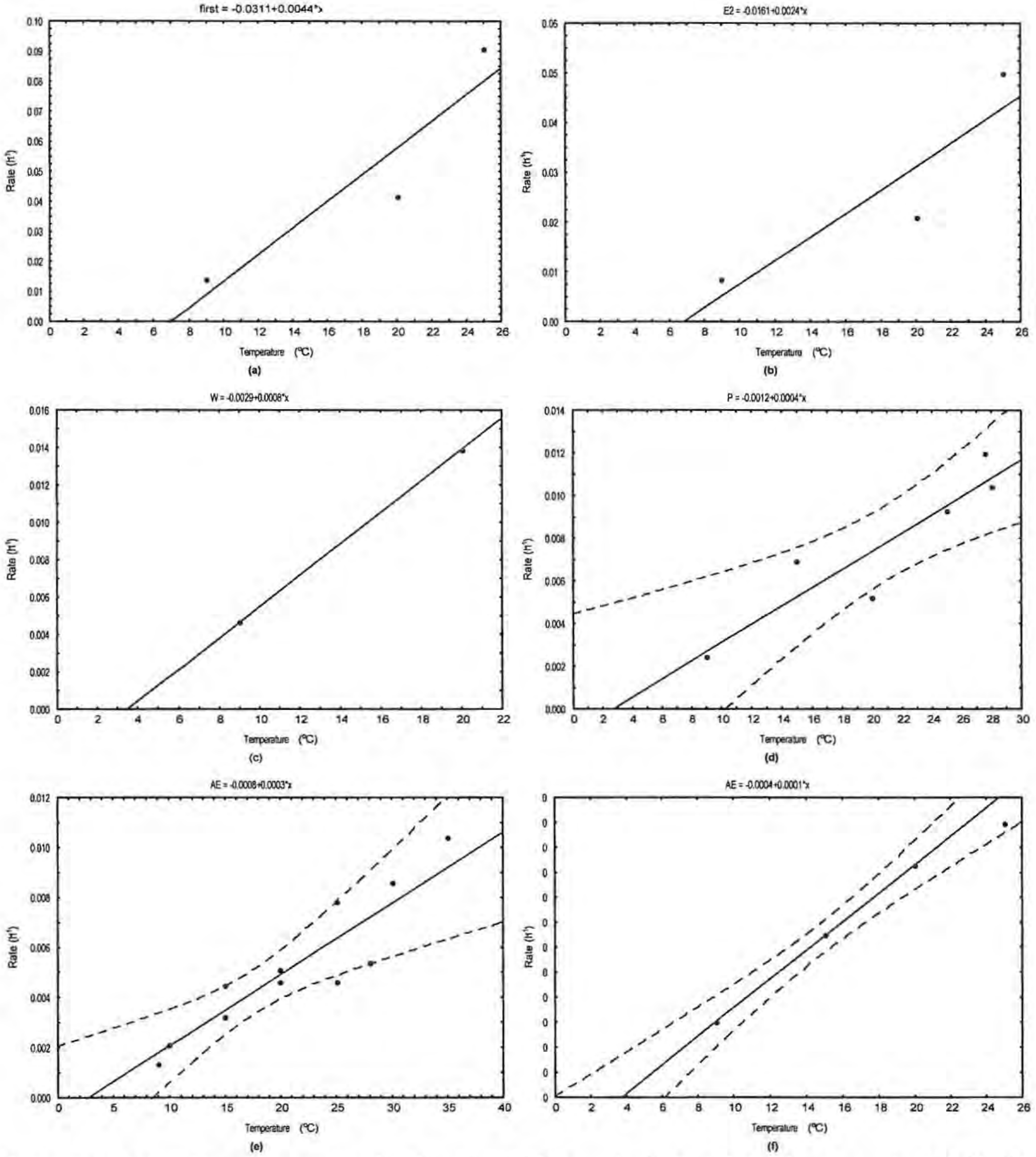


FIGURE 4.1.12 – Development to landmarks (ADD method) for *Calliphora stygia*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

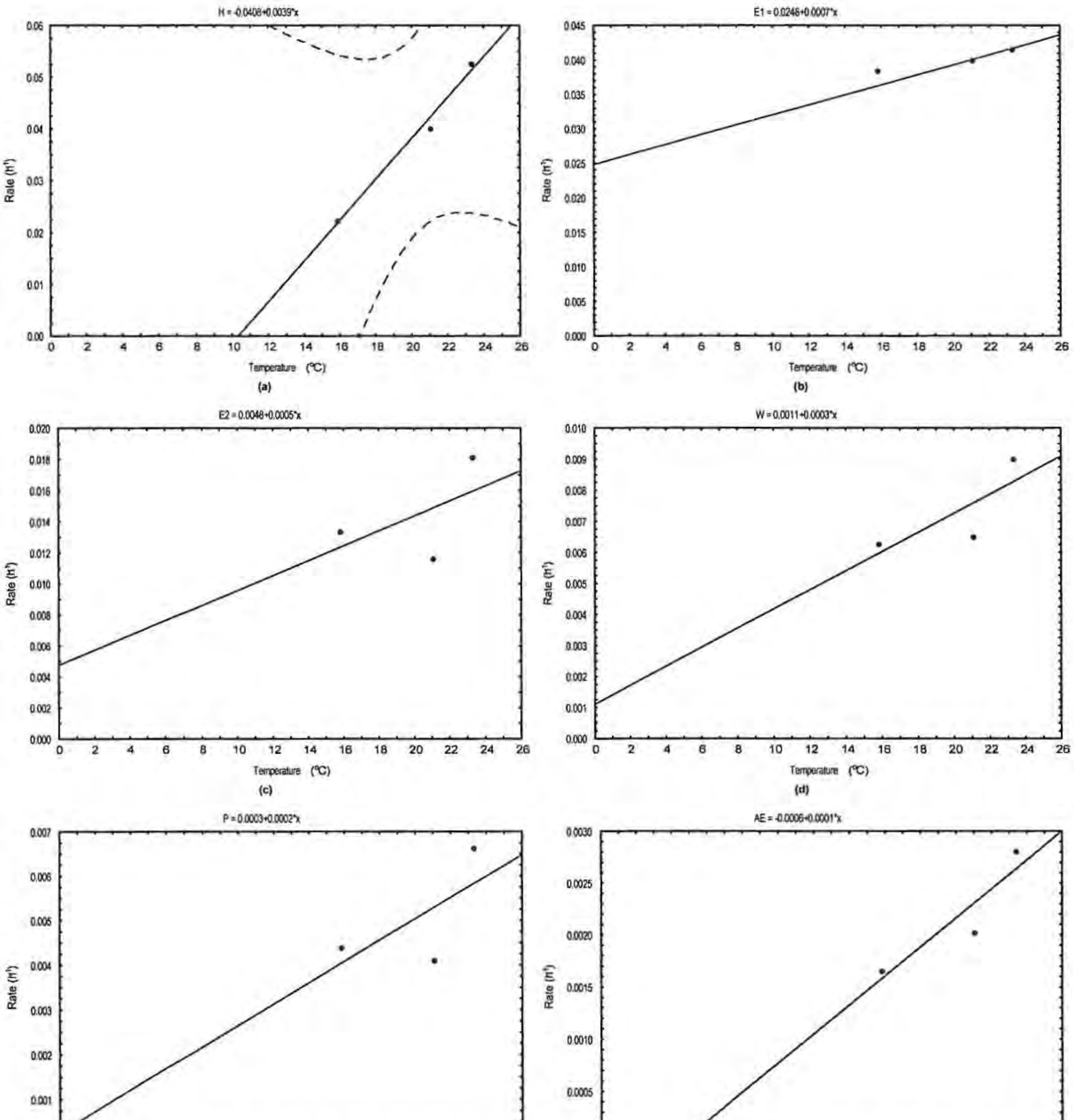


FIGURE 4.1.13 – Development to landmarks (ADD method) for *Eucalliphora latifrons*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

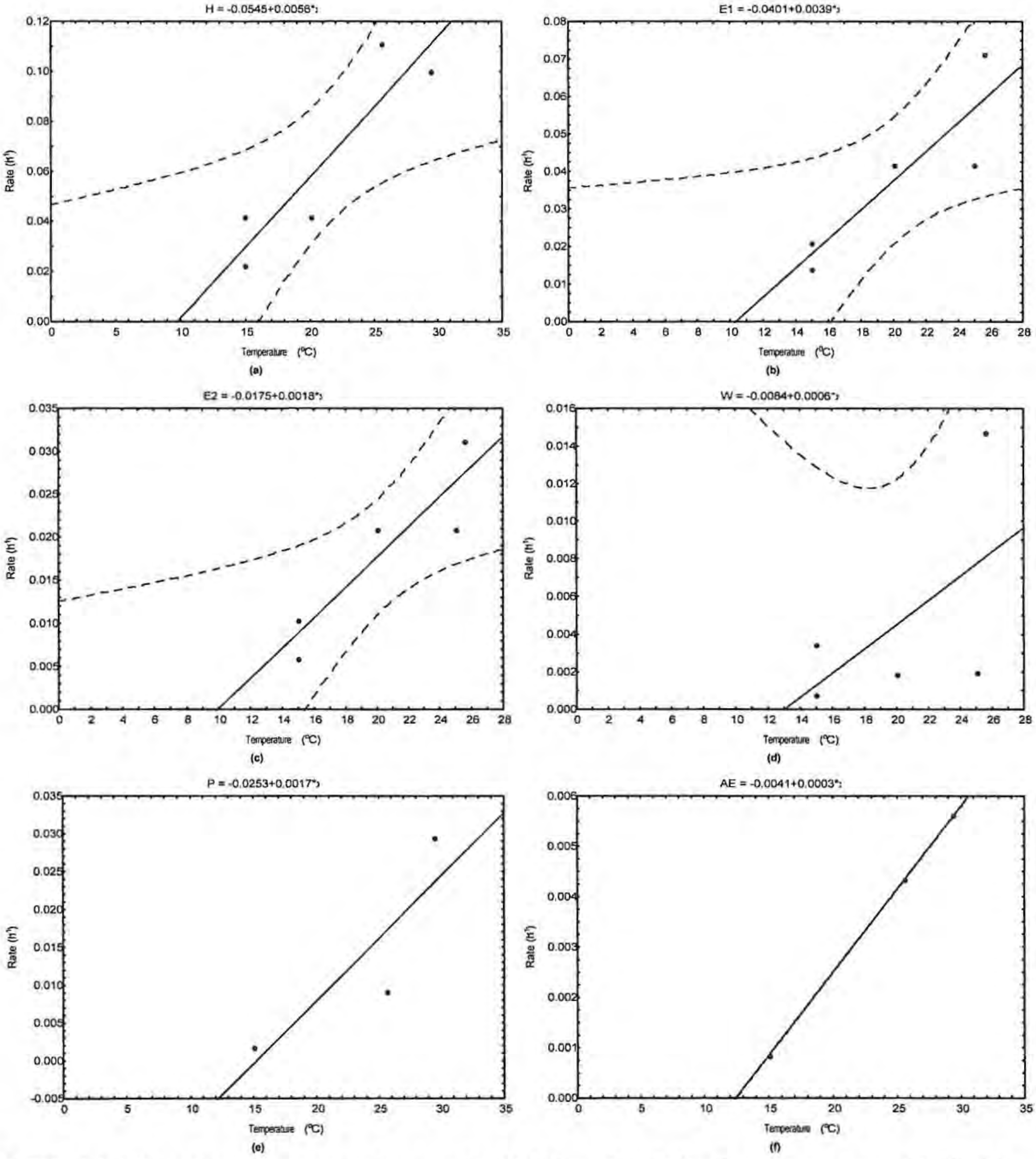


FIGURE 4.1.14 – Development to landmarks (ADD method) for *Lucilia cuprina*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

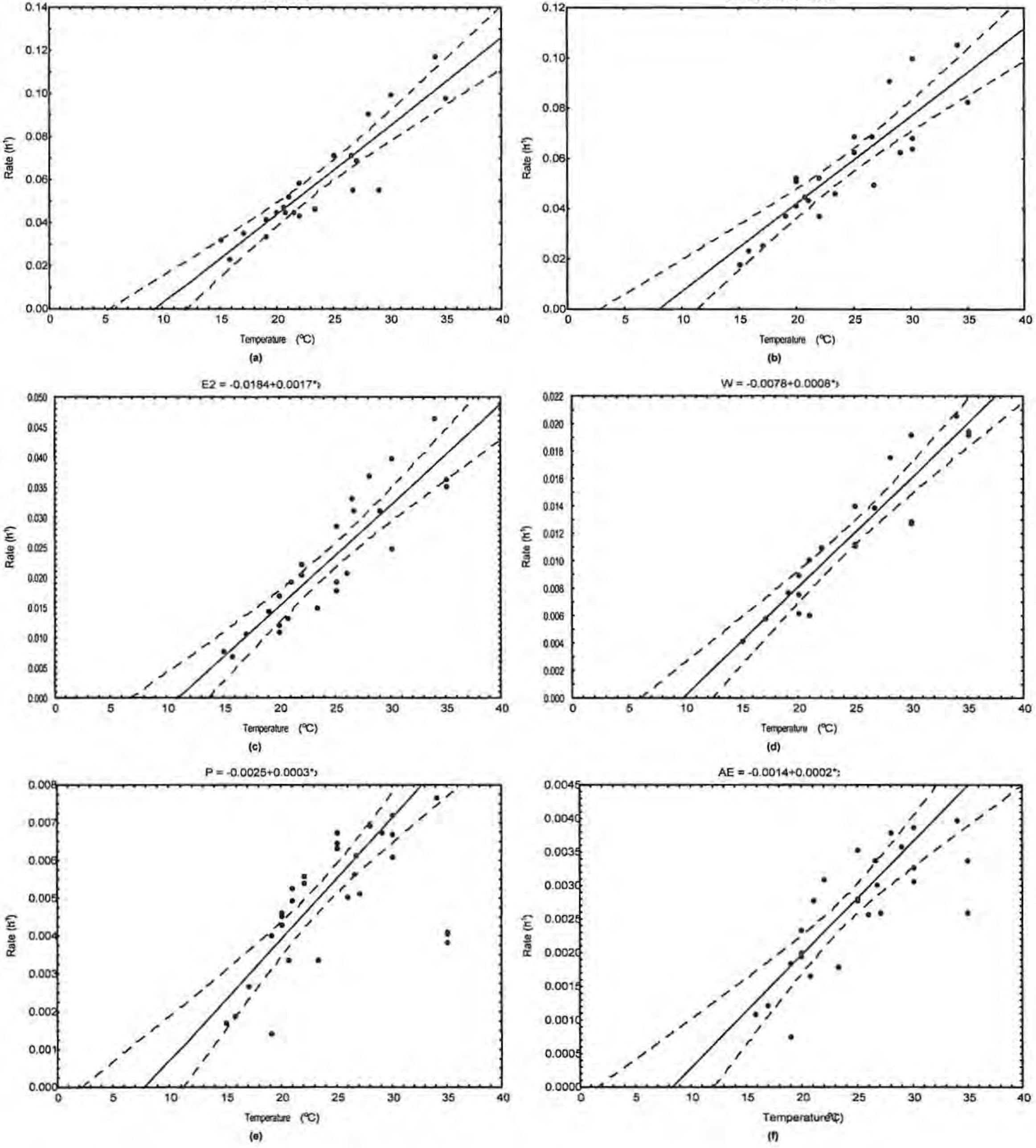


FIGURE 4.1.15 – Development to landmarks (ADD method) for *Lucilia sericata*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE

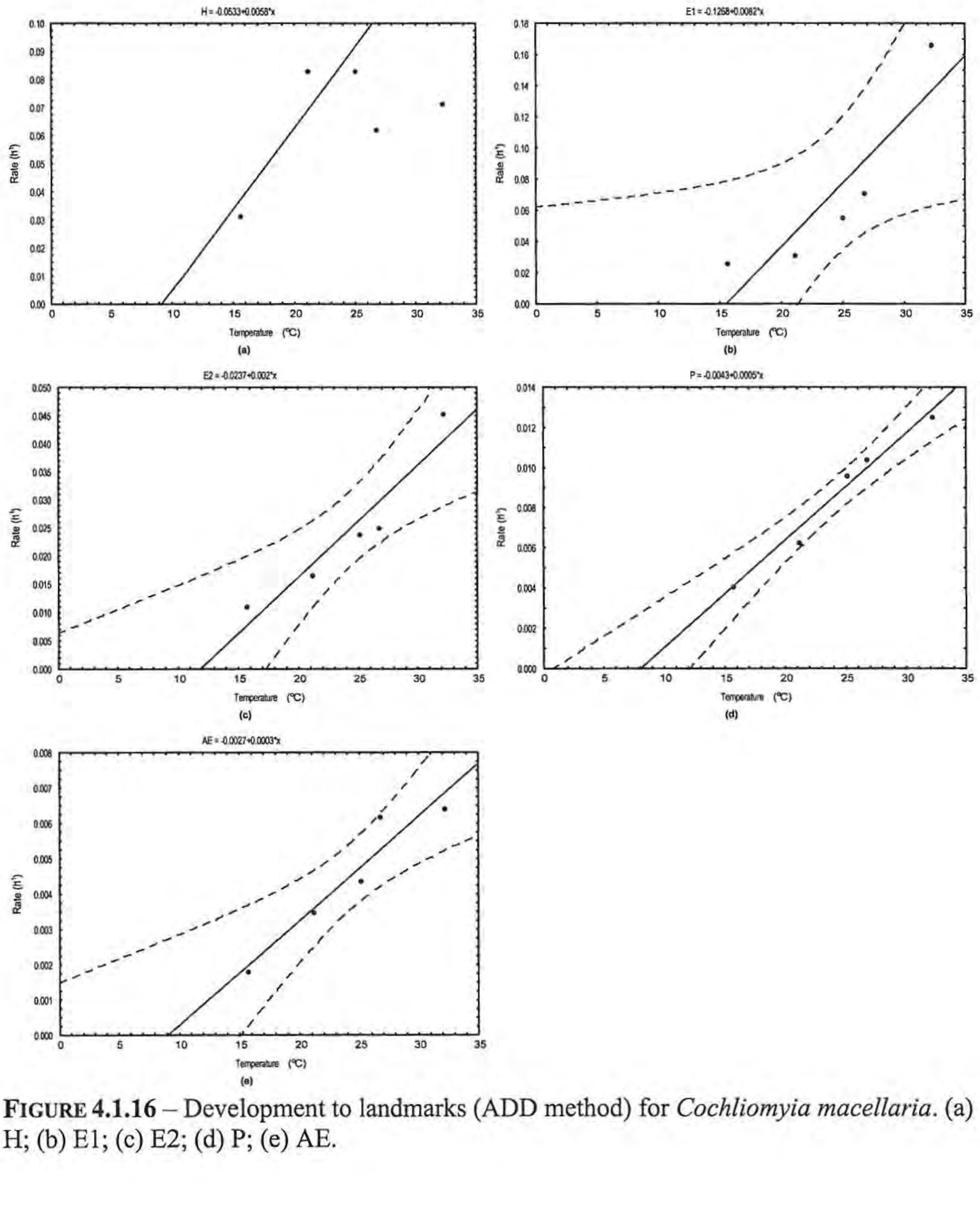


FIGURE 4.1.16 – Development to landmarks (ADD method) for *Cochliomyia macellaria*. (a) H; (b) E1; (c) E2; (d) P; (e) AE.

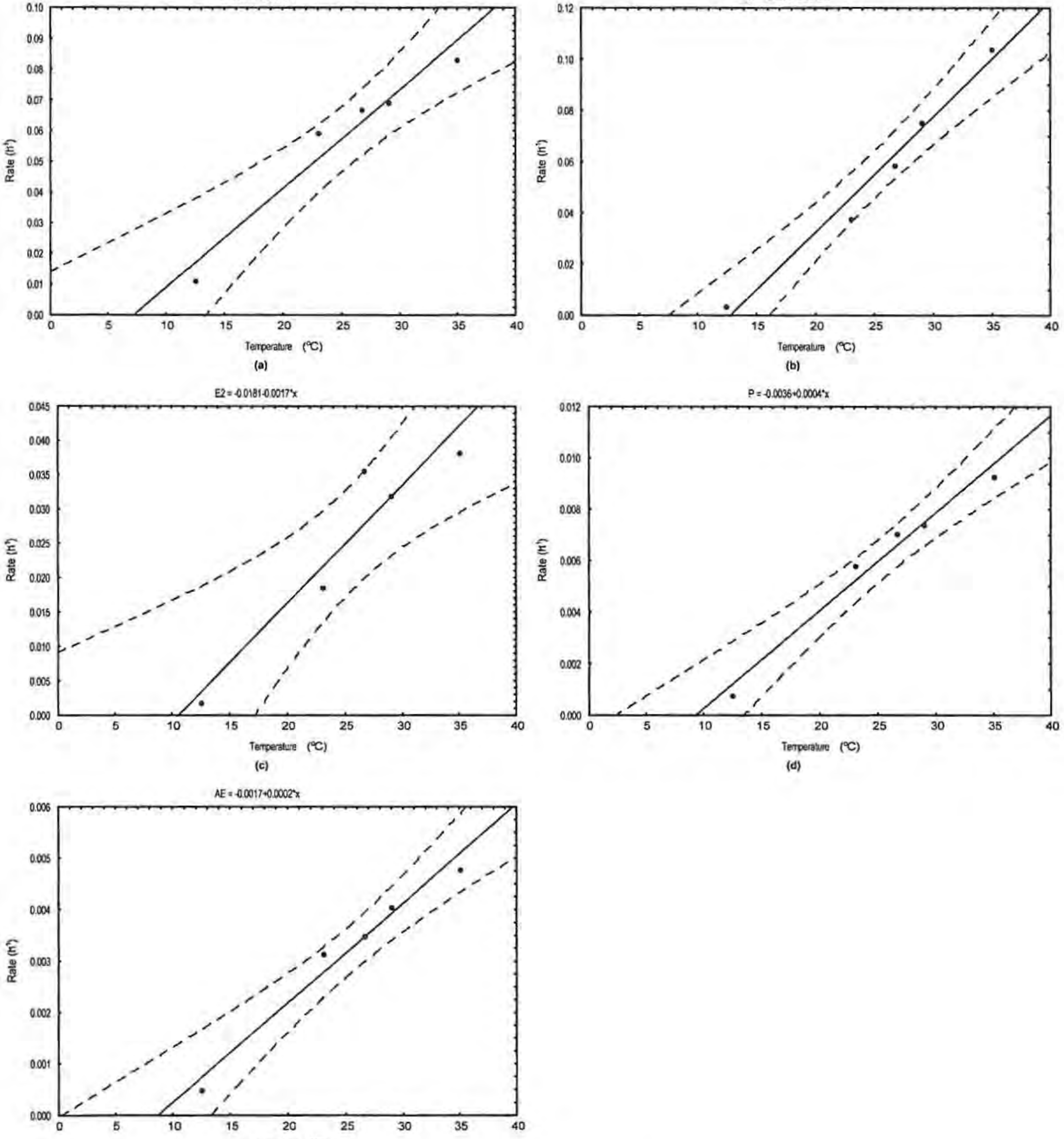


FIGURE 4.1.17– Development to landmarks (ADD method) for *Protophormia terraenovae* (a) H; (b) E1; (c) E2; (d) P; (e) AE.

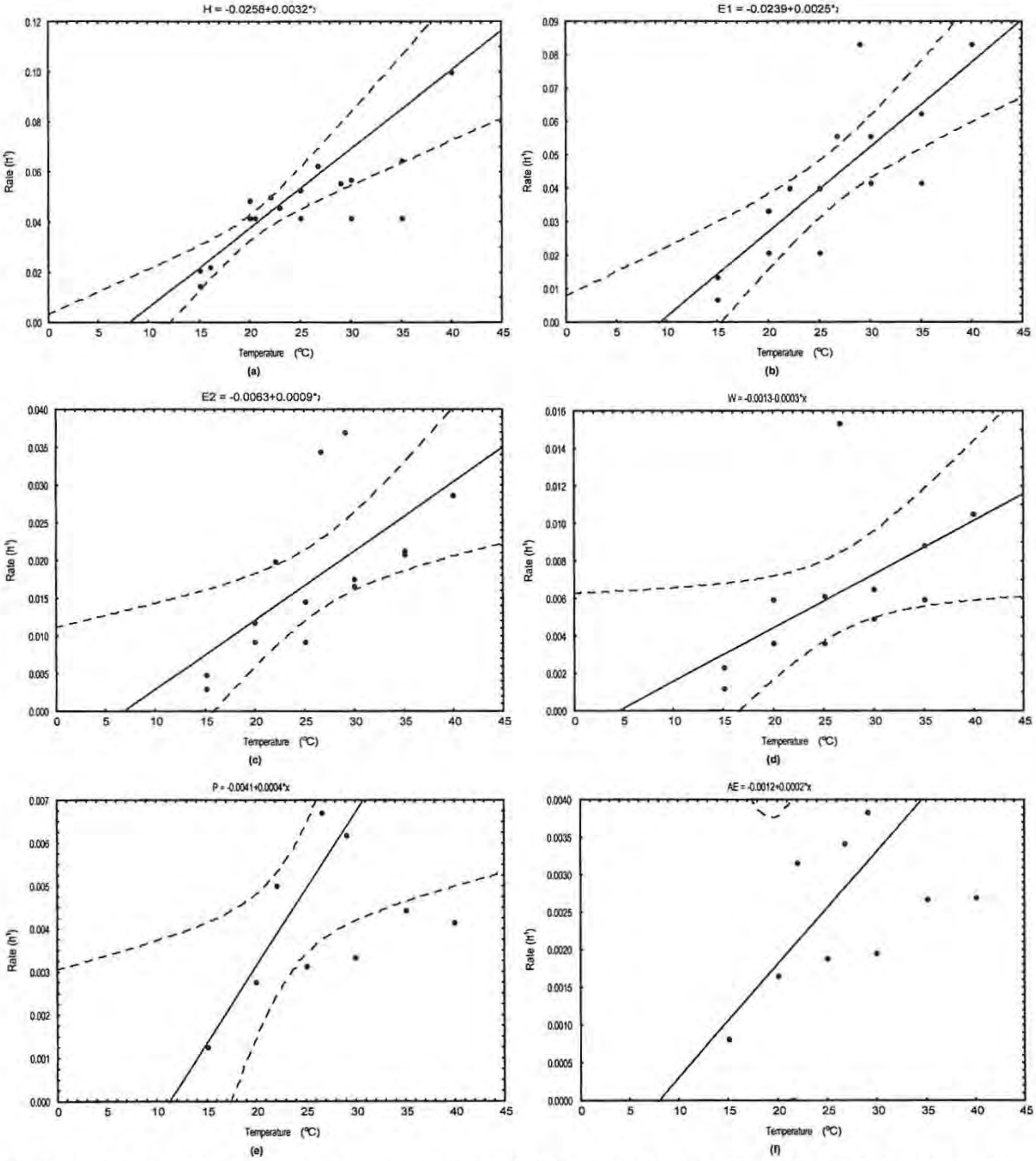


FIGURE 4.1.18 – Development to landmarks (ADD method) for *Phormia regina*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

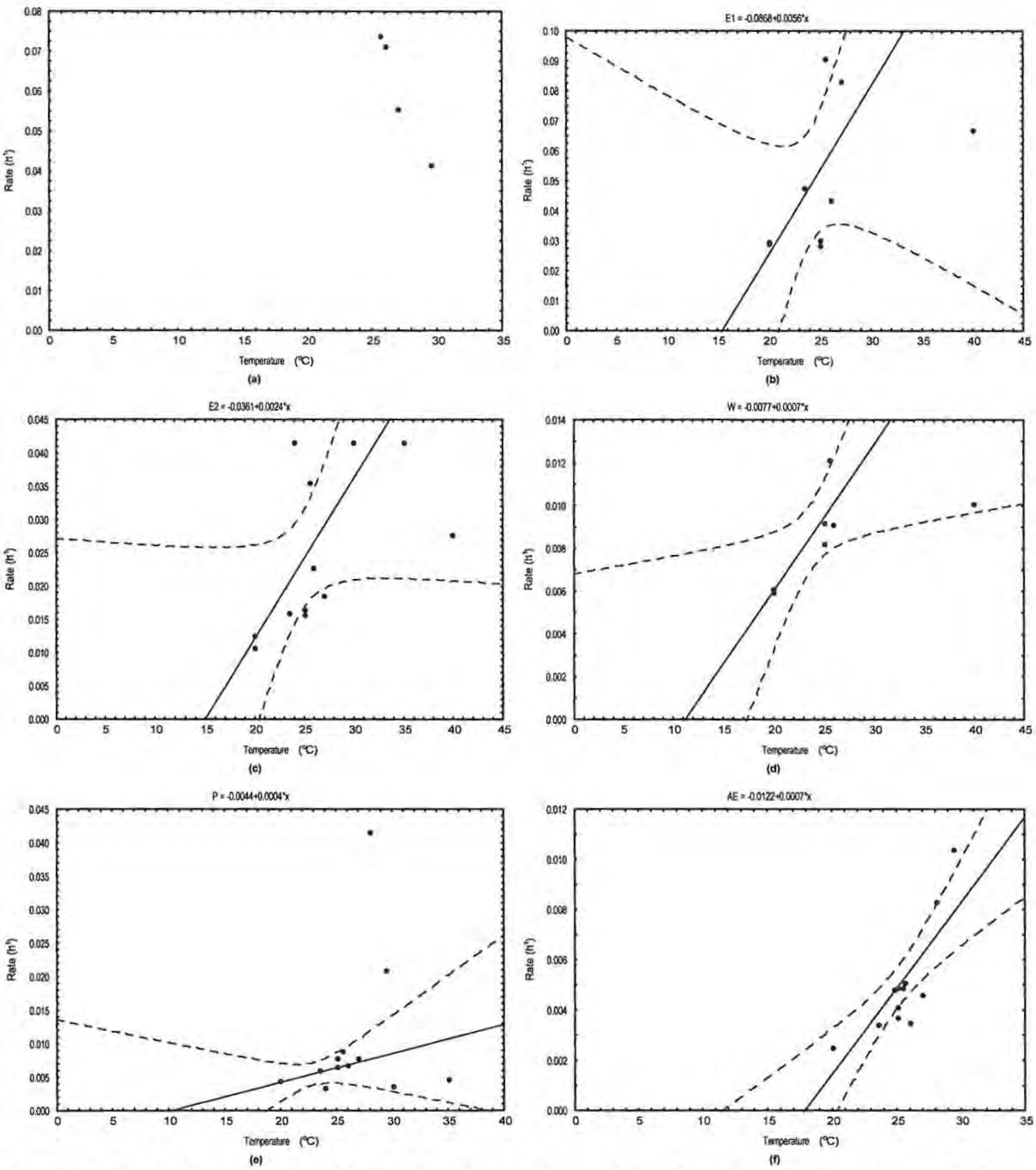


FIGURE 4.1.19 – Development to landmarks (ADD method) for *Chrysomya megacephala*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

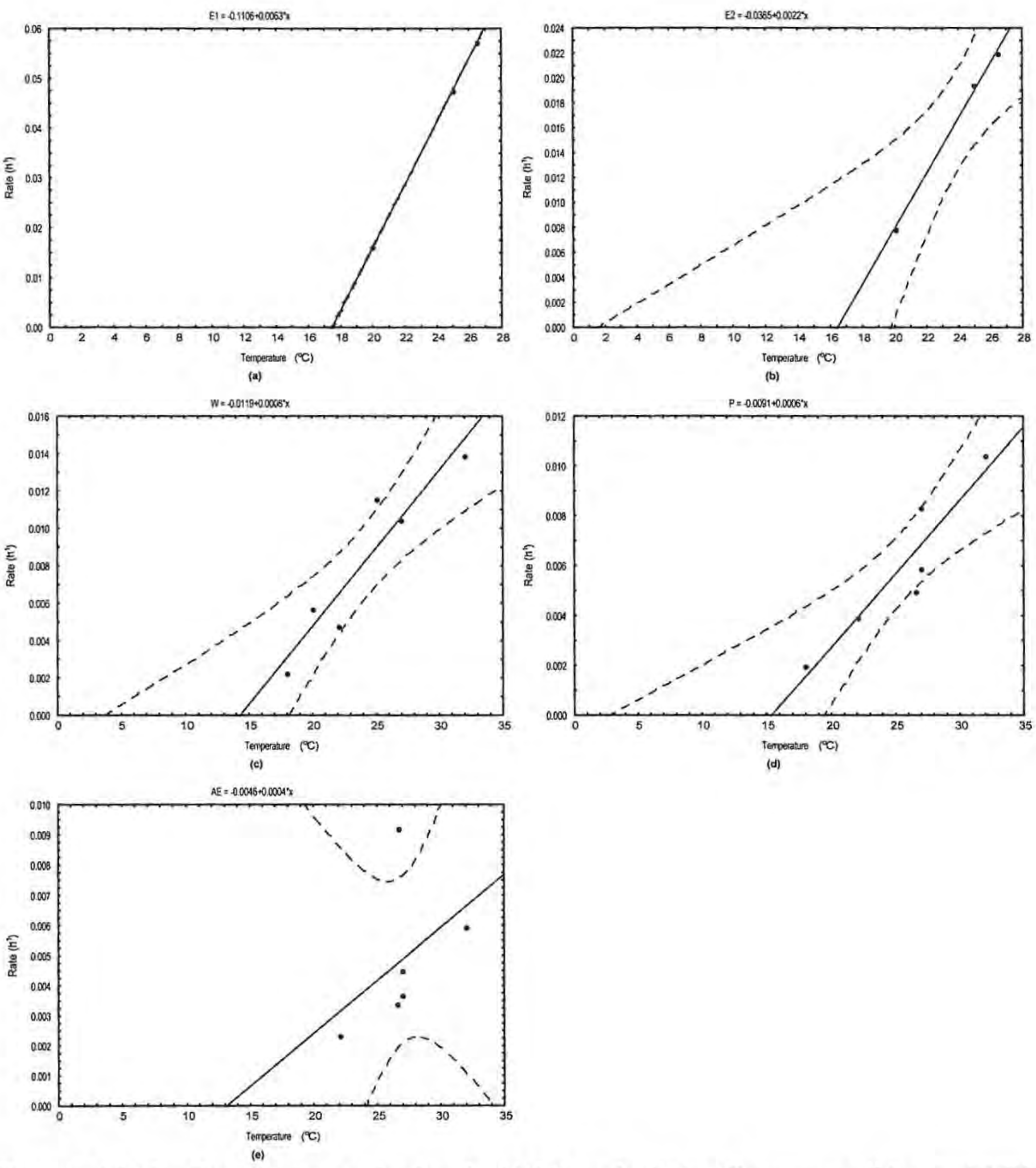


FIGURE 4.1.20 – Development to landmarks (ADD method) for *Chrysomya albiceps*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

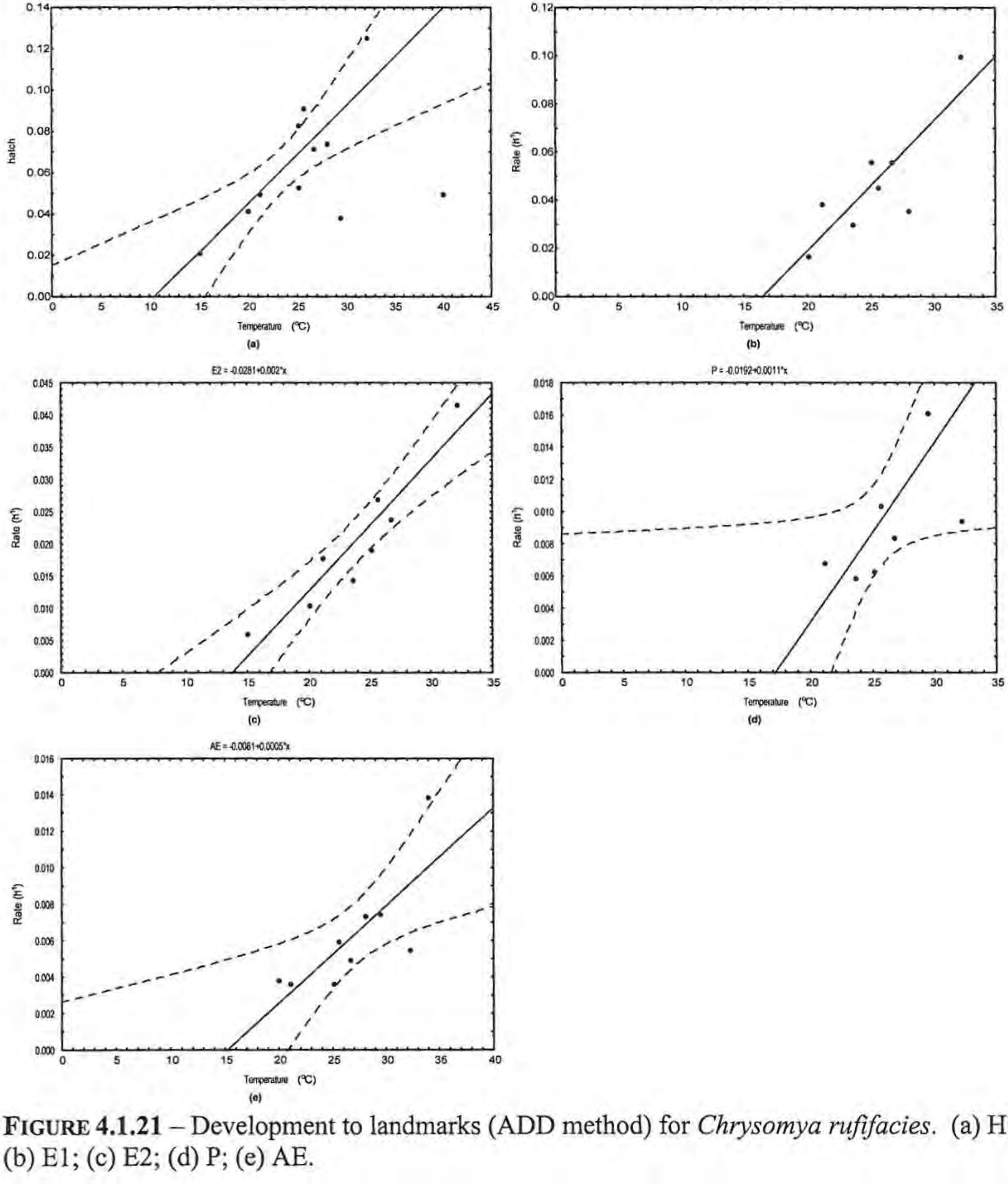


FIGURE 4.1.21 – Development to landmarks (ADD method) for *Chrysomya rufifacies*. (a) H; (b) E1; (c) E2; (d) P; (e) AE.

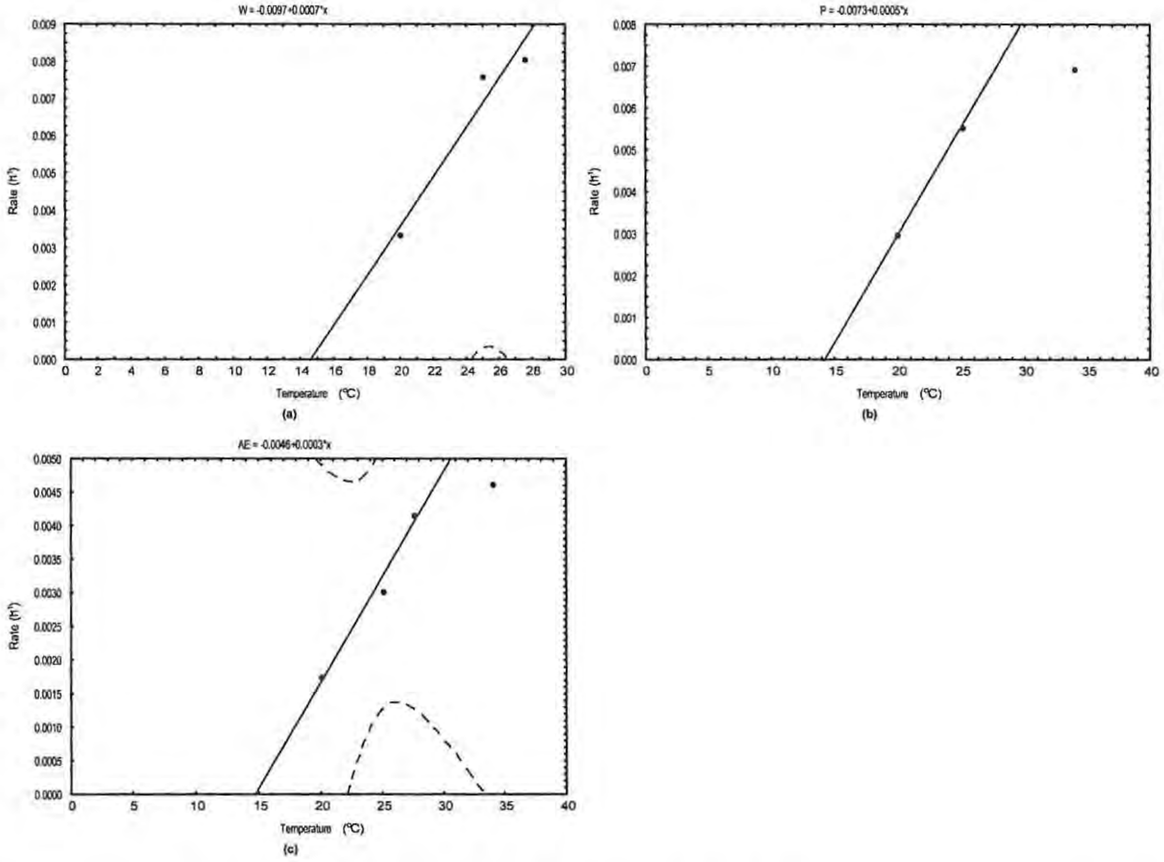


FIGURE 4.1.22 – Development to landmarks (ADD method) for *Chrysomya varipes.* (a) W; (b) P; (c) AE.

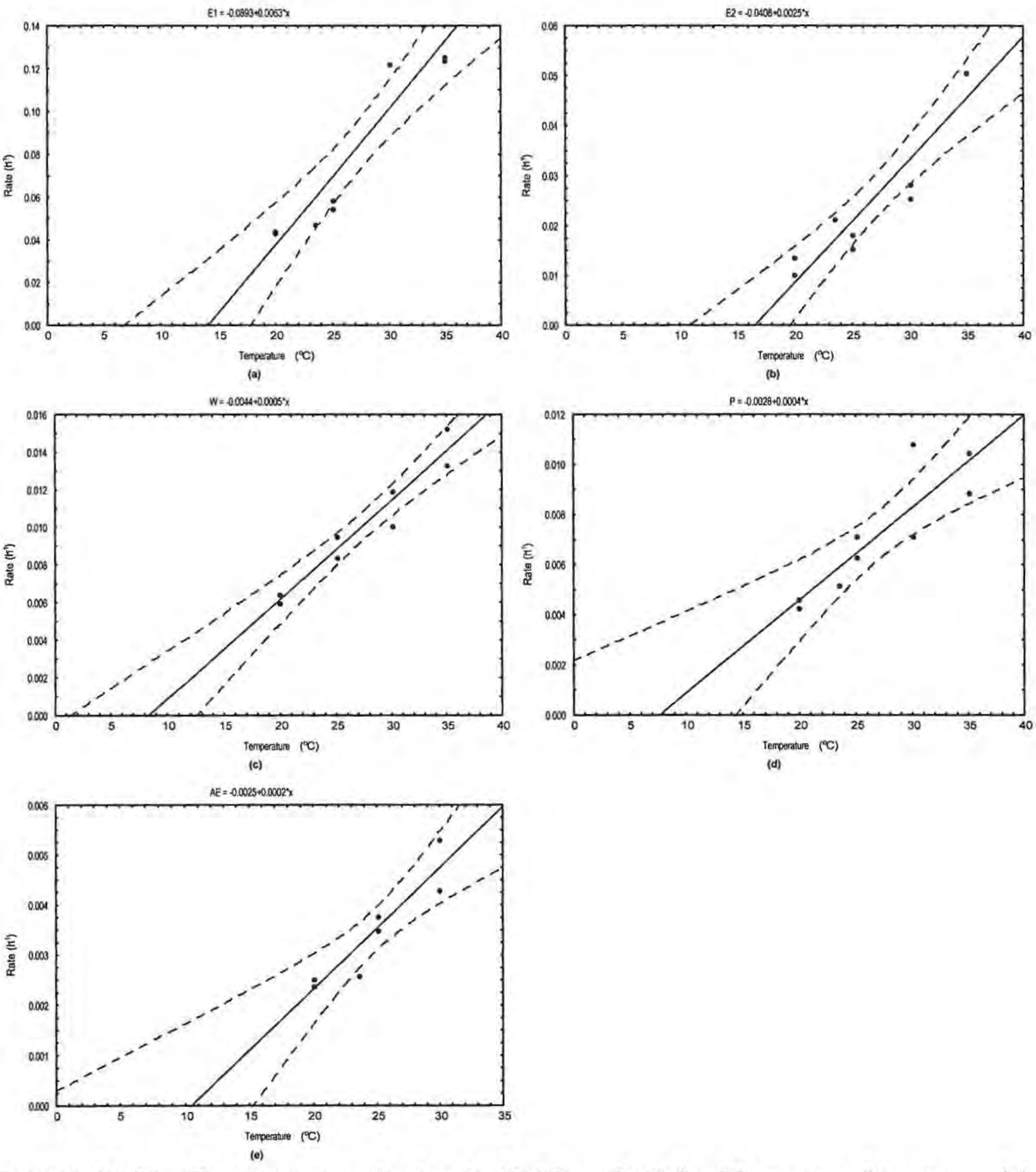


FIGURE 4.1.23 – Development to landmarks (ADD method) for *Chrysomya chloropyga*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

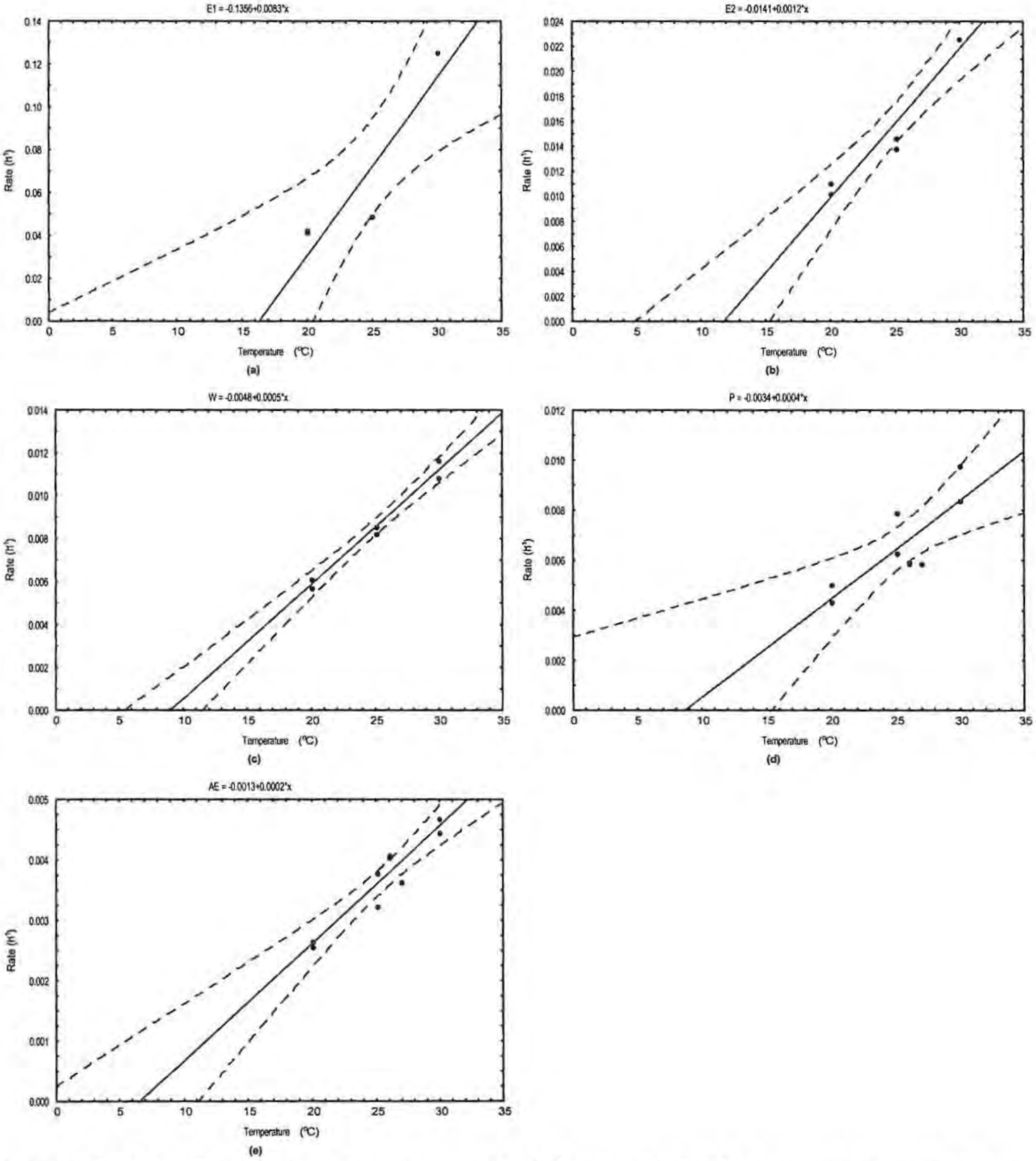


FIGURE 4.1.24 – Development to landmarks (ADD method) for *Chrysomya putoria*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

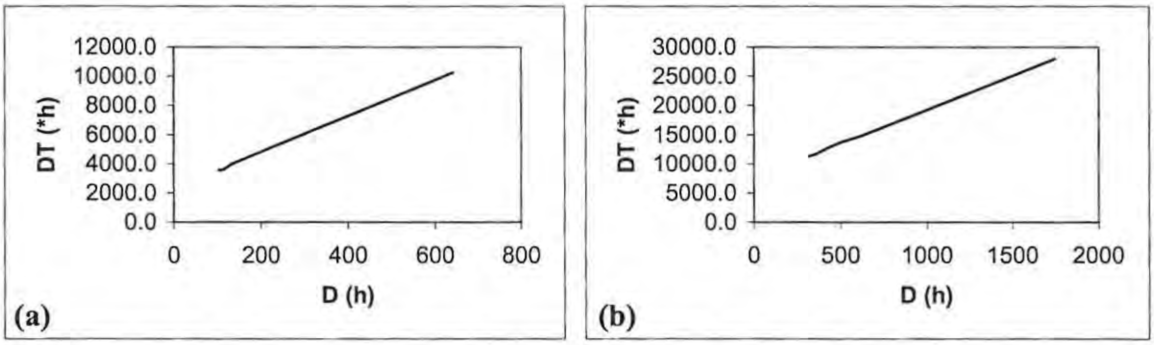


FIGURE 4.2.1 – Development to landmarks (LF method) for *Wohlfahrtia trina*. (a) P; (b) AE.

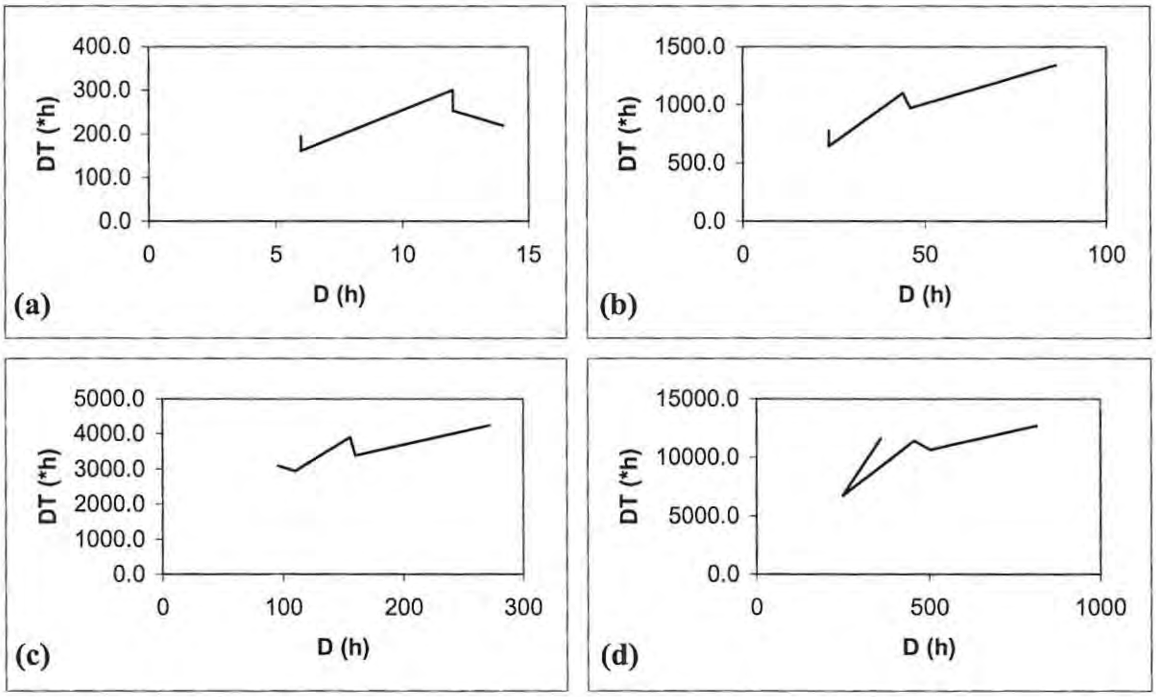


FIGURE 4.2.2 – Development to landmarks (LF method) for *Sarcophaga africa*. (a) E1; (b) E2; (c) P; (d) AE

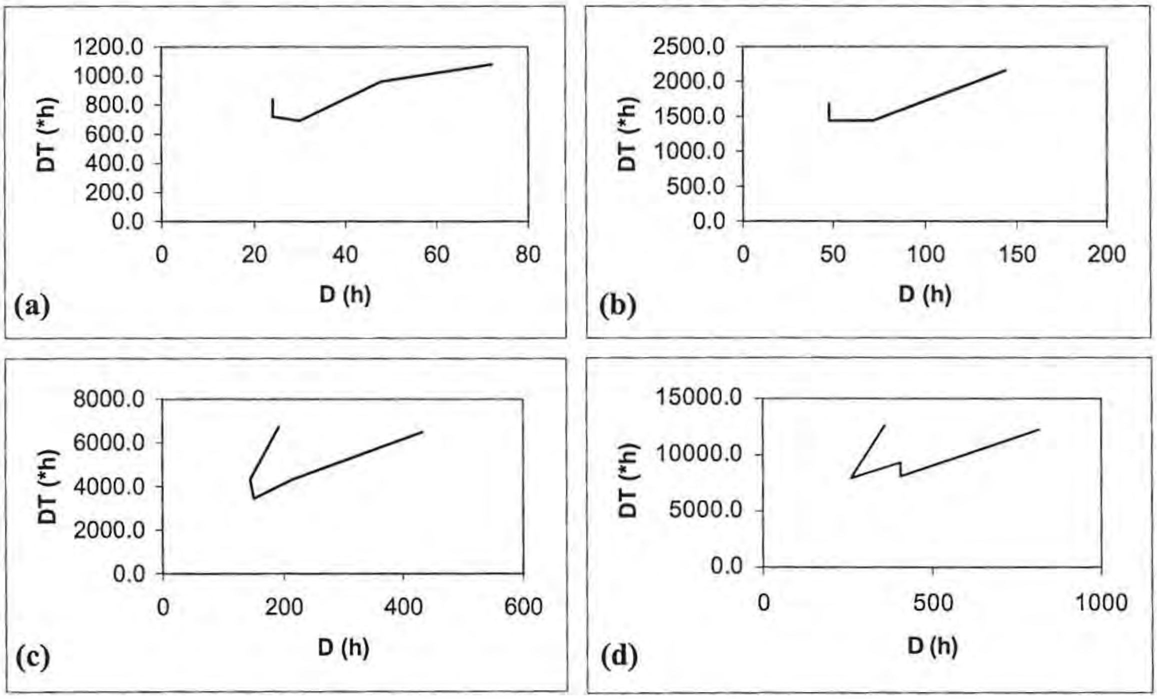


FIGURE 4.2.3 – Development to landmarks (LF method) for *Sarcophaga peregrina*. (a) E1, (b) E2, (c) P, (d) AE.

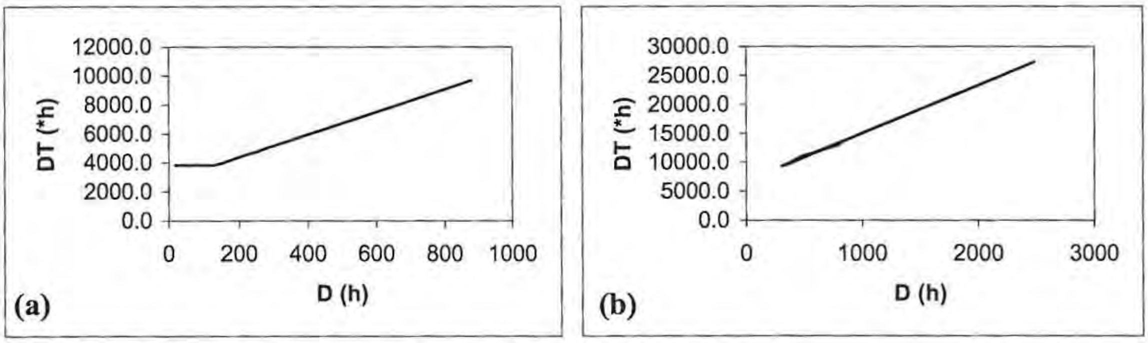


FIGURE 4.2.4 – Development to landmarks (LF method) for *Sarcophaga septentrionalis*. (a) P; (b) AE.

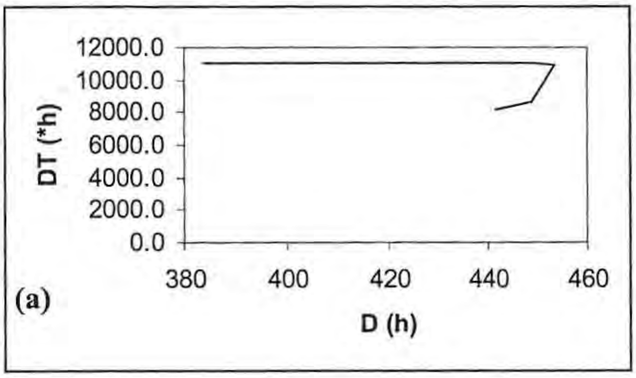


FIGURE 4.2.5 – Development to eclosion (LF method) for *Sarcophaga aegyptiaca*.

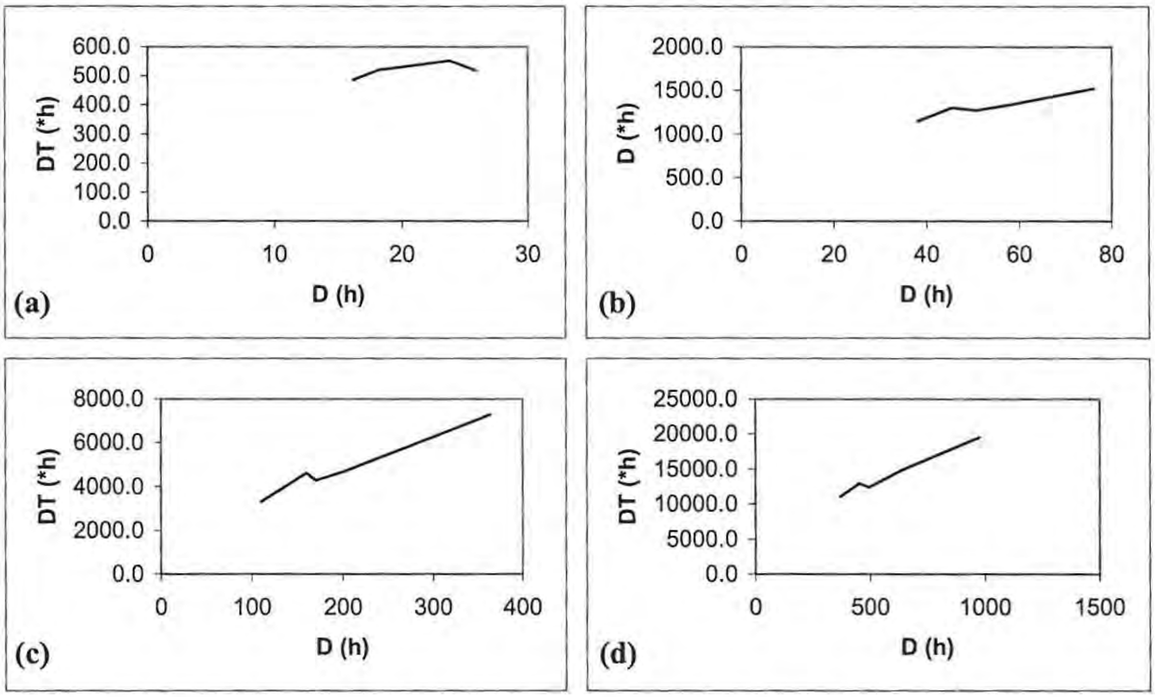


FIGURE 4.2.6 – Development to landmarks (LF method) for *Sarcophaga argyrostoma*. (a) E1; (b) E2; (c) P; (d) AE.

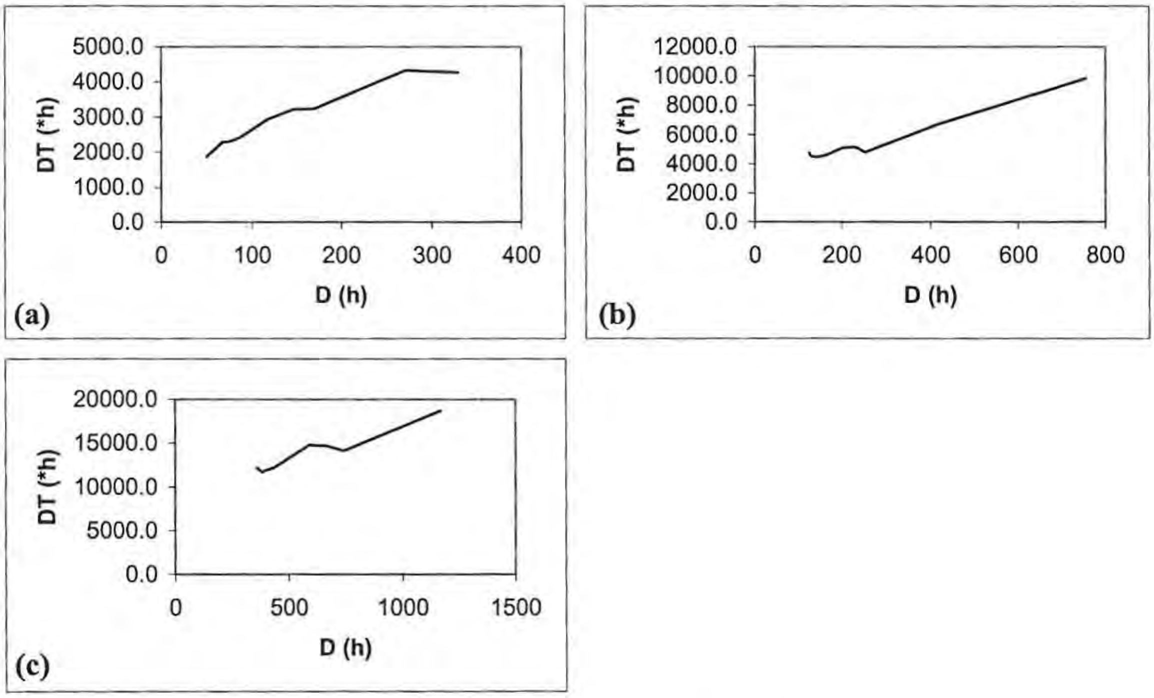


FIGURE 4.2.7 – Development to landmarks (LF method) for *Sarcophaga ruficornis*. (a) W; (b) P; (c) AE.

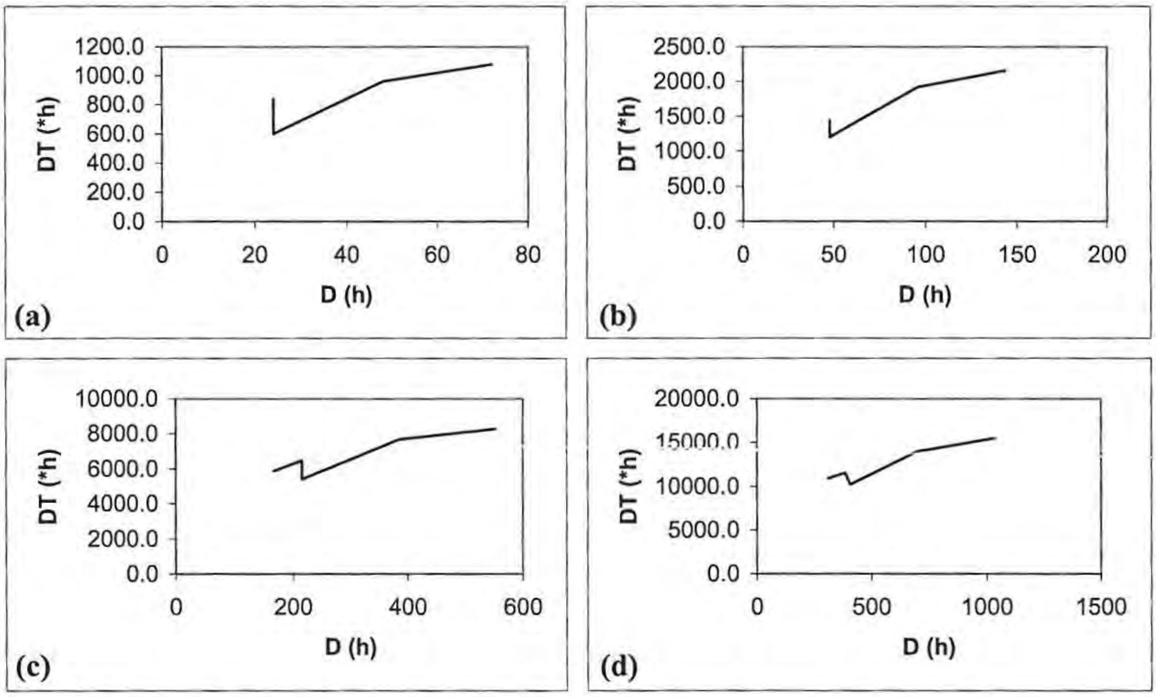


FIGURE 4.2.8 – Development to landmarks (LF method) for *Sarcophaga crassipalpis*. (a) E1; (b) E2; (c) P; (d) AE.

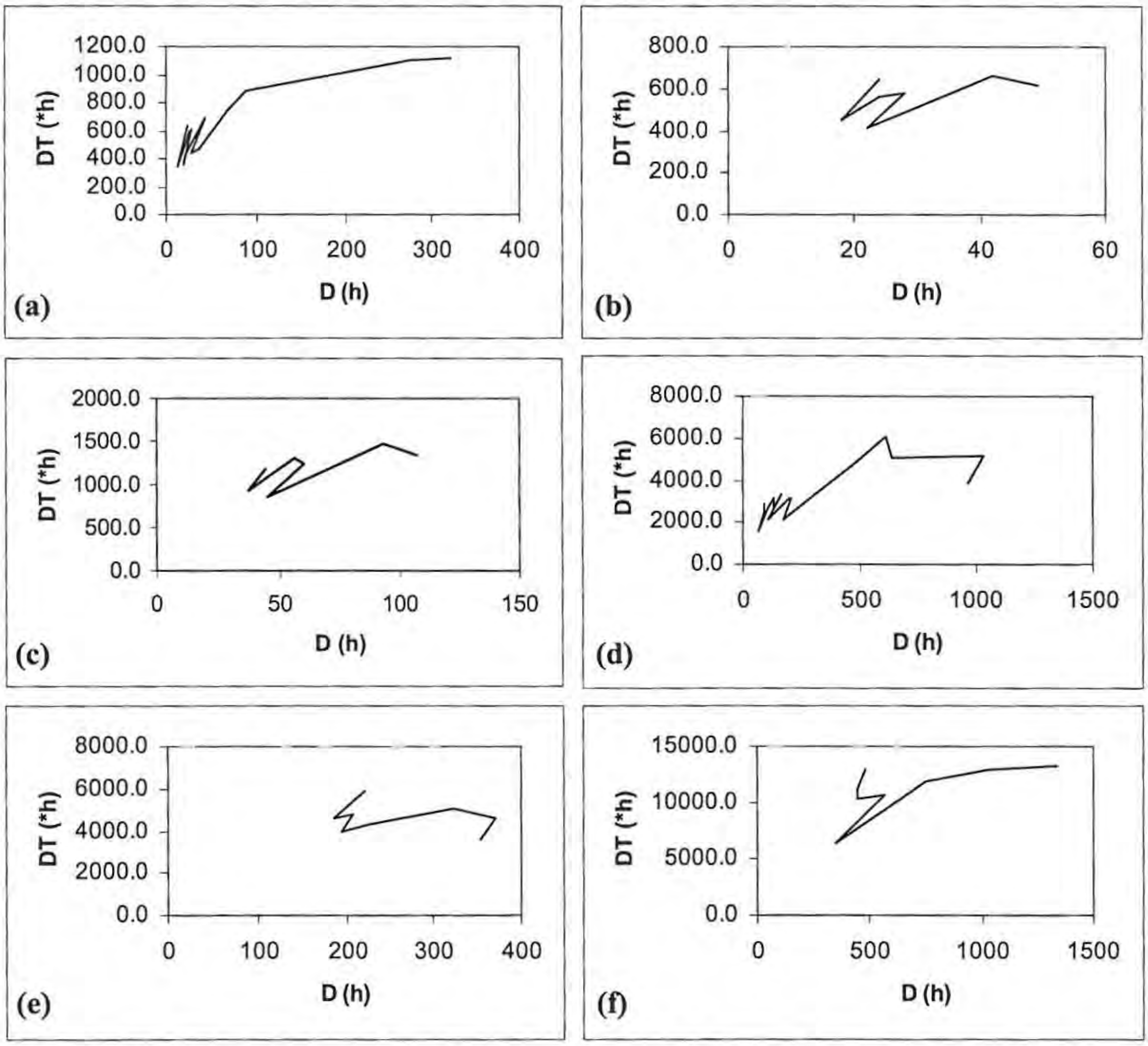


FIGURE 4.2.9 – Development to landmarks (LF method) for *Calliphora vicina*. (a) H, (b) E1, (c) E2, (d) W, (e) P, (f) AE.

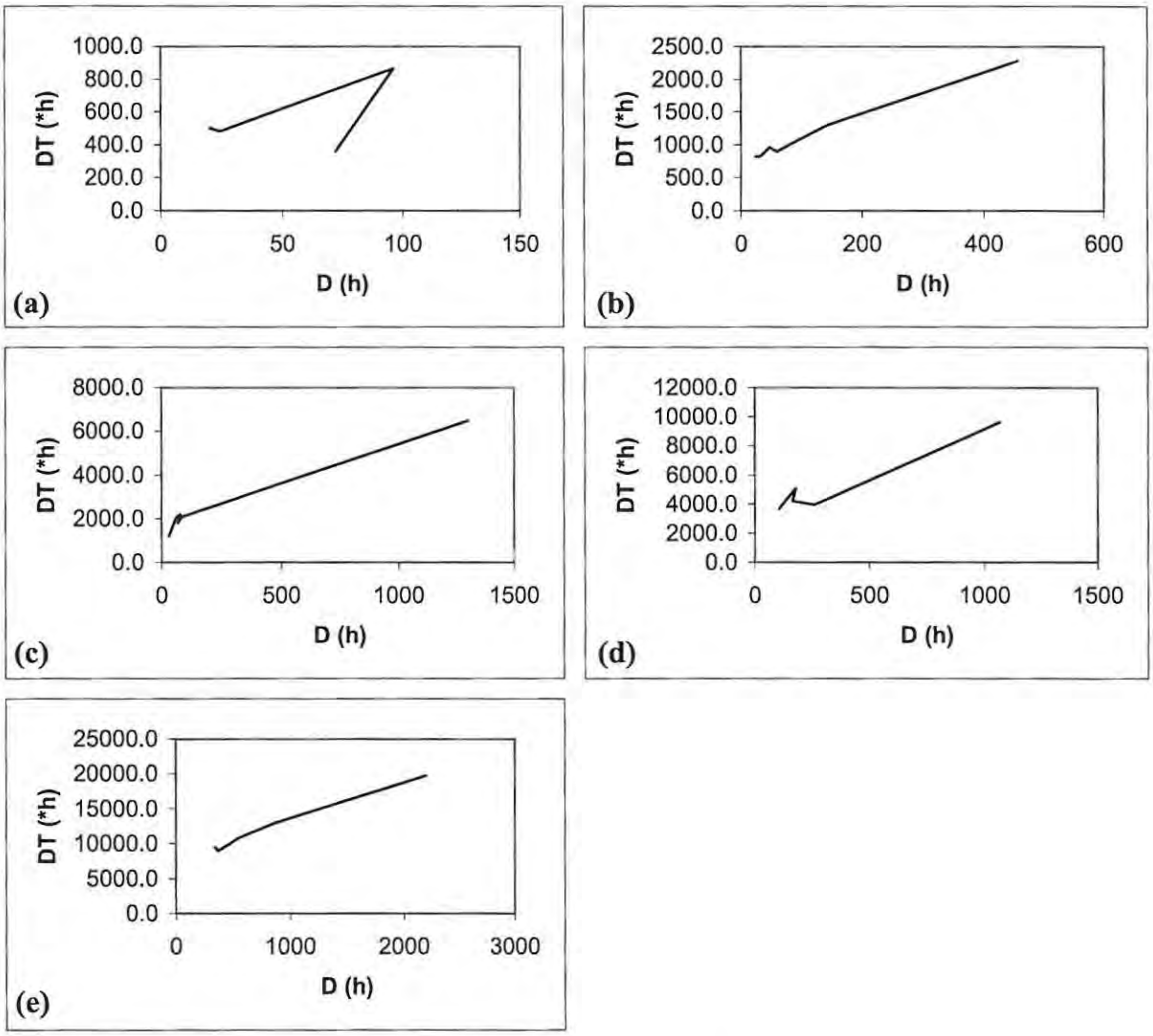


FIGURE 4.2.10 – Development to landmarks (LF method) for *Calliphora augur*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

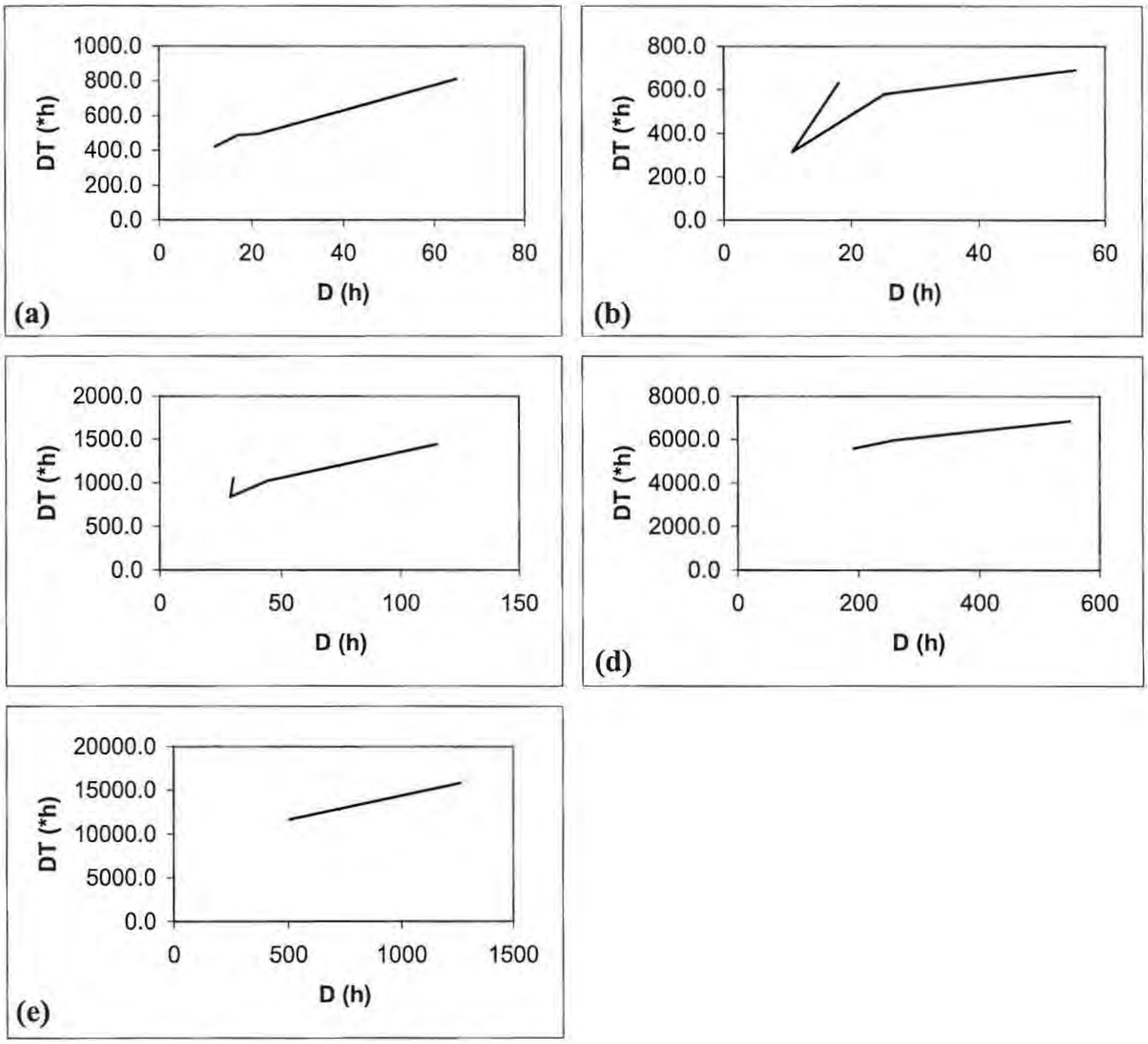


FIGURE 4.2.11 – Development to landmarks (LF method) for *Calliphora vomitoria*. (a) H, (b) E1, (c) E2, (d) P, (e) AE.

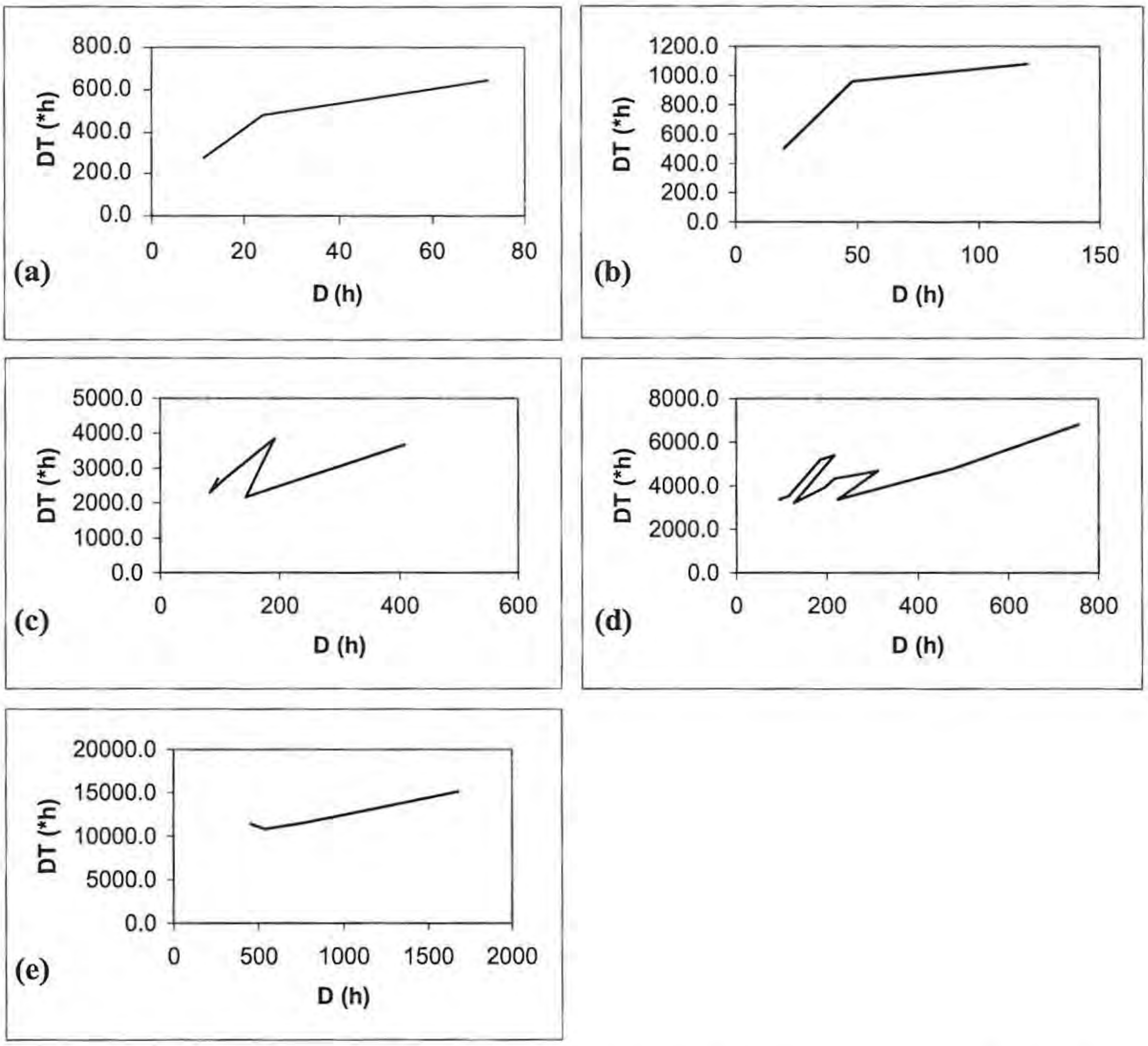


FIGURE 4.2.12 – Development to landmarks (LF method) for *Calliphora stygia*. (a) H, (b) E1, (c) W, (d) P, (e) AE.

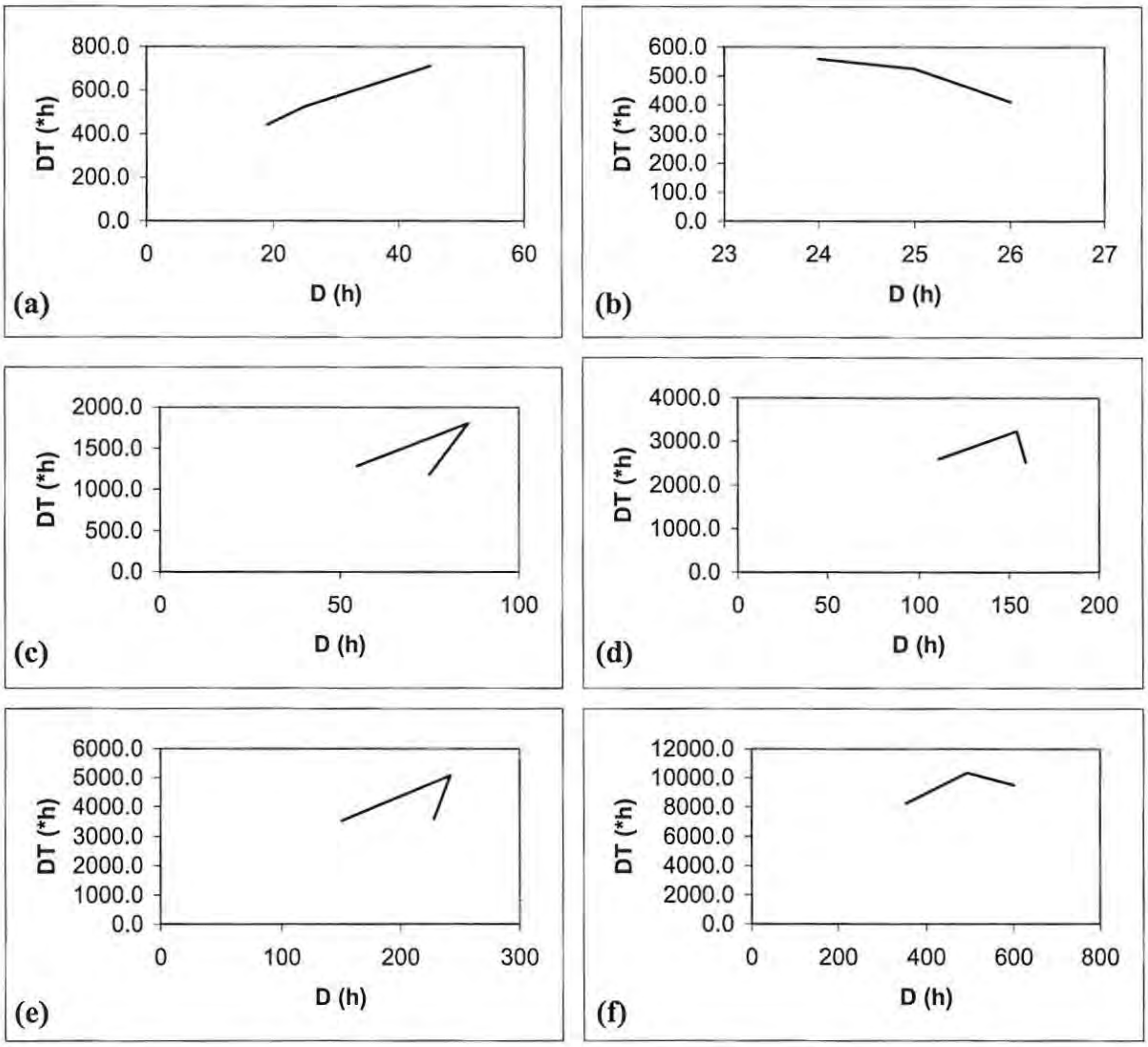


FIGURE 4.2.13 – Development to landmarks (LF method) for *Eucalliphora latifrons*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

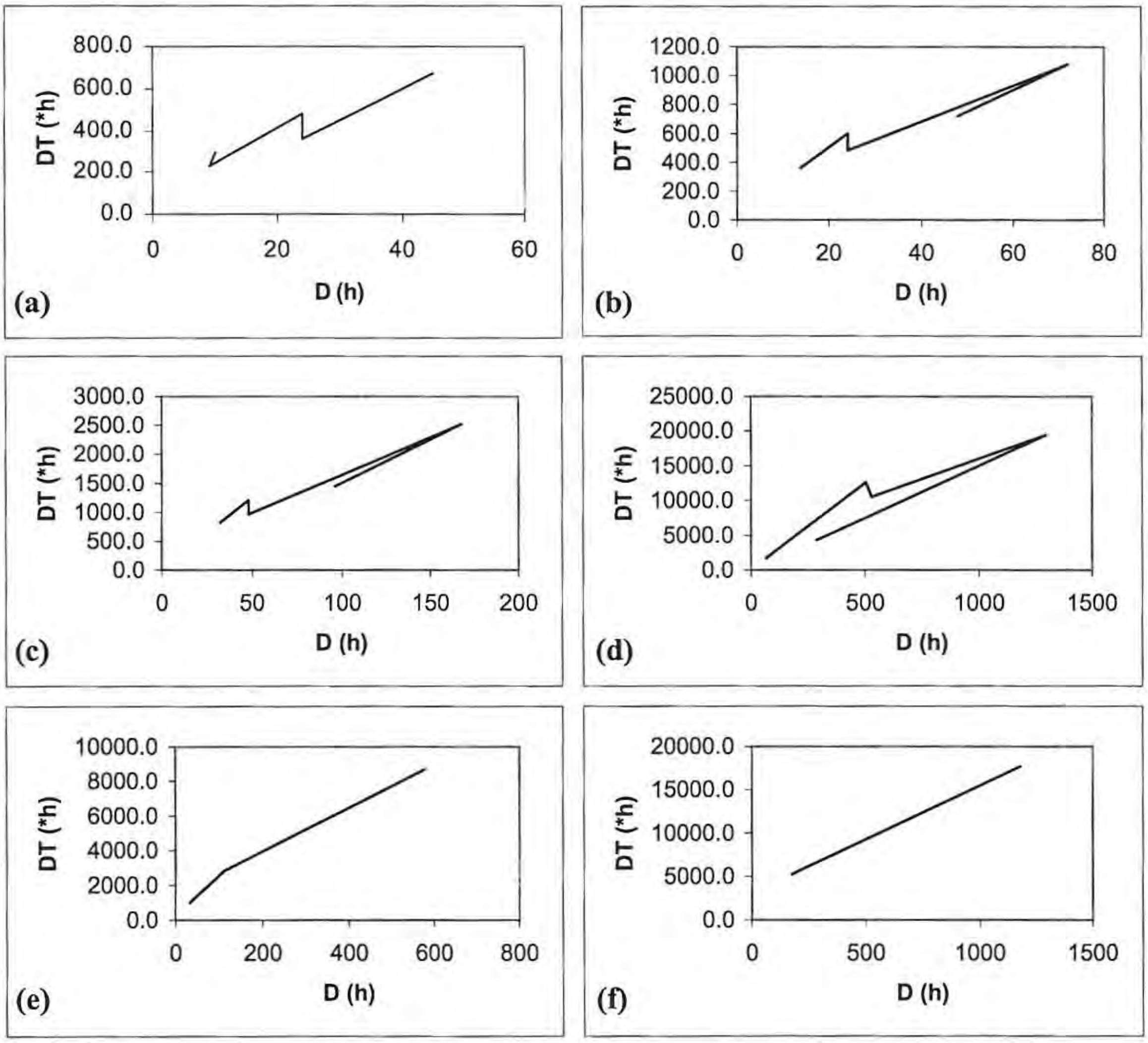


FIGURE 4.2.14 – Development to landmarks (LF method) for *Lucilia cuprina*. (a) H, (b) E1, (c) E2, (d) W, (e) P, (f) AE

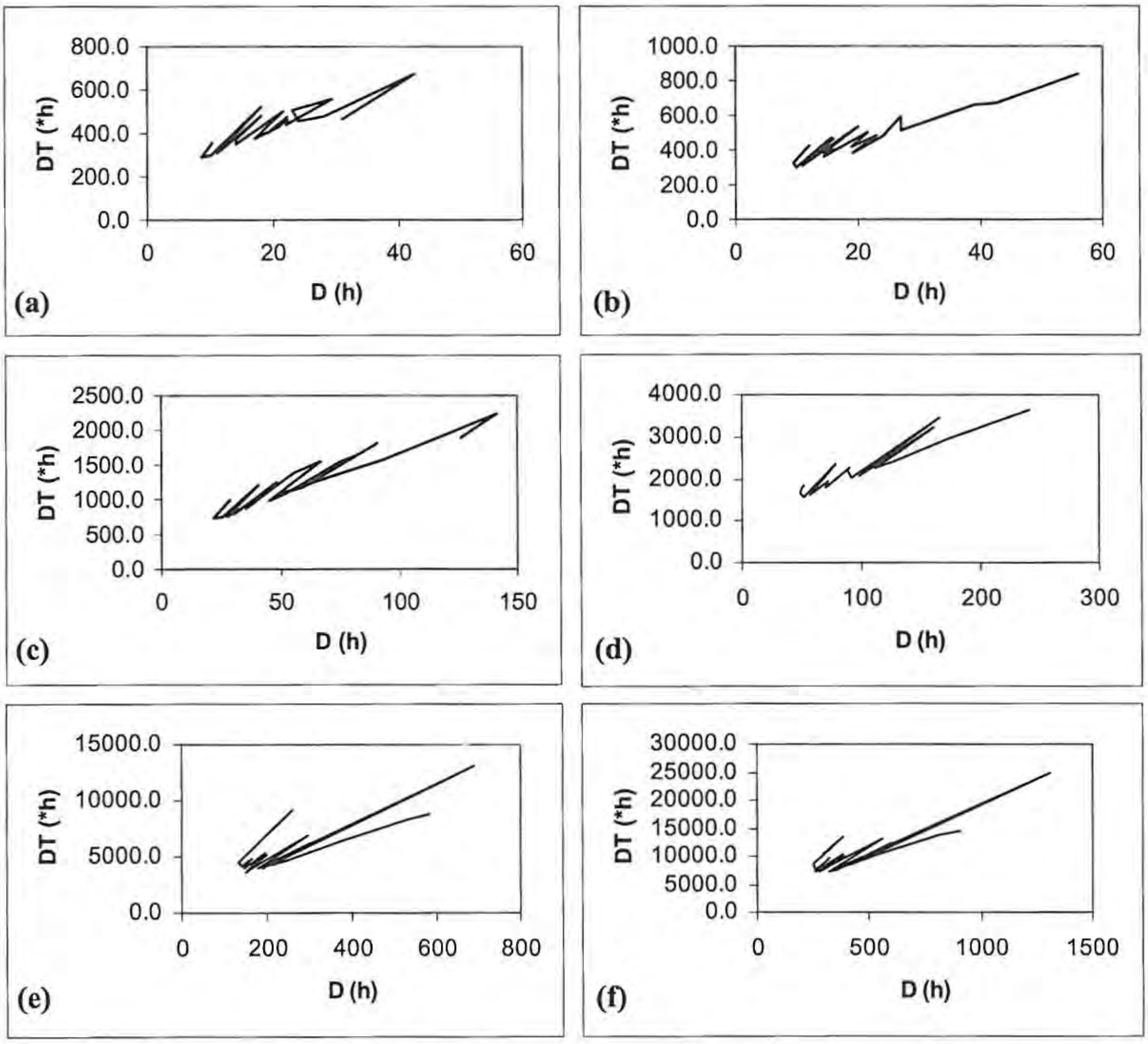


FIGURE 4.2.15 – Development to landmarks (LF method) for *Lucilia sericata*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

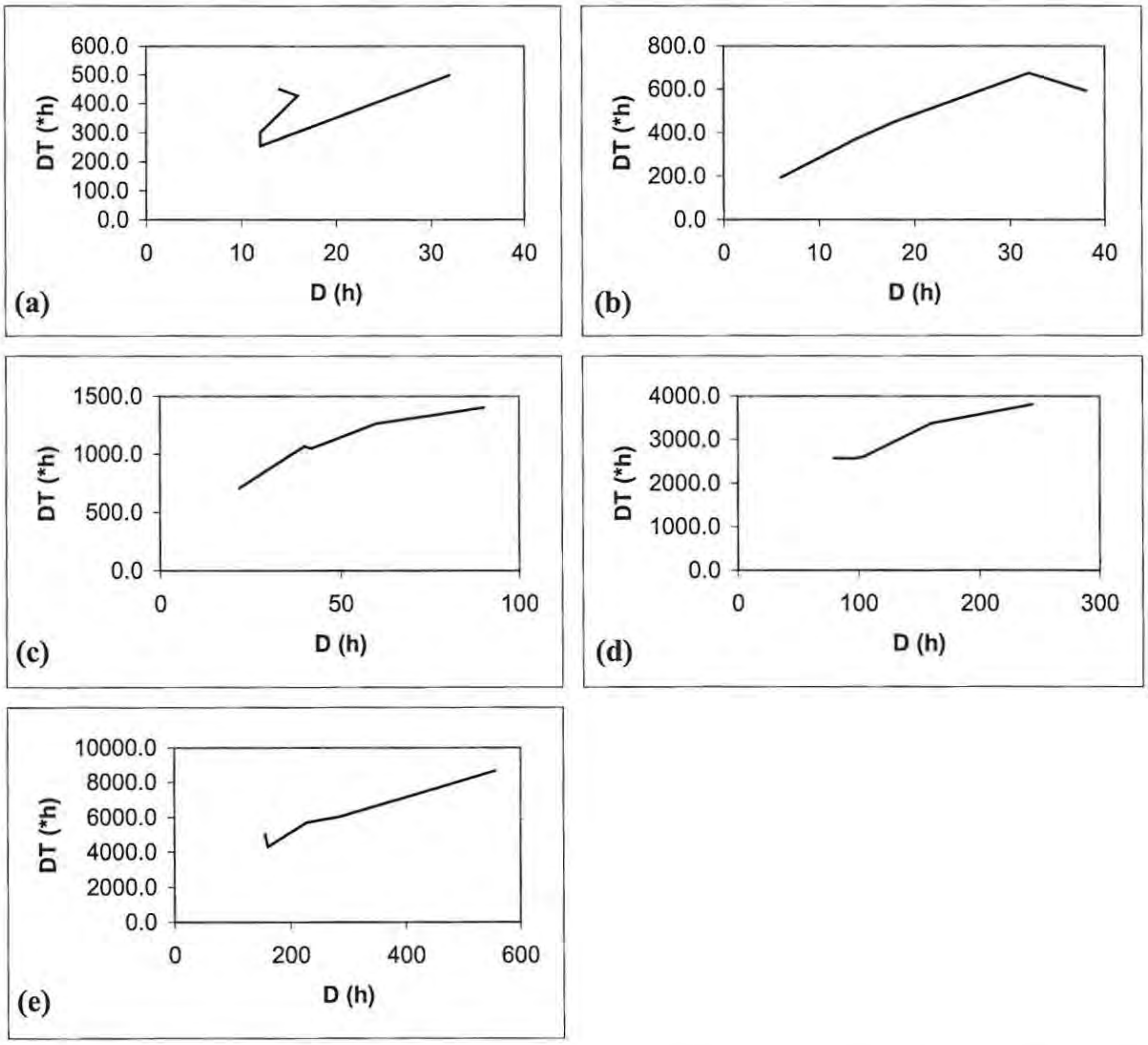


FIGURE 4.2.16 – Development to landmarks (LF method) for *Cochliomyia macellaria*. (a) H; (b) E1; (c) E2; (d) P; (e) AE.

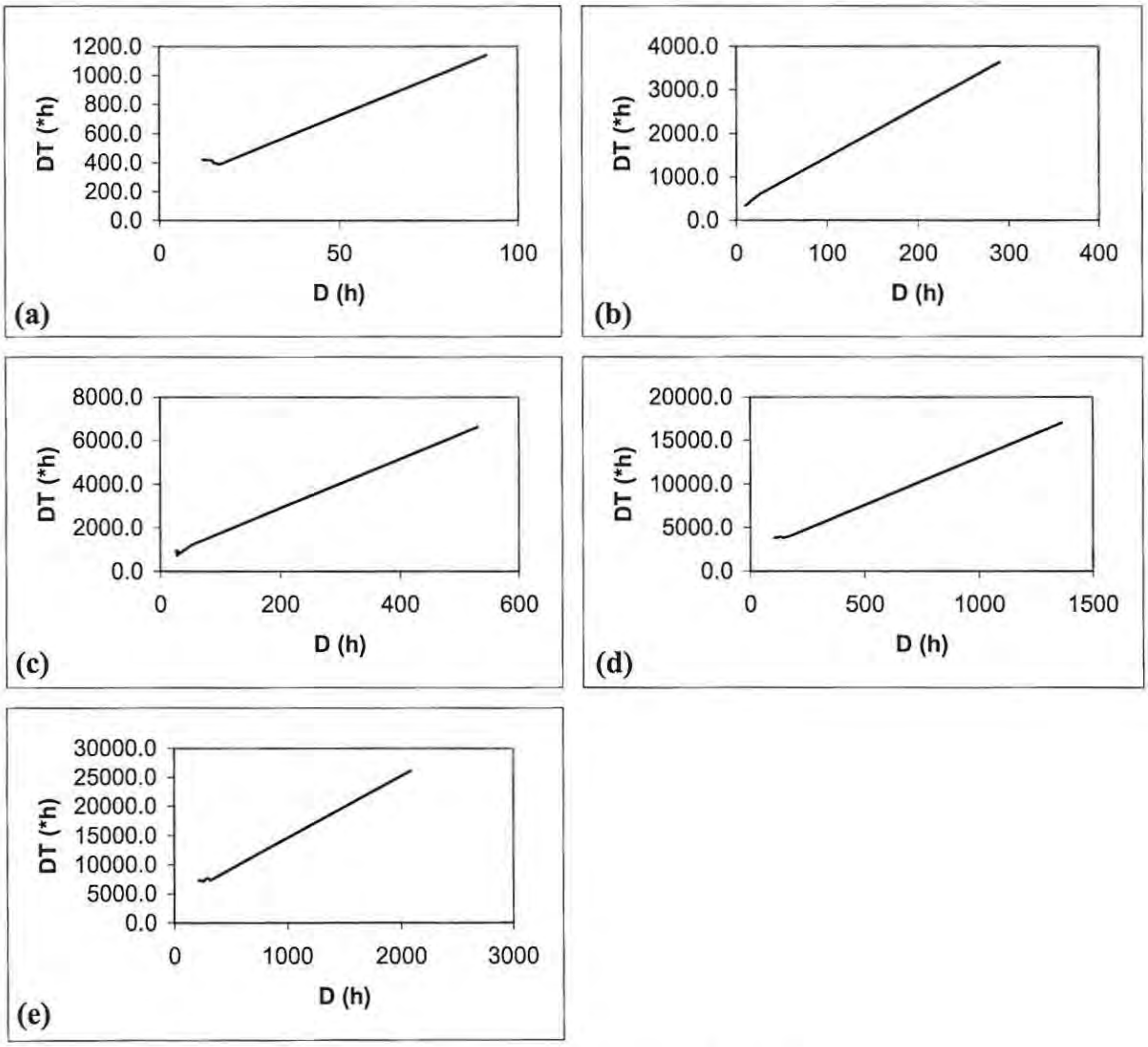


FIGURE 4.2.17 – Development to landmarks (LF method) for *Protophormia terraenovae.* (a) H; (b) E1, (c) E2, (d) P, (e) AE.

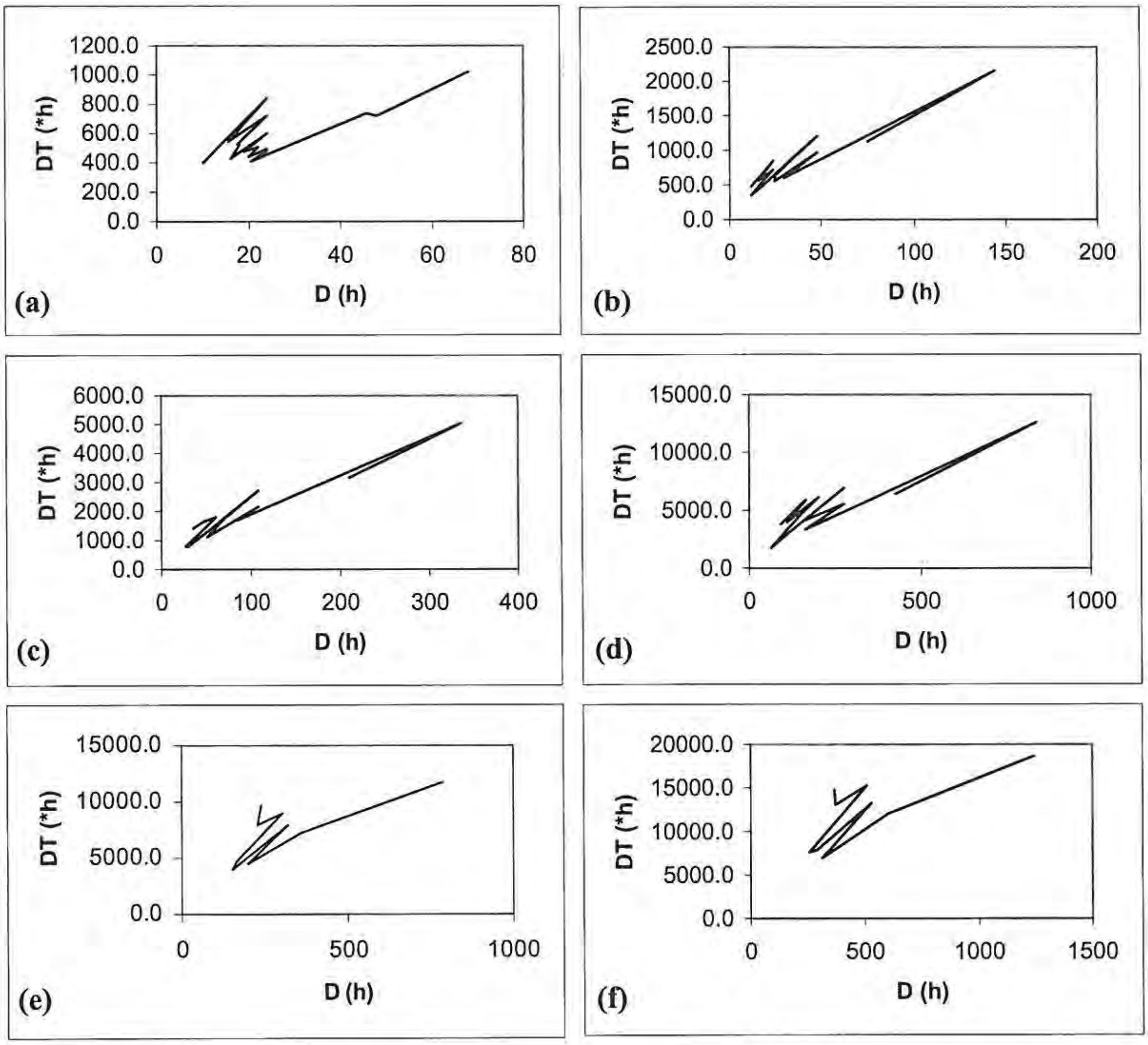


FIGURE 4.2.18 – Development to landmarks (LF method) for *Phormia regina.* (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

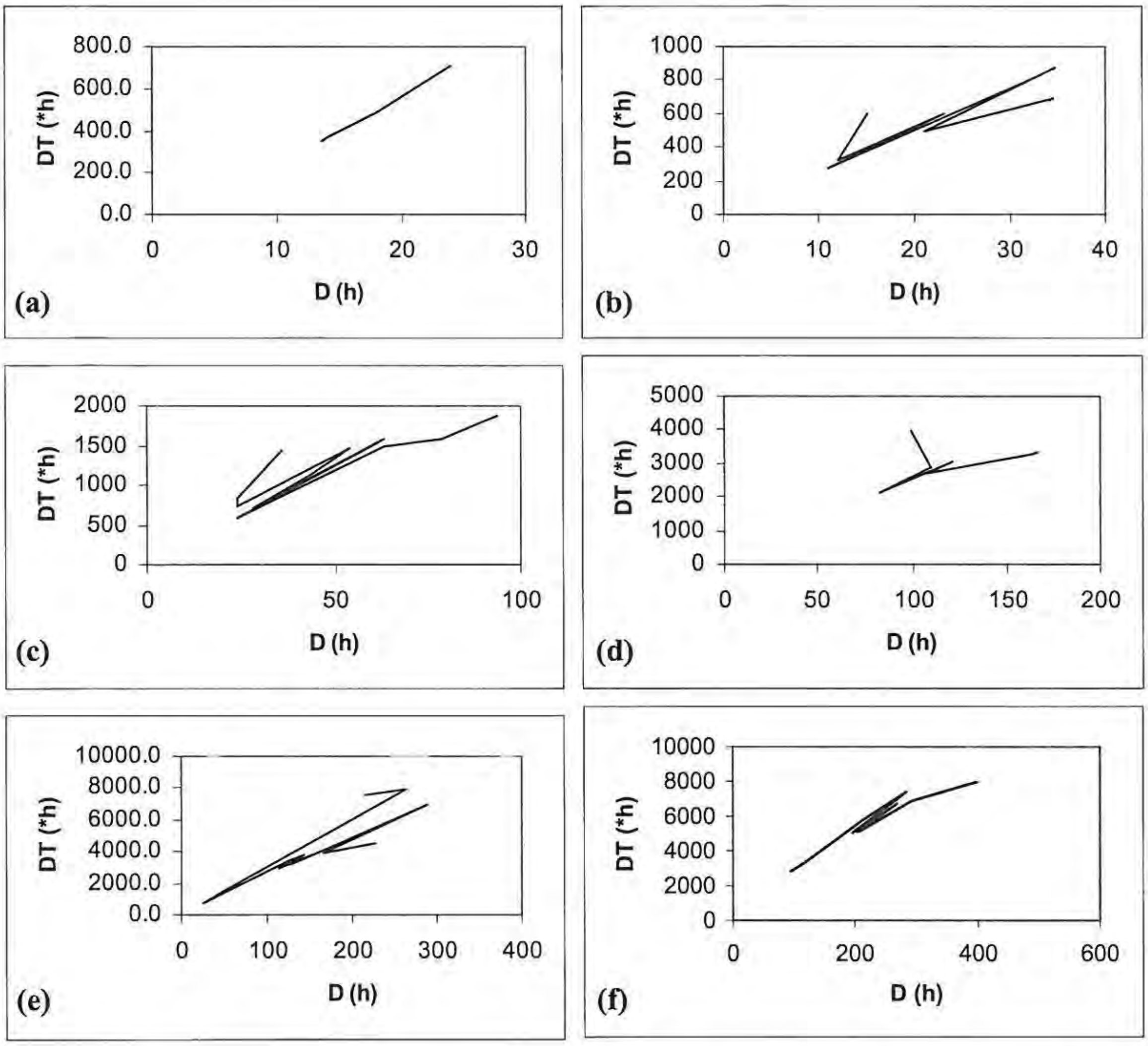


FIGURE 4.2.19 – Development to landmarks (LF method) for *C. megacephala*. (a) H; (b) E1; (c) E2; (d) W; (e) P; (f) AE.

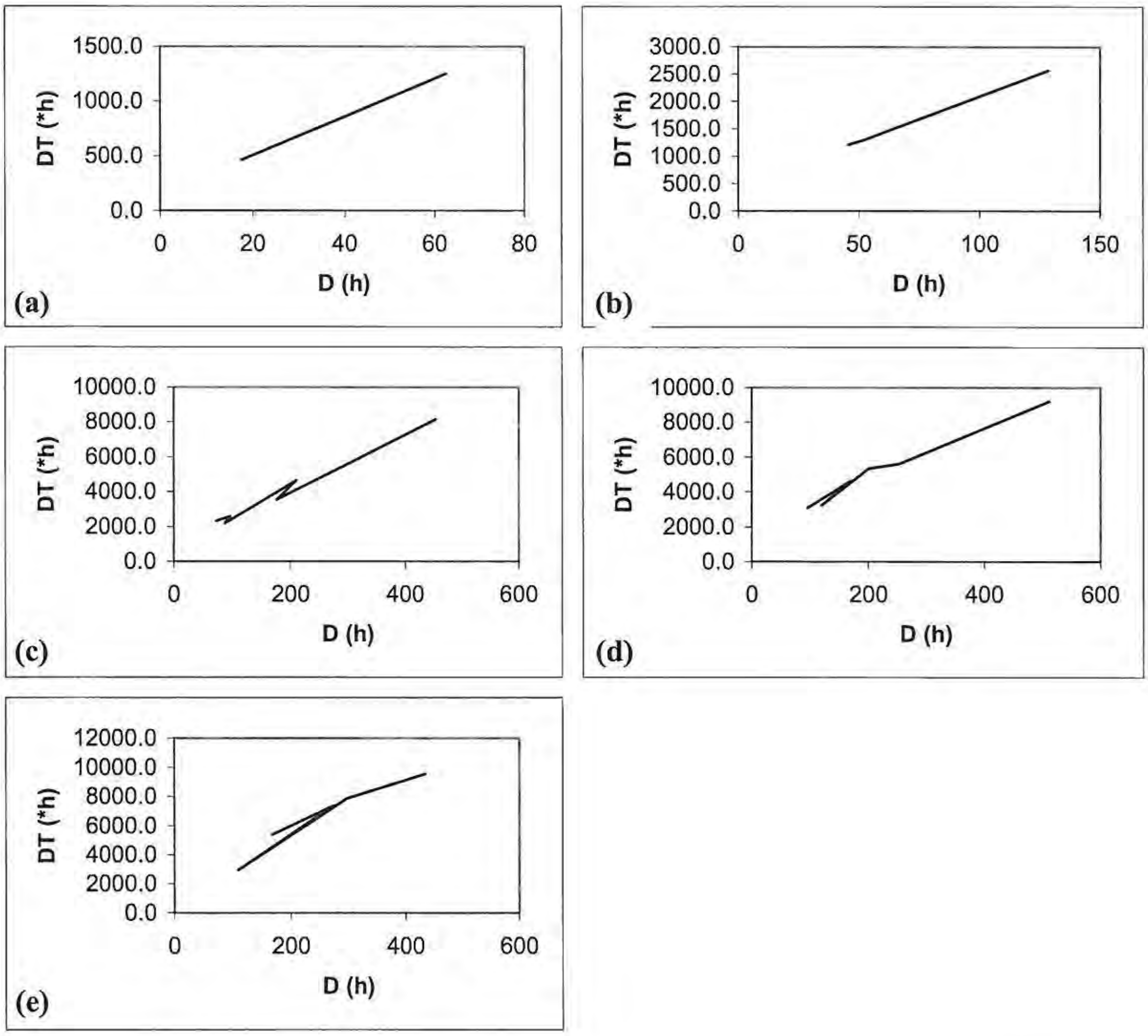


FIGURE 4.2.20 - Development to landmarks (LF method) for *Chrysomya albiceps*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.

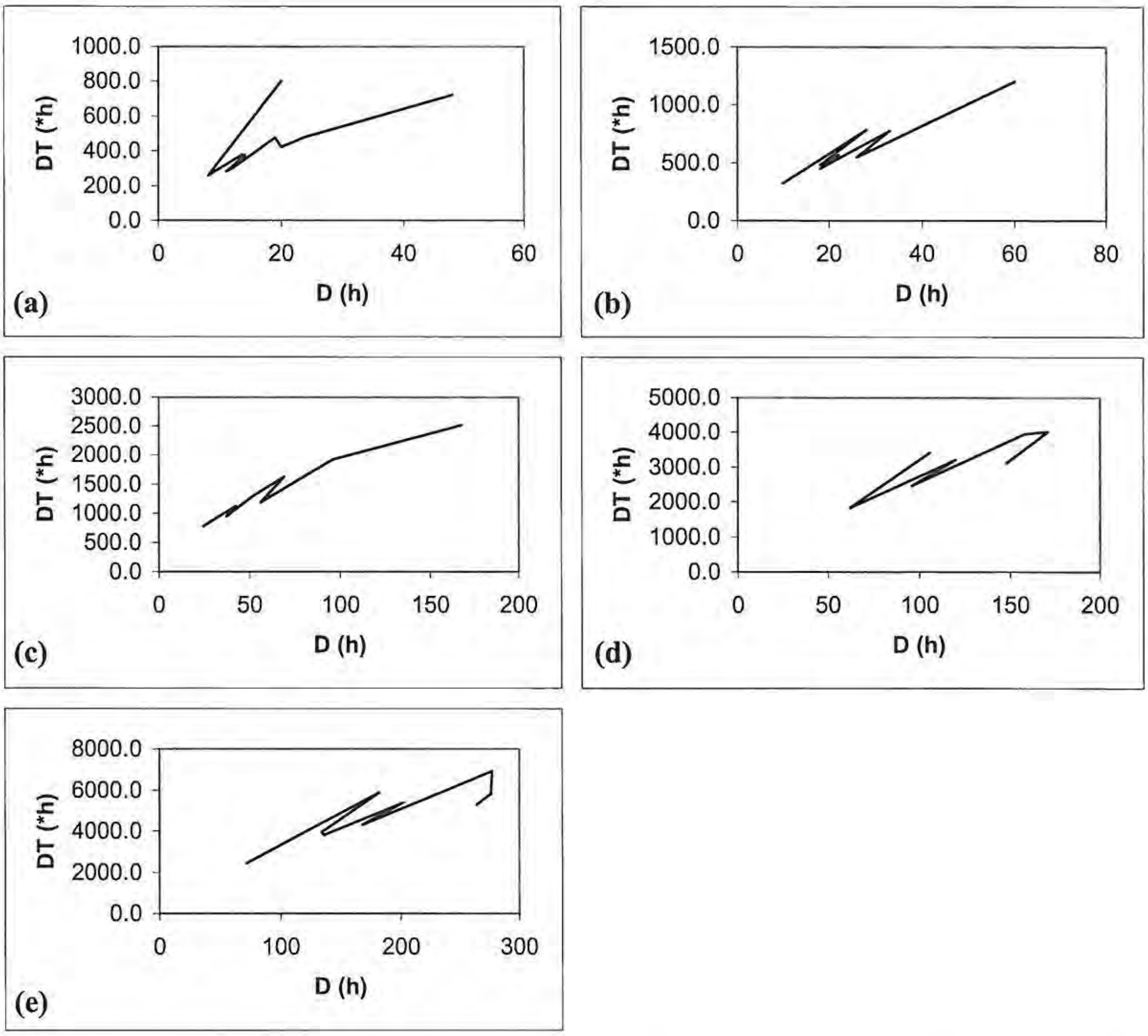


FIGURE 4.2.21 – Development to landmarks (LF method) for *C. rufifacies*. (a) H; (b) E1; (c) E2; (d) P; (e) AE.

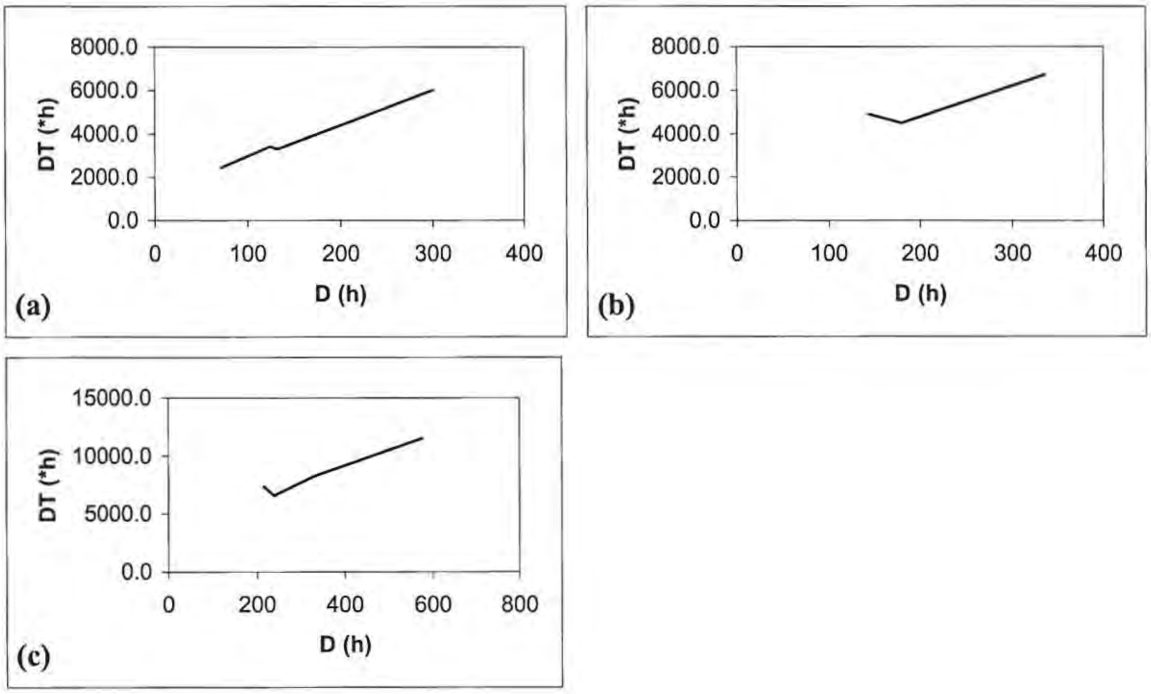


FIGURE 4.2.22 – Development to landmarks (LF method) for *Chrysomya varipes*. (a) W; (b) P; (c) AE.

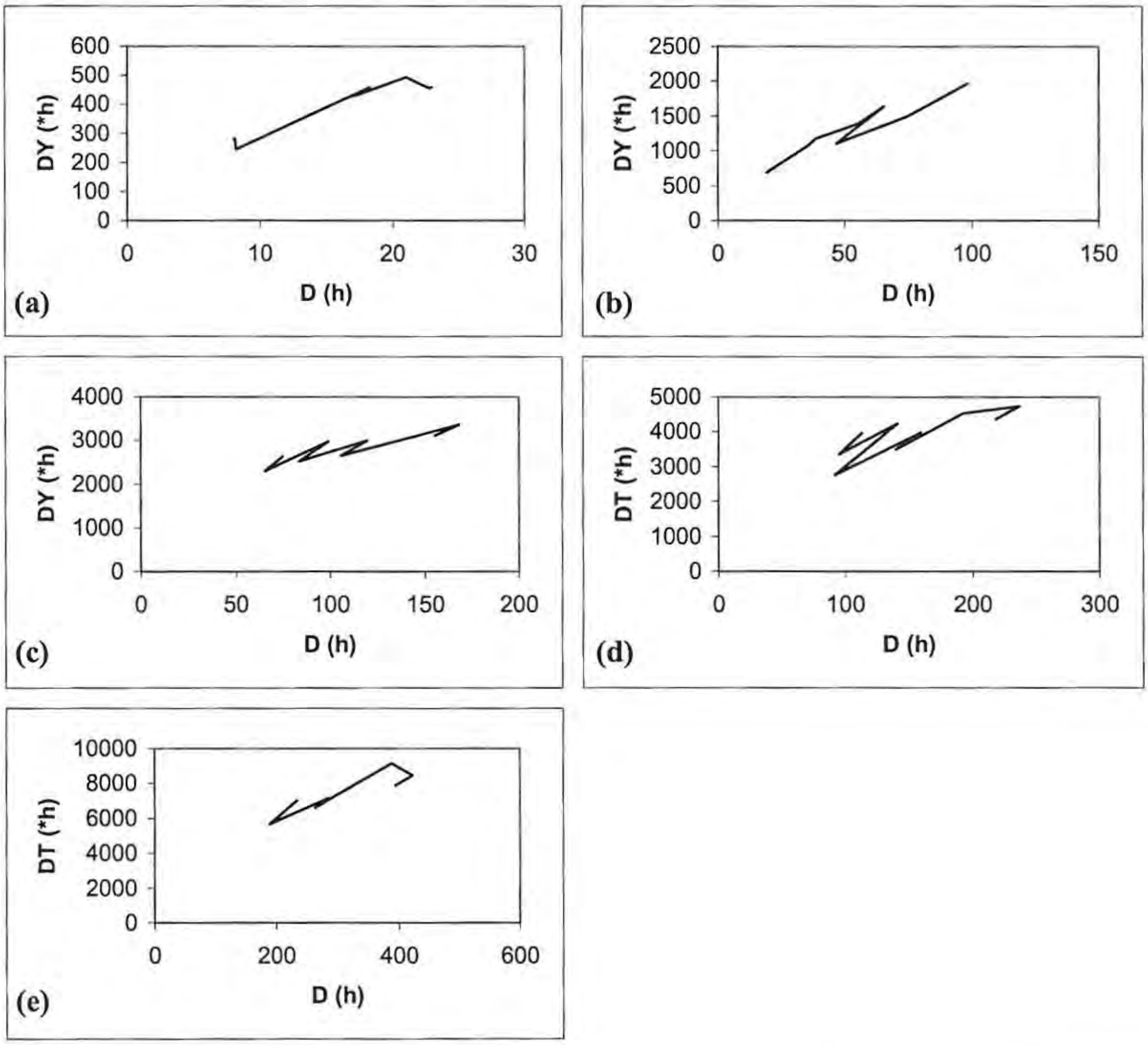


FIGURE 4.2.23 – Development to landmarks (LF method) for *C. chloropyga*. (a) E1; (b) E2; (c) W; (d) P; (e) AE

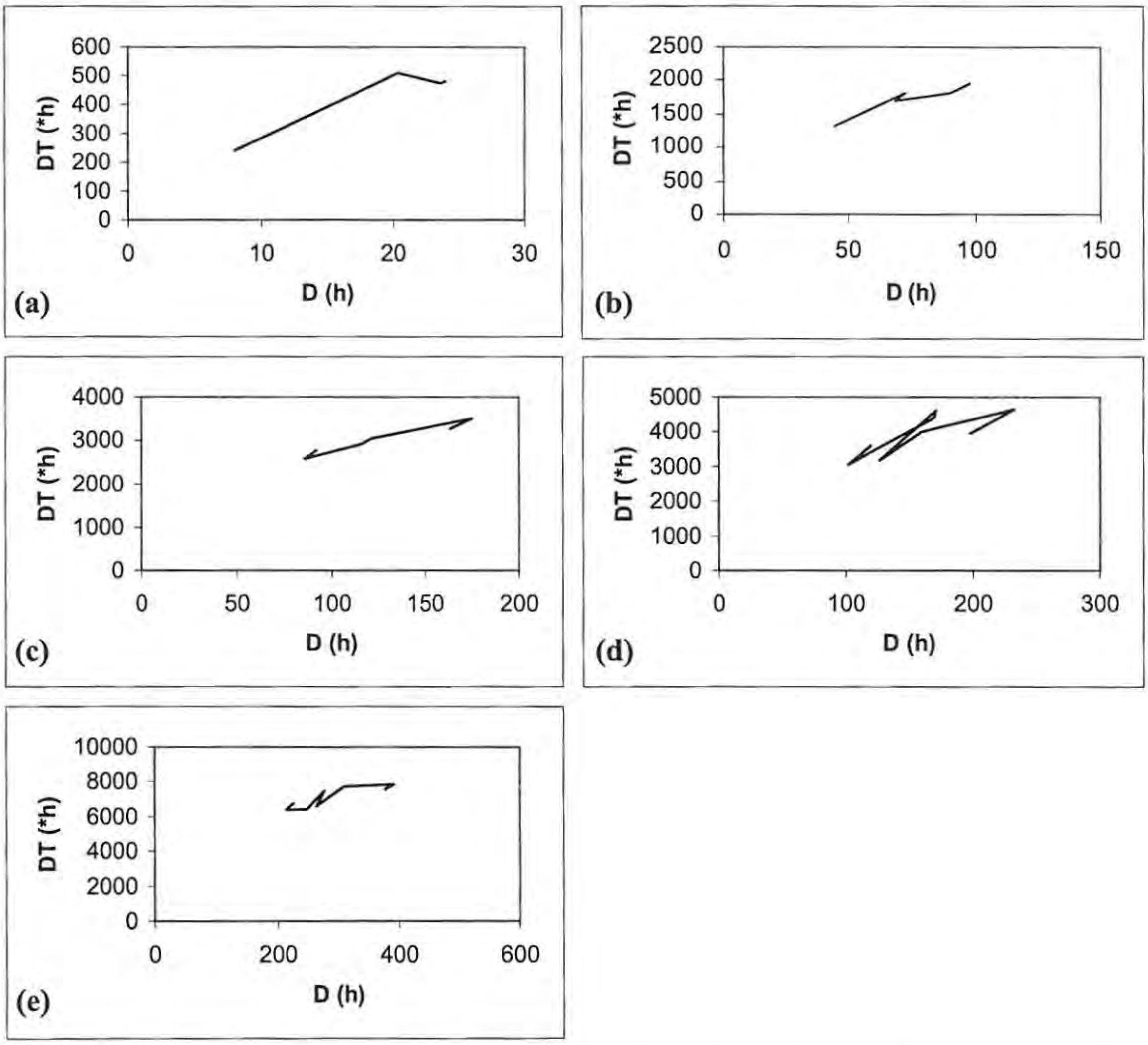
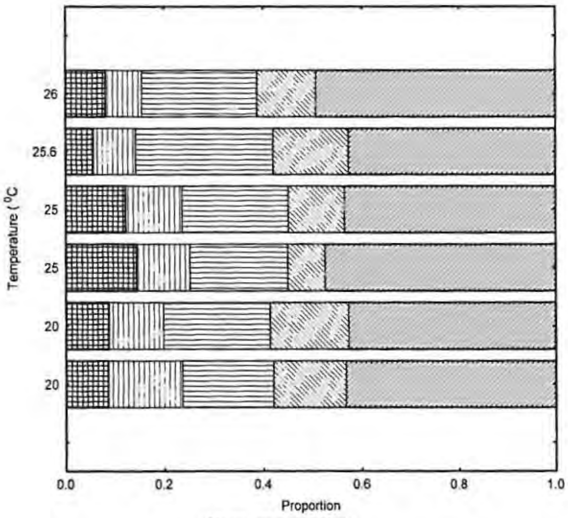
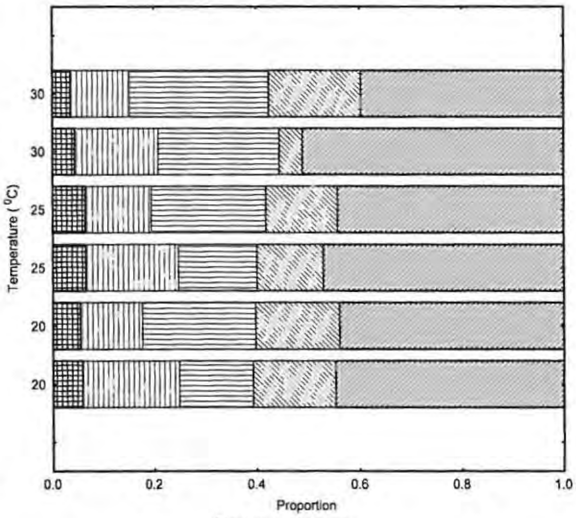


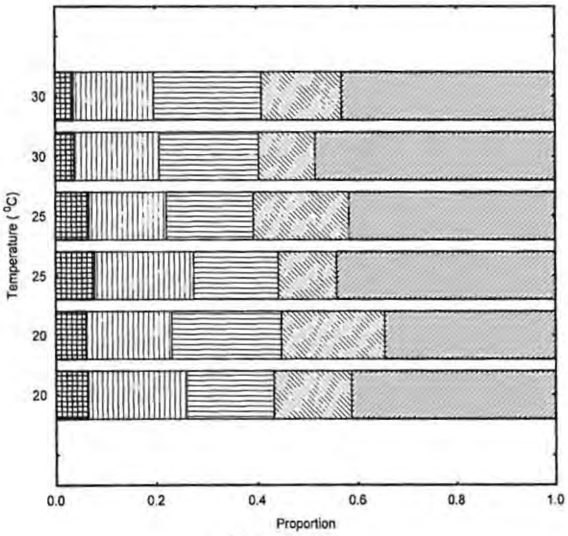
FIGURE 4.2.24 – Development to landmarks (LF method) for *C. putoria*. (a) E1; (b) E2; (c) W; (d) P; (e) AE.



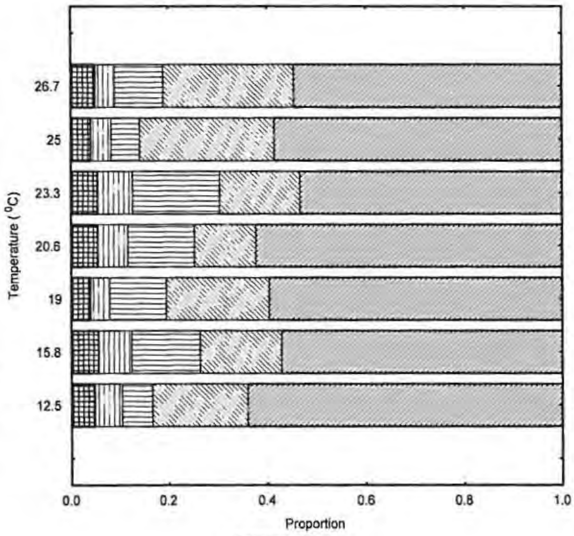
Chrysomya megacephala



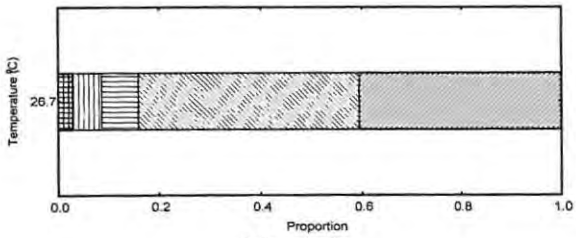
Chrysomya chloropyga



Chrysomya putoria



Calliphora vicina



Calliphora vomiton

FIGURE 4.3.1 – Proportion of total development assigned to each stadium (continued overleaf).

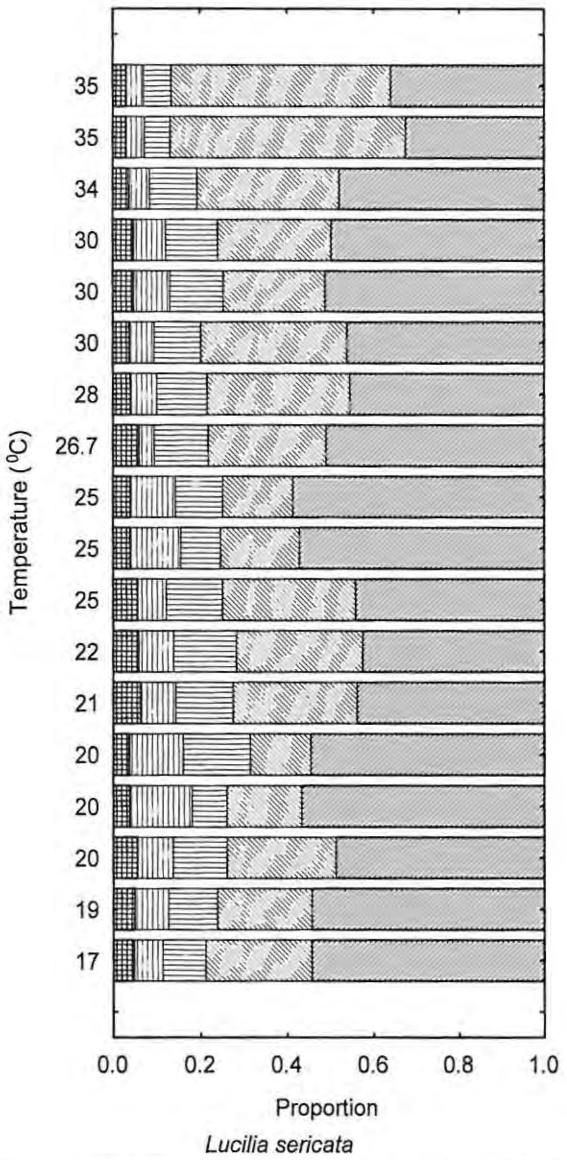


FIGURE 4.3.1 (continued) – Proportion of total development assigned to each stadium. From left: L1, L2, L3f, L3w, P. Identical temperatures from different publications or sources were treated independently to give an indication of variability.

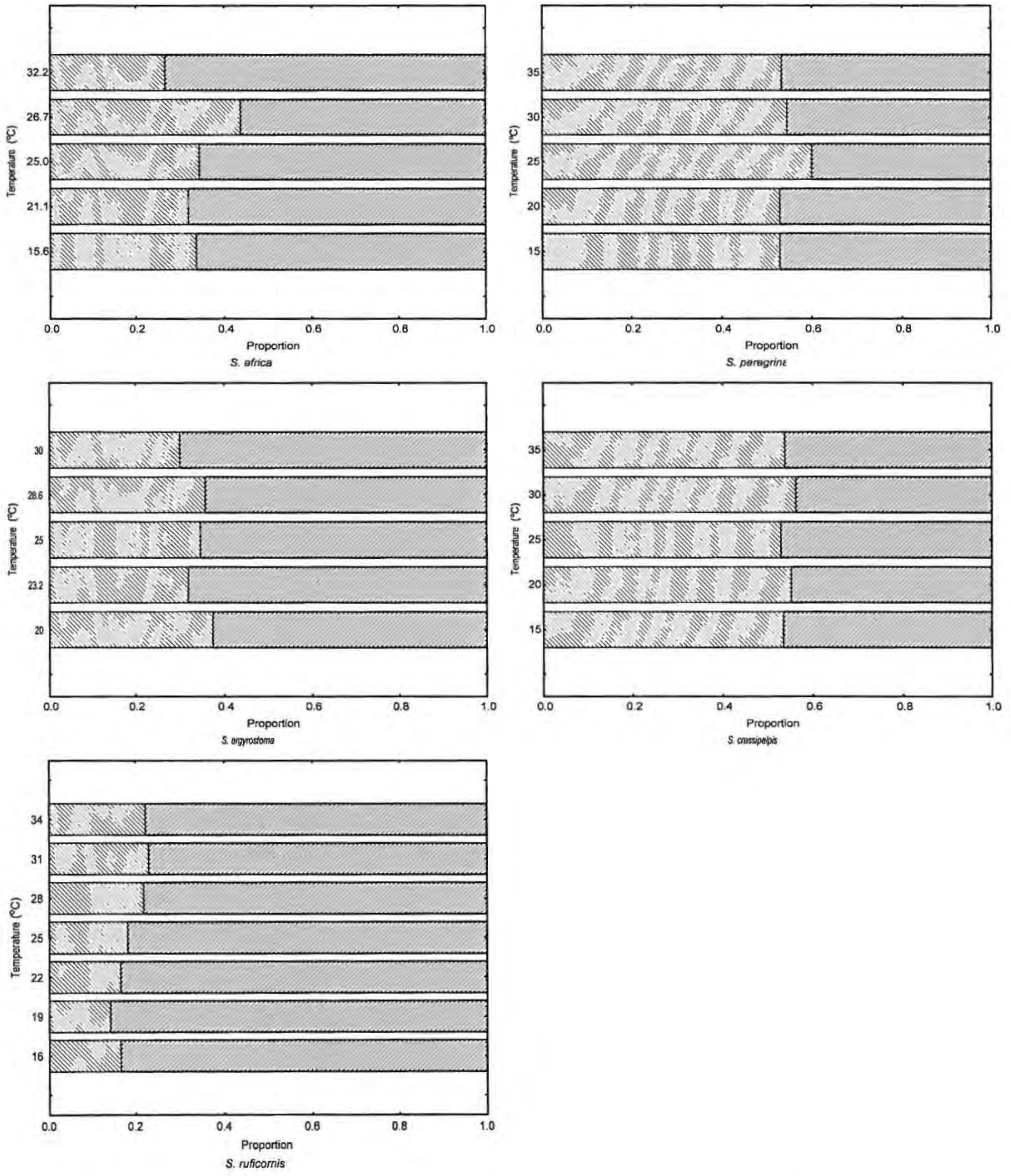


FIGURE 4.3.2A – Proportion of total development assigned to larval (red) and puparial (blue) stages for *Sarcophaga* spp.

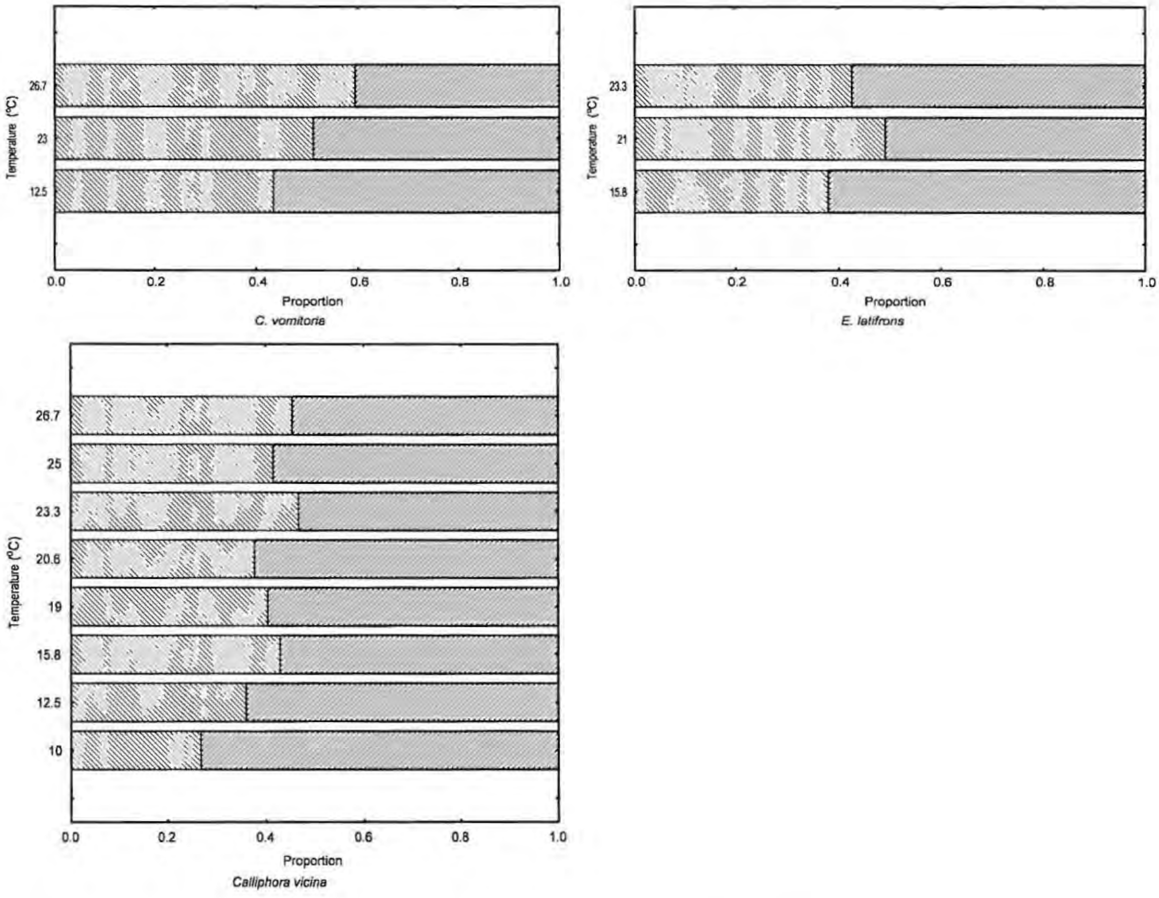


FIGURE 4.3.2B – Proportion of total development assigned to larval (red) and puparial (blue) stages in the Calliphorinae.

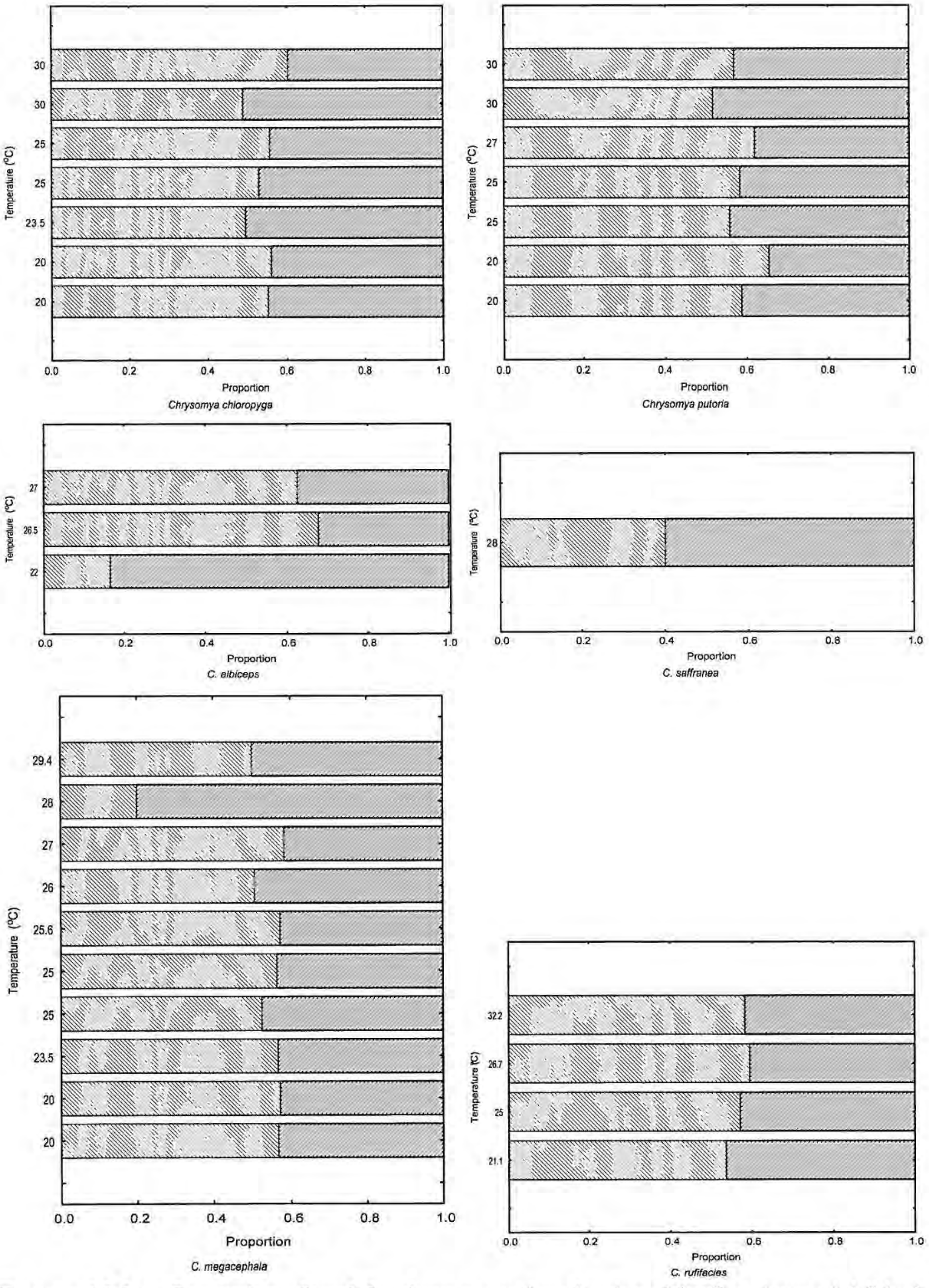


FIGURE 4.3.2C – Proportion of total development assigned to larval (red) and puparial (blue) stages in *Chrysomya.*

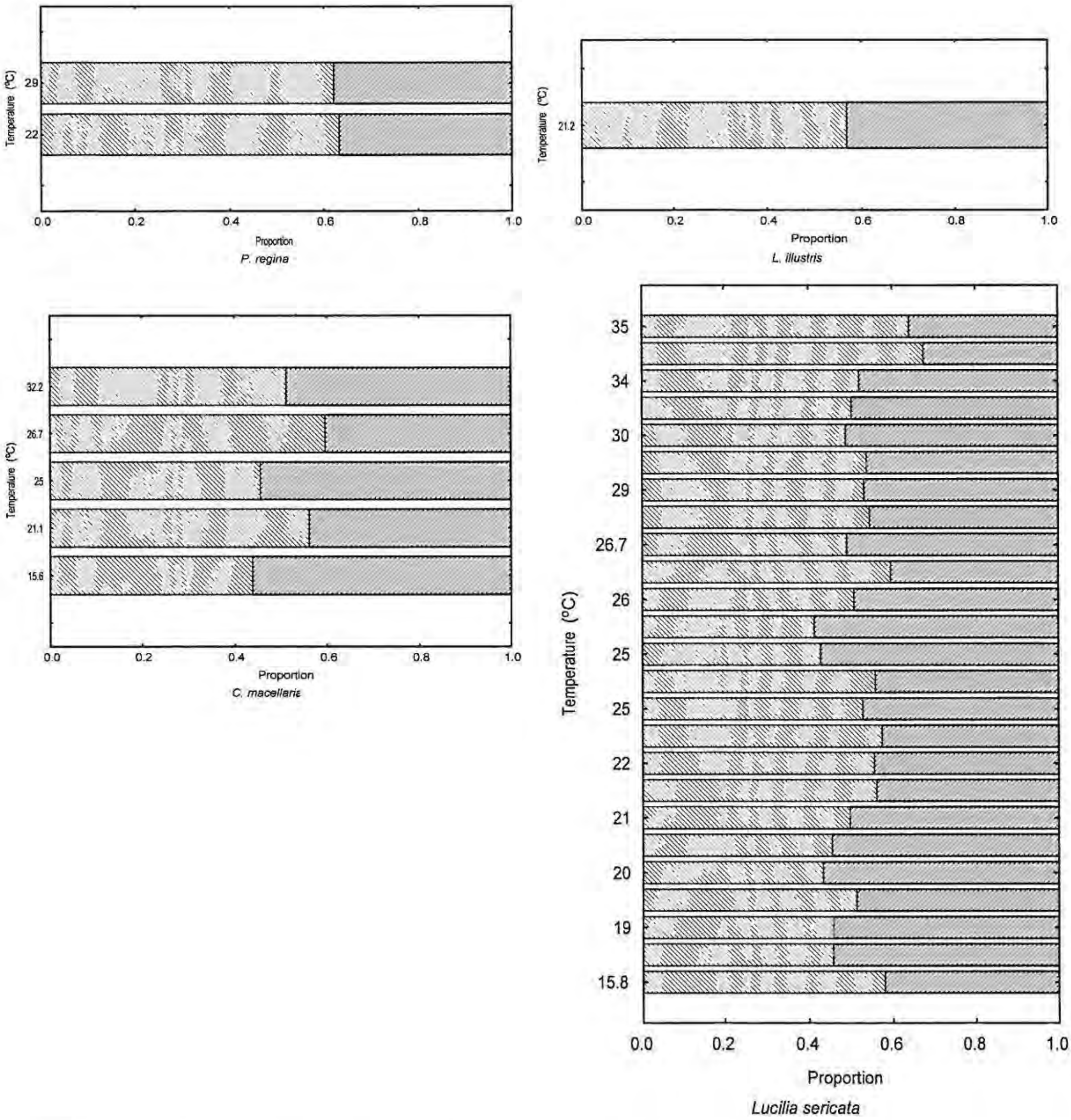


FIGURE 4.3.2D – Proportion of total development assigned to larval (red) and puparial (blue) stages for *Phormia*, *Cochliomyia*, and *Lucilia*.

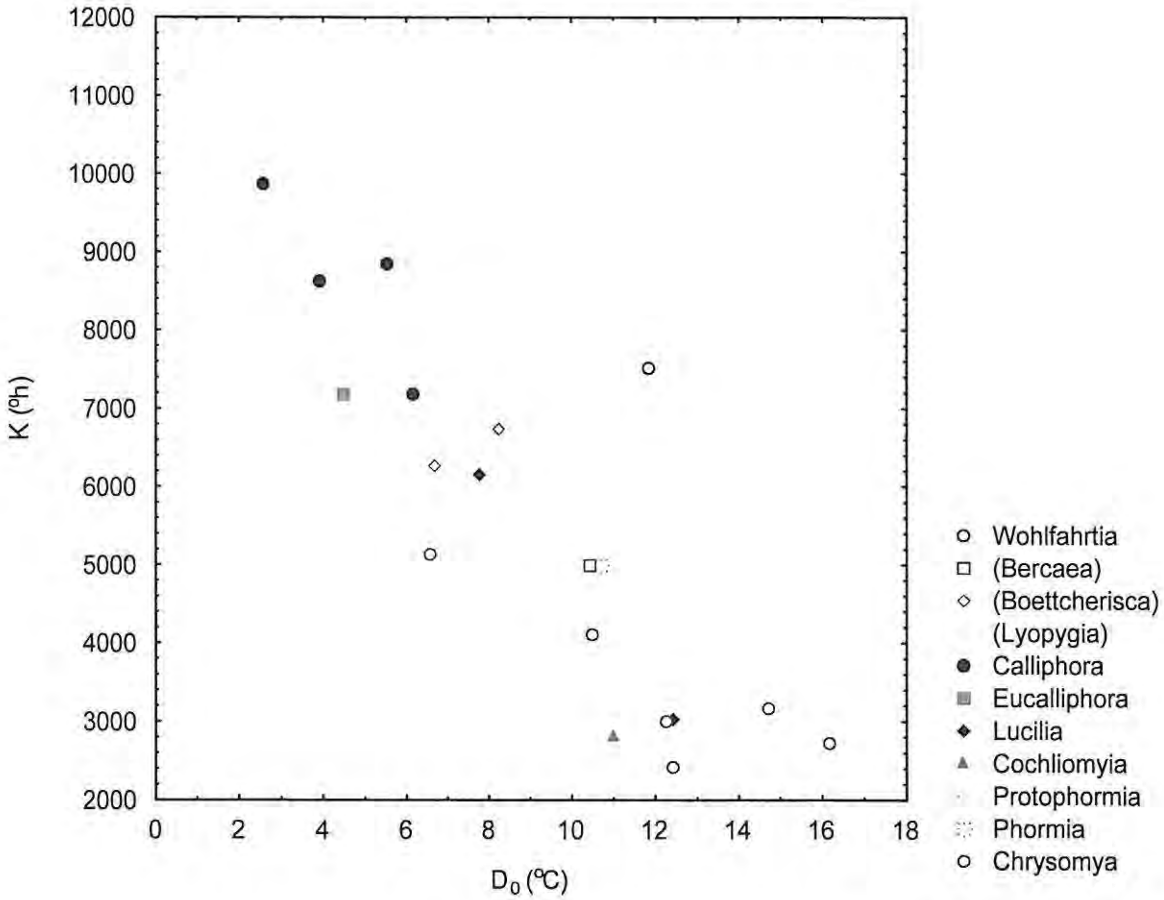


FIGURE 4.4 – Scatterplot of K against D_0, categorized by genus (Calliphoridae) and subgenus (*Sarcophaga*).

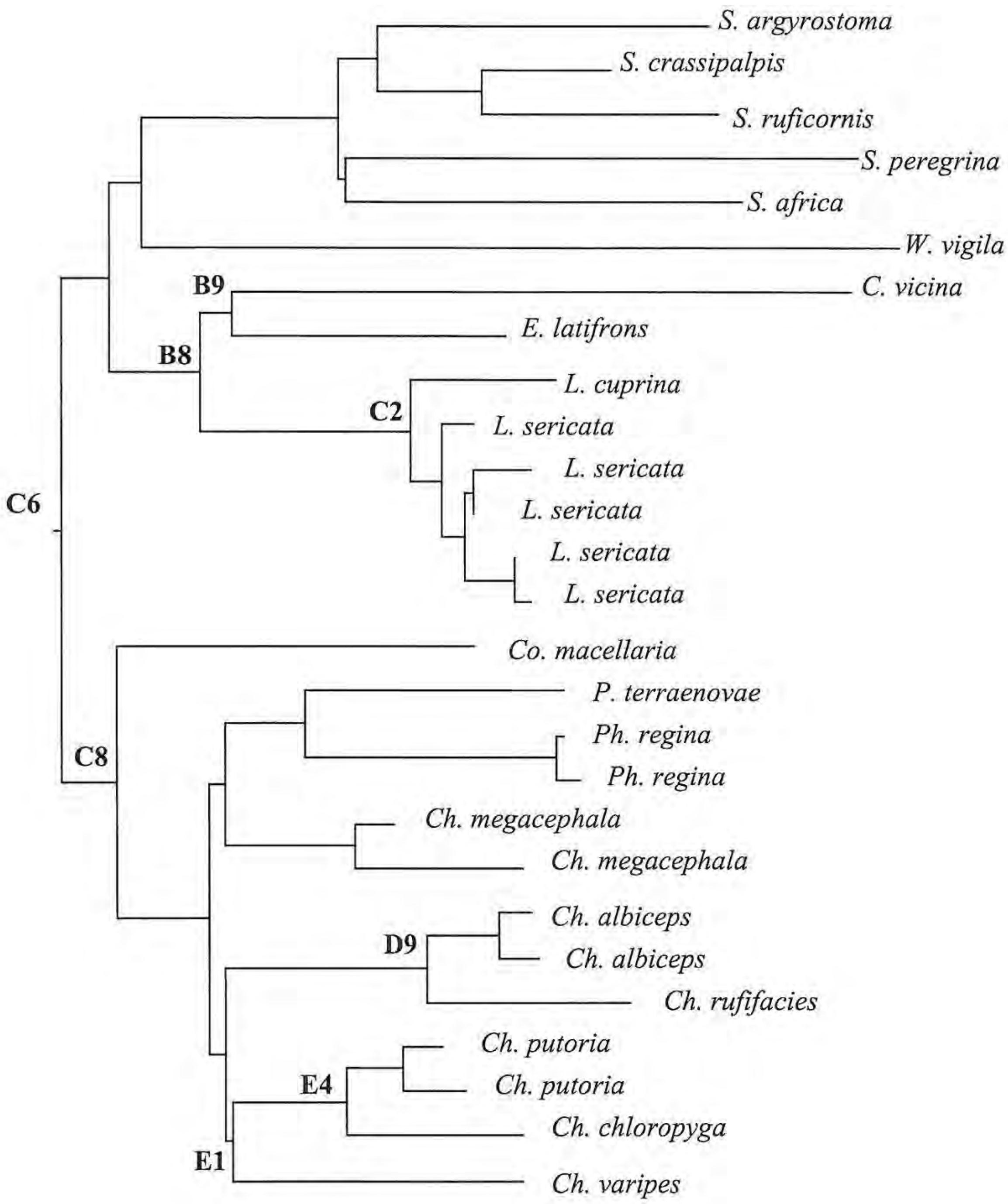


FIGURE 4.5 – Phylogeny of the specie of Sarcophagidae and Calliphoridae for which physiological data were available. Tree based on the neighbour-joining tree of the conservative data set (Fig. 2.2.1). Re-rooting node identifiers (see text) appear above branches. Genus abbreviations: *C.* = *Calliphora*; *Ch.* = *Chrysomya*; *Co.* = *Cochliomyia*; *E.* = *Eucalliphora*; *L.* = *Lucilia*; *Ph.* = *Phormia*; *P* = *Protophormia*; *S.* = *Sarcophaga*.

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Appendix A

Key To The Forensically Important Calliphorid Species In Southern Africa

This key is designed only for identification of necrophagous calliphorids that are known or suspected to be of forensic importance in southern Africa. Only reliable external morphological characters have been included, to allow rapid identification of flies. Characters relating to adult body colour have often been found to be misleading or subjective (Ullyett, 1945; pers. obs.), so undue reliance should not be placed on such characters. Information has been gathered from personal observation and experience of adult flies, communication with A. Bownes (author of the IdentiFly software), and from the literature. Diagnostic characters for species described in each couplet are in **boldface**, characters that may be misleading are marked with an asterisk.

Detailed descriptions of immature and adult stages of a variety of calliphorids and sarcophagids can be found in Smith (1933), Knipling (1936), Hafez (1940), Ullyett (1945), Zumpt (1956, 1965), Kitching (1976), Prins (1982), Greenberg & Szyska (1984), Smith (1986), Liu & Greenberg (1989), Aspoas (1991), Holloway (1991), Meskin (1991), Rognes (1991, 1997b), and Greenberg & Singh (1995).

Key to adult blowflies of South Africa

1	a)	**Prothoracic spiracle white or yellow.**	**... 2**
	b)	Prothoracic brown or black.	**... 6**
2	a)	Costa of wing darkly infuscated or blackened for most of length.	**... 3**
	b)	Costa of wing not darkly infuscated or blackened for most of length.	**...4**
3	a)	**Only the costa of wing darkly infuscated**, buccae bright yellow or orange. Body metallic blue, adult > 1.2cm in length. **Female: frons and vertex bright yellow or orange;** **Male: larger upper eye facets sharply demarcated from smaller lower facets.**	***CHRYSOMYA MARGINALIS***

	b)	**Part of the subcosta also infuscated**, buccae yellow or orange. Body metallic blue, adult >1 cm in length. **Female: frons and vertex reddish-brown**; Male: no sharp demarcation between upper and lower eye facets.	***CHRYSOMYA INCLINATA***
4	a)	**Buccae with yellow/orange pollinosity**. Body colour uniform bright green or coppery green.	***CHRYSOMYA ALBICEPS***
5	a)	**Black "⌋ ⌊" markings on pronotum**, pronotum lighter blue (almost turquoise) than remainder of thoracic nota, **4th abdominal sclerite bright copper**. Body colour greenish-blue, buccae with white pollinosity.	***CHRYSOMYA CHLOROPYGA***
	b)	**Faint "- -" markings on pronotum,** pronotum colour same colour as remainder of thoracic nota, 4th abdominal sclerite same colour as remainder of abdomen. Body colour variable (coppery-green, bright green, blue or purple), buccae with white pollinosity.	***CHRYSOMYA PUTORIA***
6	a)	Body colour black, with or without a metallic blue sheen.	**... 7**
	b)	Body colour metallic coppery-green, green or blue. No checkerboard pattern on abdomen (*c.f. Sarcophaga* spp.).	**... 8**
7	a)	**Buccae dark** (black).	***CALLIPHORA CROCEIPALPIS***
	b)	**Buccae yellow or orange**.	***CALLIPHORA VICINA***
8	a)	**Buccae orange/yellow**. Body colour blue or greenish-blue, heavy bodied adult of > 1.2 cm length. **Prothoracic spiracle brown or black.** Male: eyes large and red.	***CHRYSOMYA MEGACEPHALA*** **(cf. *C.*** ***BEZZIANA*** – an obligate parasite)
	b)	**Buccae dark or with slight whitish pollinosity. Body colour bright green or coppery, adult length ± 1cm. Prothoracic spiracle brown.** **Male: eyes red but not conspicuously large.**	**... 9**
9	a)	**Long, dense hairs on supraspiracular convexity**. Body colour metallic green or copper. Uncommon in carcasses, usually in low densities.	***HEMIPYRELLIA FERNANDICA***
	b)	Supraspiracular convexity with short hairs only. Body colour bright metallic green or copper.	**... 10**

10	a)	**Paravertical setulae usually 1+1; 15 – 25 setulae on quadrant between anterior margin and discal setae of scutellum**; body colour variable, *fore-femora bright metallic green*. **Female: pre-, outer- and inner-vertical setae form a right-angle.**	***LUCILIA CUPRINA***
	b)	**Paravertical setulae usually 2+2; 35 – 55 setulae on quadrant between anterior margin and discal setae of scutellum**; *body colour variable, fore-femora brown or black*. **Female: pre-, outer- and inner-vertical setae form an obtuse angle.**	***LUCILIA SERICATA***

Appendix B

Aligned Sequences For Species Included In The Phylogenetic Analysis (Chapter 2).

Missing data are denoted as "-", gaps are coded as "?"

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                                                                          60
M. domestica          ATTTAATCGC AACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTT
P. silenus            ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum           ---------- ---------- ---------- ---------- ---------- ----------
H. bovis              ---------- ---------- ---------- ---------- ---------- ----------
O. ovis               ---------- ---------- ---------- ---------- ---------- ----------
W. vigila             ATTTAATCGC GACAGTGGTT ATTCTCTACT AATCATAAAG ATATTGGTAC TTTATATTTT
G. intestinalis       ---------- ---------- ---------- ---------- ---------- ----------
B. devia              ATTTAATCGC AACAGTGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
R. lherminieri        ATTTAATCGC GACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGGAC TTTATATTTT
B. plinthopyga        ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATACTTC
P. chrysostoma        ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC ATTATATTTC
S. peregrina          ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATACTTT
S. crassipalpis       ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
S. cooleyi            ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
S. bullata            ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
S. africa             ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
S. ruficornis         ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
S. argyrostoma        ATTTAATCGC AACAATGGTT ATTCTCTACT AATCATAAAG ATATTGGAAC TTTATATTTT
C. cadaverina         ATTTAATCGC GACAATGGTT ATTCTCAACT AATCATAAAG ATATTGGTAC TTTATACTTT
E. latifrons          ATTTAATCGC GACAATGATT ATTCTCAACT AATCATAAAG ATATTGGTAC TTTATACTTT
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          ATTTAATCGC AACAATGGTT ATTTTCAACT AATCATAAAG ATATTGGTAC TTTATACTTT
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     ATTTAATCGC AACAATGGTT ATTTTCAACT AATCATAAAG ATATTGGAAC TTTATATTTT
P. sialia             ATTTAATCGC AACAATGGCT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. callipes           ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. macellaria         ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
P. regina "1"         ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTT
P. regina "2"         ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTT
P. terraenovae        ATTTAATCGC GACAATGATT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. bezziana           ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. megacephala "1"    ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            ATTTAATCGC GACAATGATT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. varipes            ATTTAATCGC GACAATGATT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTT
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. putoria "USA"      ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     ATTTAATCGC GACAATGGTT ATTTTCTACT AATCATAAAG ATATTGGTAC TTTATATTTC

                                                                         120
M. domestica          ATCTTCGGAG CATGATCTGG TATAGTAGGA ACATCATTAA GAATTTTAAT TCGAGCTGAA
P. silenus            ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum           ---------- ---------- ---------- ---------- ---------- ----------
H. bovis              ---------- ---------- ---------- ---------- ---------- ----------
O. ovis               ---------- ---------- ---------- ---------- ---------- ----------
W. vigila             CTATTTGGAG CTTGATCAGG AATAGTAGGA ACTTCATTAA GAATTTTAAT TCGAGCAGAA
G. intestinalis       ---------- ---------- ---------- ---------- ---------- ----------
B. devia              ATCTTCGGAA TTTGATCAGG AATAATTGGA ACTTCTTTAA GTATCTTAAT TCGAACTGAA
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R. lherminieri        ATTTTTGGTG CTTGATCAGG AATAGTAGGA ACTTCTTTAA GAATTCTTAT TCGAGCAGAA
B. plinthopyga        ATTTTTGGAG CTTGATCCGG AATAGTAGGA ACTTCGTTAA GAATTCTTAT TCGAGCTGAA
P. chrysostoma        ATTTTTGGAG CTTGAGCAGG TATAGTAGGA ACATCTCTAA GAATTCTTAT TCGAGCCGAA
S. peregrina          ATTTTCGGAG CTTGAGCAGG TATAGTAGGA ACTTCATTAA GAATTCTTAT TCGAGCAGAA
S. crassipalpis       ATCTTCGGAG CTTGAGCAGG AATAGTAGGA ACTTCACTAA GAATTCTTAT TCGAGCAGAA
S. cooleyi            ATTTTCGGAG CTTGAGCAGG TATAGTAGGA ACTTCATTAA GAATTCTTAT TCGAGCAGAA
S. bullata            ATTTTCGGAG CTTGAGCAGG TATAGTAGGA ACTTCATTAA GAATTCTTAT TCGAGCAGAA
S. africa             ATTTTCGGAG CTTGAGCAGG TATAGTAGGA ACTTCATTAA GAATTCTTAT TCGAGCAGAA
S. ruficornis         ATCTTCGGAG CTTGAGCAGG AATAGTAGGA ACTTCACTAA GAATTCTTAT TCGAGCAGAA
S. argyrostoma        ATTTTCGGAG CTTGAGCAGG AATAGTAGGA ACTTCACTAA GAATCCTAAT TCGAGCAGAA
C. cadaverina         ATTTTTGGAG CTTGATCAGG AATGATCGGA ACTTCATTAA GAATTTTAAT TCGAGCCGAA
E. latifrons          ATTTTTGGAG CTTGATCAGG AATAATTGGA ACTTCATTAA GAATTTTAAT TCGAGCTGAA
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          ATTTTTGGAG CTTGATCCGG TATAATCGGA ACTTCATTAA GAATTTTAAT TCGAGCTGAA
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     ---------- CTTGATCCGG AATAATTGGA ACTTCTTTAA GAATTCTAAT TCGAGCTGAA
P. sialia             ATCTTTGGAG CTTGATCAGG AATAATTGGA ACTTCACTAA GAATCCTAAT TCGAGCAGAA
C. callipes           ATTTTTGGAG CCTGATCAGG AATAGTAGGG ACTTCATTAA GAATTTTAAT TCGAGCAGAA
C. macellaria         ATTTTTGGAG CTTGATCTGG AATAGTAGGA ACTTCTCTAA GAATTCTAAT TCGAGCAGAA
P. regina "1"         ATTTTCGGAG CTTGATCTGG AATAATTGGA ACTTCATTAA GAATTCTAAT TCGAGCTGAA
P. regina "2"         ATTTTCGGAG CTTGATCTGG AATAATTGGA ACTTCATTAA GAATTCTAAT TCGAGCTGAA
P. terraenovae        ATTTTCGGAG CTTGATCCGG AATAGTAGGA ACTTCTTTAA GAATCCTAAT TCGAGCCGAA
C. bezziana           ATTTTCGGAG CCTGATCCGG AATAGTAGGA ACTTCATTAA GAATTTTAAT TCGAGCTGAA
C. megacephala "1"    ATTTTCGGAG CTTGATCCGG AATAGTAGGA ACTTCATTAA GTATTTTAAT TCGAGCTGAA
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            ATTTTCGGAG CTTGATCTGG AATAGTAGGA ACTTCATTAA GTATCCTAAT TCGAGCTGAA
C. varipes            ATTTTCGGAG CTTGATCCGG AATAGTAGGA ACTTCACTAA GTATCCTAAT TCGAGCTGAA
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      ATTTTCGGAG CTTGATCTGG AATAGTAGGG ACTTCATTAA GAATTCTAAT TCGAGCTGAA
C. putoria "USA"      ATTTTCGGAG CTTGATCCGG AATAGTAGGA ACTTCATTAA GTATTTTAAT TCGAGCCGAA
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         ATTTTCGGAG CTTGATCTGG AATAGTAGGA ACTTCTTTAA GAATCCTAAT TCGAGCTGAA
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     ATTTTCGGAG CTTGATCTGG AATAGTAGGA ACTTCTTTAA GAATTCTAAT TCGAGCTGAA

                                                                    180
M. domestica          TTAGGACACC CTGGTGCTCT AATTGGAGAC GATCAAATTT ATAATGTTAT TGTAACAGCT
P. silenus            ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum           ---------- ---------- ---------- ---------- ---------- ----------
H. bovis              ---------- ---------- ---------- ---------- ---------- ----------
O. ovis               ---------- ---------- ---------- ---------- ---------- ----------
W. vigila             TTAGGACATC CTGGAGCTTT AATTGGTAAT GATCAAATTT ATAACGTAAT TGTTACAGCC
G. intestinalis       ---------- ---------- ---------- ---------- ---------- ----------
B. devia              TTAGGACATC CAGGAGCATT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTAACAGCT
R.lherminieri         TTAGGACATC CAGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTTAT TGTTACAGCT
B. plinthopyga        TTAGGACATC CAGGTGCACT TATTGGTGAC GATCAAATTT ATAATGTAAT CGTTACAGCT
P. chrysostoma        TTAGGTCATC CAGGAGCTCT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTCACAGCT
S. peregrina          TTAGGTCACC CTGGTGCATT AATTGGAGAT GACCAAATTT ATAACGTAAT TGTTACAGCT
S. crassipalpis       TTAGGTCATC CTGGTGCATT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTTACAGCT
S. cooleyi            CTGGGTCACC CTGGTGCATT AATTGGAGAT GATCAAATTT ATAACGTAAT TGTTACAGCT
S. bullata            CTAGGCCATC CGGGTGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTTACAGCC
S. africa             TTAGGTCACC CTGGTGCATT AATTGGTGAT GATCAAATTT ATAATGTAAT TGTTACAGCC
S. ruficornis         TTAGGTCATC CTGGTGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTTACAGCT
S. argyrostoma        CTAGGTCACC CTGGTGCATT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTTACAGCT
C. cadaverina         CTAGGACACC CTGGAGCATT AATTGGTGAT GACCAAATCT ATAATGTAAT TGTTACAGCT
E. latifrons          CTAGGACACC CTGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTTACAGCC
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          TTAGGACACC CTGGTGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTTACAGCT
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     TTAGGACATC CTGGAGCTTT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTTACAGCT
P. sialia             CTAGGGCACC CTGGAGCATT AATCGGAGAT GATCAAATTT ATAACGTAAT TGTAACAGCT
C. callipes           TTAGGACACC CTGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCT
C. macellaria         TTAGGACATC CTGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTTACAGCT
P. regina "1"         CTAGGGCACC CTGGAGCTCT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCT
P. regina "2"         CTAGGGCACC CTGGAGCTCT AATTGGAGAT GACCAAATTT ATAACGTAAT TGTAACAGCT
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P. terraenovae          TTAGGGCACC CTGGAGCACT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACGGCT
C. bezziana             TTAGGACACC CTGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCT
C. megacephala "1"      TTAGGACACC CTGGAGCATT AATTGGAGAC GACCAAATTT ATAATGTAAT TGTAACAGCT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TTAGGACACC CTGGAGCACT AATTGGAGAT GACCAAATTT ATAATGTAAT CGTAACAGCT
C. varipes              TTAGGACACC CTGGAGCTTT AATTGGAGAC GACCAAATCT ATAATGTAAT TGTAACAGCT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TTAGGTCACC CTGGAGCATT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCA
C. putoria "USA"        TTAGGACACC CTGGAGCACT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCT
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           TTAGGACATC CTGGAGCACT AATTGGAGAT GATCAAATTT ATAATGTAAT TGTAACAGCT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       TTAGGACATC CTGGAGCACT AATTGGAGAT GACCAAATTT ATAATGTAAT TGTAACAGCT

                                                                          240
M. domestica            CATGCTTTTA TTATAATTTT CTTTATAGTA ATACCTATTA TAATTGGAGG GTTTGGAAAT
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               CATGCTTTTA TTATGATTTT TTTCATAGTA ATACCTATTA TAATCGGAGG TTTCGGAAAT
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                CATGCTTTCA TTATAATTTT CTTTATAATT ATACCAATTA TAATTGGAGG ATTTGGAAAT
R. lherminieri          CATGCTTTTA TTATAATTTT CTTTATAGTA ATACCTATTA TAATTGGAGG ATTTGGAAAT
B. plinthopyga          CATGCTTTTA TTATAATTTT CTTCATAGTA ATACCTATTA TAATTGGAGG ATTTGGAAAT
P. chrysostoma          CATGCTTTTA TTATAATTTT CTTTATAGTA ATGCCAATTA TAATTGGTGG ATTTGGAAAT
S. peregrina            CATGCCTTTA TTATAATTTT TTTTATAGTA ATGCCAATTA TAATTGGAGG ATTTGGAAAT
S. crassipalpis         CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TGATTGGAGG ATTTGGAAAC
S. cooleyi              CATGCTTTTA TTATAATTTT TTTTATAGTA ATGCCAATTA TAATTGGAGG GTTTGGAAAT
S. bullata              CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
S. africa               CATGCTTTCA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
S. ruficornis           CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAC
S. argyrostoma          CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATCA TAATTGGAGG ATTTGGAAAC
C. cadaverina           CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGTAAT
E. latifrons            CATGCCTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGTAAT
C. vicina               ---------- ---------- ---------- ---------- -----TGTGG GTTTGGAAAT
L. sericata "1"         ---------- ---------- ---------- ---------- ------GGGG GTTTGGAAAT
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            CATGCTTTTA TTATAATTTT CTTTATAGTA ATGCCAATTA TAATTGGAGG ATTTGGAAAT
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- GTTTGGAAAT
L. sericata "17"        ---------- ---------- ---------- ---------- ------GGGG GTTTGGAAAT
L. sericata "34"        ---------- ---------- ---------- ---------- -------GGG GTTTGGAAAT
L. sericata "USA"       CATGCTTTTA TTATAATTTT TTTTATAGTA ATGCCAATTA TAATTGGAGG ATTTGGAAAT
P. sialia               CATGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
C. callipes             CACGCTTTTA TTATAATTTT TTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
C. macellaria           CACGCTTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
P. regina "1"           CATGCTTTTA TTATAATTTT CTTTATAGTT ATACCAATTA TAATTGGAGG ATTTGGAAAT
P. regina "2"           CATGCTTTTA TTATAATTTT CTTTATAGTT ATACCAATTA TAATTGGAGG ATTTGGAAAT
P. terraenovae          CACGCTTTTA TTATAATTTT CTTTATAGTA ATACCGATTA TAATTGGAGG ATTTGGAAAT
C. bezziana             CACGCTTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
C. megacephala "1"      CACGCTTTTA TTATAATTTT CTTTATAGTA ATGCCAATTA TAATTGGAGG ATTTGGAAAT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ------AAAT
C. norrisi              CATGCTTTTA TTATGATTTT CTTTATAGTA ATGCCAGTTA TAATTGGAGG ATTTGGAAAT
C. varipes              CATGCCTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        CATGCTTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAC
C. putoria "USA"        CACGCTTTTA TTATAATTTT CTTTATAGTA ATGCCAATTA TAATTGGAGG ATTTGGAAAT
C. putoria "SA"         ---------- ---------- ---------- ---------- --------GG GTTTGGAAAT
C. chloropyga           ---------- ---------- ---------- ---------- -----GGAGG GTTTGGAAAT
C. rufifacies           CATGCTTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------G GTTTGGAAAT
C. albiceps "USA"       CATGCCTTTA TTATAATTTT CTTTATAGTA ATACCAATTA TAATTGGAGG ATTTGGAAAT

                                                                          300
M. domestica            TGATTAGTTC CTTTAATATT AGGAGCTCCA GATATAGCAT TCCCTCGAAT GAATAATATA
P. silenus              ---------- ---------- ---------- --TATAGCAT TCCCACGAAT AAATAATATA
H. lineatum             ---------- ---------- ---------- --TATAGCAT TCCCACGAAT AAATAACATA
H. bovis                ---------- ---------- ---------- --TATAGCAT TCCCACGAAT AAATAACATA
O. ovis                 ---------- ---------- ---------- --TATAGCAT TCCCACGAAT AAATAACATA
W. vigila               TGATTAGTTC CTTTAATGTT AGGGGCCCCA GATATAGCAT TCCCTCGAAT AAATAATATA
G. intestinalis         ---------- ---------- ---------- --TATAGCAT TCCCACGAAT AAATAATATA
B. devia                TGATTAGTAC CTTTAATATT AGGAGCTCCA GACATAGCAT TTCCTCGAAT AAATAATATA
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R. lherminieri        TGATTGGTTC CAATTATACT TGGTGCTCCA GATATAGCTT TCCCTCGAAT AAATAATATA
B. plinthopyga        TGATTAGTTC CAATTATACT TGGAGCACCA GATATAGCTT TCCCTCGAAT AAATAATATA
P. chrysostoma        TGACTAGTAC CAATTATATT AGGAGCCCCA GATATAGCTT TCCCTCGAAT AAATAATATA
S. peregrina          TGACTGGTAC CAATTATATT AGGAGCCCCA GATATAGCTT TTCCTCGAAT AAATAATATA
S. crassipalpis       TGATTAGTTC CAATTATACT AGGAGCTCCA GATATAGCCT TTCCTCGAAT AAATAATATA
S. cooleyi            TGATTAGTAC CAATTATACT AGGAGCTCCA GACATAGCTT TCCCTCGAAT AAATAATATA
S. bullata            TGATTAGTAC CAATTATACT AGGAGCCCCA GACATAGCTT TCCCTCGAAT AAATAATATA
S. africa             TGATTAGTGC CAATTATACT AGGAGCTCCA GATATAGCCT TCCCTCGGAT AAACAATATA
S. ruficornis         TGACTAGTTC CAATTATATT AGGAGCTCCA GATATAGCTT TTCCTCGAAT AAATAATATA
S. argyrostoma        TGACTAGTTC CAATTATACT AGGAGCTCCA GATATAGCTT TCCCTCGAAT AAATAATATA
C. cadaverina         TGATTAGTCC CTTTAATGTT AGGAGCCCCA GATATAGCTT TCCCTCGGAT AAACAATATA
E. latifrons          TGATTAGTTC CTTTAATGCT AGGAGCTCCA GATATAGCCT TCCCTCGAAT AAATAATATA
C. vicina             TGATTAGTGC CTTTAATATT AGGAGCTCCA GATATAGCCT TCCCTCGGAT ATACAATATA
L. sericata "1"       TGATTAGTGC CATTAATACT AGGAGCTCCA GATATAGCAT CTCCTCGAAT AAATAATATA
L. cuprina            ---------- -ATTAATACG AGGAGCTCCA GATATAGCAT TCCCTCGAAT AAATAATATA
L. illustris          TGATTAGTTC CTTTAATATT AGGAGCTCCA GATATAGCAT TCCCTCGAAT AAATAATATA
L. sericata "41"      TGATTAGTGC CATTAATACT AGGAGCTCCA GATATAGCAT TCCCTCGAAT AAATAATATA
L. sericata "17"      TGATTAGTGC CATTAATACT AGGAGCTCCA GATATAGCAT CCCCTCGAAT AAATAATATA
L. sericata "34"      TGATTAGTGC CATTAATATT AGGAGCTCCA GATATGGGGT TCCCTCGAAT AAATAATATA
L. sericata "USA"     TGATTAGTTC CATTAATACT AGGAGCTCCA GATATAGCAT TCCCTCGAAT AAATAATATA
P. sialia             TGATTAGTTC CTCTAATATT AGGAGCTCCA GATATAGCTT TCCCCCGAAT GAATAATATA
C. callipes           TGATTAGTTC CTTTAATGTT AGGAGCTCCT GATATGGCTT TCCCACGAAT AAATAATATA
C. macellaria         TGATTAGTTC CTTTAATACT AGGAGCTCCT GATATAGCTT TCCCACGAAT AAATAATATA
P. regina "1"         TGATTAGTTC CTTTAATATT AGGGGCTCCT GATATAGCTT TCCCACGAAT AAACAATATA
P. regina "2"         TGATTAGTTC CTTTAATATT AGGGGCTCCT GATATAGCTT TCCCACGAAT AAACAATATA
P. terraenovae        TGACTAGTTC CCCTTATATT AGGGGCTCCT GATATAGCAT TCCCTCGAAT AAATAATATA
C. bezziana           TGATTAGTTC CCTTAATATT AGGAGCTCCA GATATAGCTT TCCCACGAAT AAATAATATA
C. megacephala "1"    TGACTAGTTC CTTTAATGTT AGGAGCTCCA GATATAGCTT TCCCACGAAT AAATAATATA
C. megacephala "2"    TGATTAGCGC CTTTAATGTA AGGAGCTCCA TATATAGCTA CCCCACGAAT AAATAATATA
C. norrisi            TGATTAGTCC CTCTAATATT AGGAGCTCCA GATATAGCTT TCCCACGAAT AAATAATATA
C. varipes            TGATTAGTTC CATTAATACT AGGAGCTCCA GACATAGCTT TTCCACGAAT AAATAATATA
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ----GCCACC AAGAAAATGG GCGGTTCCCA ACCCACGAAT AAACATTATA
C. semimetallica      TGATTAGTAC CTCTAATATT AGGAGCTCCA GATATAGCAT TTCCTCGACT AAATAATATA
C. putoria "USA"      TGATTAGTTC CTTTAATACT AGGAGCTCCA GATATAGCTT TCCAACGAAT AAATAATATA
C. putoria "SA"       TGATTAGTGC CTTTAATAGT AGGAGCTCCA TATATAGCTT TCCCACGAAT AAATAATATA
C. chloropyga         TGATTAGTGC CTTTAATACT AGGAGCTCCA GATATAGCTT TCCCACGAAT AAATAATATA
C. rufifacies         TGACTAGTCC CTCTAATACT AGGAGCCCCA GATATGGCTT TCCCACGAAT AAATAATATA
C. albiceps "SA"      TGATTAGTGC CTTTAATATT AGGAGCCCCA GATATAGCTT TCCCACGAAT AAATAATATA
C. albiceps "USA"     TGACTAGTTC CTTTAATATT AGGAGCCCCA GATATAGCTT TCCCACGAAT AAATAATATA

                                                                   360
M. domestica          AGTTTTTGAT TATTACCTCC TGCATTAACT CTATTATTAG TAAGAAGTAC AGTAGAAAAG
P. silenus            AGATTTTGGC TTCTTCCTCC TTCATTATCT CTTTTACTAG TTAGAAGTAC AGTGGAAAAT
H. lineatum           AGATTTTGAT TATTACCTCC ATCATTAACA TTACTATTAG TAAGAAGAAT AGTGGAAAAC
H. bovis              AGATTTTGAT TATTACCTCC ATCATTAACA TTACTATTAG TAAGAAGAAT AGTGGAAAAC
O. ovis               AGATTTTGAT TATTACCTCC ATCATTAACA TTACTATTAG TAAGAAGAAT AGTGGAAAAC
W. vigila             AGTTTTTGAT TACTCCCTCC TGCATTAACA TTATTATTAG TAAGTAGTAT AGTAGAAAAC
G. intestinalis       AGTTTTTGAC TTCTTCCACC AGCTCTAACT CTTCTTCTAA TGAGAAGAAT AGTAGAAAAC
B. devia              AGTTTTTGAT TACTACCCCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTAGAAAAC
R. lherminieri        AGTTTTTGAT TACTTCCTCC AGCTCTTACA TTATTACTAG TAAGTAGTAT AGTAGAAAAC
B. plinthopyga        AGTTTTTGAC TTCTTCCTCC AGCTTTAACA TTATTACTAG TAAGTAGTAT AGTAGAAAAT
P. chrysostoma        AGTTTCTGAC TTTTACCTCC AGCATTAACA TTACTTTTAG TAAGTAGTAT AGTAGAAAAT
S. peregrina          AGTTTTTGAC TTTTACCTCC AGCATTAACA CTACTTCTAG TAAGCAGCAT AGTAGAAAAT
S. crassipalpis       AGTTTTTGAC TTTTACCCCC AGCATTAACA TTGCTTCTAG TAAGTAGTAT AGTAGAAAAT
S. cooleyi            AGTTTTTGAC TTTTACCTCC AGCATTAACA TTACTTCTAG TAAGTAGTAT AGTAGAAAAC
S. bullata            AGTTTTTGAC TTTTACCCCC AGCATTAACA TTACTTCTAG TAAGTAGTAT AGTAGAAAAC
S. africa             AGTTTTTGAC TTTTACCTCC TGCATTAACA TTGCTTCTAG TAAGTAGTAT AGTAGAAAAT
S. ruficornis         AGTTTTTGAC TTTTACCTCC AGCATTAACA TTACTTCTAG TAAGTAGCAT AGTAGAAAAC
S. argyrostoma        AGATTTTGAC TTTTACCTCC TGCATTAACA TTACTACTAG TAAGTAGTAT AGTAGAAAAT
C. cadaverina         AGTTTCTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTGGAAAAC
E. latifrons          AGTTTTTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAC
C. vicina             AGTTTTTGAC TTTTACCTCA TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAC
L. sericata "1"       AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
L. cuprina            AGTTTTGGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
L. illustris          AGATTTTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAC
L. sericata "41"      AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
L. sericata "17"      AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
L. sericata "34"      AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
L. sericata "USA"     AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TTAGTAGTAT AGTAGAAAAC
P. sialia             AGTTTTTGAC TACTACCTCC TGCATTAACC TTATTATTAG TAAGTAGCAT AGTAGAAAAC
C. callipes           AGTTTTTGAC TTTTACCTCC TGCACTAACT CTATTATTAG TAAGTAGTAT AGTAGAAAAC
C. macellaria         AGTTTTTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTAAAAAAC
P. regina "1"         AGTTTTTGAC TTTTACCTCC TGCATTAACT CTACTGTTAG TTAGTAGTAT AGTAGAAAAT
P. regina "2"         AGTTTTTGAC TTTTACCTCC TGCATTAACT CTATTGTTAG TTAGTAGTAT AGTAGAAAAT
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P. terraenovae          AGTTTCTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTAGAAAAT
C. bezziana             AGTTTCTGAC TTTTACCTCC TGCATTAACT TTATTGTTAG TAAGTAGTAT AGTAGAAAAT
C. megacephala "1"      AGTTTCTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTACAAAAT
C. megacephala "2"      AGTTTCTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTAGAAAAT
C. norrisi              AGTTTCTGAC TTTTACCTCC TGCATTAACT TTATTATTAG TAAGTAGTAT AGTAGAAAAT
C. varipes              AGTTTTTGAC TTTTACCTCC TGCTCTAACA CTATTGTTAG TAAGTAGTAT AGTAGAAAAT
C. inclinata            ---------- -TTTACCACC TGCATTAACT CTATTATTAG TAAGAAGTAT AGTAGAAAAT
C. marginalis           AGTTTCTGAC TTTTACCTCC TGCTTTAACT CTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. semimetallica        AGTTTTTGAC TTTTACCTCC TGCTTTAACT CTATTATTAG TAAGTAGTAT AGTAGAAAAT
C. putoria "USA"        AGTTTCTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. putoria "SA"         AGTTTCTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. chloropyga           AGTTTTTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. rufifacies           AGTTTTTGAC TTTTACCCCC TGCACTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. albiceps "SA"        AGTTTCTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT
C. albiceps "USA"       AGTTTCTGAC TTTTACCTCC TGCATTAACT TTACTATTAG TAAGTAGTAT AGTAGAAAAT

                                                                              420
M. domestica            GGAGCTGGAA CAGGTTGAAC TGTTTATCCA CCTTTATCAT CAATTATTGC TCATGGTGGA
P. silenus              GGAGCTGGTA CAGGATGAAC TGTTTATCCT CCTTTATCAG CTAATATCGC TCATGGAGGA
H. lineatum             GGAGCTGGAA CAGGATGAAC AGTTTATCCA CCTTTATCCT CAAATATCGC TCATGGAGGC
H. bovis                GGAGCTGGAA CAGGATGAAC AGTTTATCCA CCTTTATCCT CAAATATCGC TCATGGAGGC
O. ovis                 GGAGCTGGAA CAGGATGAAC AGTTTATCCA CCTTTATCCT CAAATATCGC TCATGGAGGC
W. vigila               GGAGCTGGAA CTGGTTGAAC TGTTTACCCT CCACTTTCAG CTAATATTGC TCATAGAGGA
G. intestinalis         GGAGCCGGAA CTGGTTGAAC AGTTTACCCT CCCCTATCTG CTACAATTGC CCACAGCGGT
B. devia                GGAGCTGGGA CAGGATGAAC TGTTTACCCT CCCCTATCTT CTAATATTGC TCATGGAGGA
R. lherminieri          GGAGCTGGAA CTGGATGAAC TGTTTACCCA CCATTATCTT CTAATATTGC TCATGGAGGA
B. plinthopyga          GGAGCTGGAA CAGGTTGAAC TGTTTACCCT CCTTTATCTT CTAATATTGC CCATGGAGGA
P. chrysostoma          GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCATTATCTT CTAATATTGC TCATGGAGGG
S. peregrina            GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCTT CTAATATTGC CCATGGAGGT
S. crassipalpis         GGAGCTGGAA CGGGGTGAAC TGTTTACCCT CCTTTATCTT CTAATATTGC TCATGGAGGA
S. cooleyi              GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCAT CTAATATTGC TCATGGAGGA
S. bullata              GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCTT CTAACATCGC CCATGGAGGA
S. africa               GGAGCTGGAA CAGGTTGAAC TGTATACCCT CCTTTATCTT CTAATATTGC TCATGGAGGA
S. ruficornis           GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCAT CTAATATTGC TCATGGAGGA
S. argyrostoma          GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCAT CTAATATTGC TCATGGAGGA
C. cadaverina           GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATCGC TCATGGAGGA
E. latifrons            GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCCTTATCTT CTAATATTGC CCATGGAGGA
C. vicina               GGAGCTGGAA CAGGATGAGC TGTTTACCCT CCTTTATCTT CTAATATCGC TCATGGAGGA
L. sericata "1"         GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCATGGAGGA
L. cuprina              GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCACGGAGGA
L. illustris            GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTTTATCAT CTAATATCGC TCATGGAGGA
L. sericata "41"        GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCATGGAGGA
L. sericata "17"        GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCATGGAGGA
L. sericata "34"        GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCATGGAGGA
L. sericata "USA"       GGAGCTGGAA CAGGATGAAC AGTTTACCCT CCTCTATCTT CTAATATTGC TCATGGAGGA
P. sialia               GGAGCTGGAA CAGGATGAAC TGTTTACCCC CCTCTATCAT CTAATATTGC TCATGGAGGA
C. callipes             GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCTT CTAATATTGC TCATGGAGGA
C. macellaria           GGAGCTGGAA CAGGATGAAC TGTTTACCCT CCTTTATCTT CTAATATTGC TCACGGAGGA
P. regina "1"           GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCATGGAGGA
P. regina "2"           GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCATGGAGGA
P. terraenovae          GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCTT CTAATATTGC TCACGGAGGA
C. bezziana             GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCTT CTAATATTGC TCACGGAGGA
C. megacephala "1"      GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCTT CTAATATTGC TCATGGAGGA
C. megacephala "2"      GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCTT CTAATATTGC TCATGGAGGA
C. norrisi              GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCACGGAGGA
C. varipes              GGGGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCACGGAGGA
C. inclinata            GGGGTTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCACGGGGGG
C. marginalis           GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCAT CTAATATTGC TCATGGAGGC
C. semimetallica        GGAGCTGGAA CAGGATGAAC TGTTTACCCA CCTTTATCTT CTAATATCGC TCATGGAGGA
C. putoria "USA"        GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCTTTATCAT CTAATATTGC CCATGGTGGT
C. putoria "SA"         GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCTTTATCAT CTAATATTGC CCATGGTGGT
C. chloropyga           GGGGCTGGAA CAGGATGAAC AGTTTATCCA CCTTTATCAT CTAATATTGC CCATGGTGGA
C. rufifacies           GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCTTTATCAT CTAATATTGC ACATGGTGGA
C. albiceps "SA"        GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCTTTATCAT CTAATATTGC TCATGGTGGA
C. albiceps "USA"       GGAGCTGGAA CAGGATGAAC TGTTTATCCA CCTTTATCAT CTAATATTGC TCATGGTGGA

                                                                              480
M. domestica            GCTTCAGTTG ATTTAGCTAT TTTCTCTCTT CACTTAGCAG GAATTTCTTC AATTTTAGGA
P. silenus              CCTTCAGTTG ACTTAGCTAT TTTTTCACTT CATCTTGCAG GAATTTCATC TATTTTAGGA
H. lineatum             CCATCTGTTG ATTTAGCAAT TTTTTCTTTA CATCTAGCAG GTATTTCATC TATCTTAGGT
H. bovis                CCATCTGTTG ATTTAGCAAT TTTTTCTTTA CATCTAGCAG GTATTTCATC TATCTTAGGT
O. ovis                 CCATCTGTTG ATTTAGCAAT TTTTTCTTTA CATCTAGCAG GTATTTCATC TATCTTAGGT
W. vigila               GCTTCTGTGG ATTTAGCAAT CTTCTCTCTT CATTTGGCTG GAATTTCTTC TATTTTAGGG
G. intestinalis         CCATCAGTAG ATCTAGCAAT TTTCTCTCTA CATTTAGCAG GAGTATCTTC TATTCTGGGT
B. devia                GCCTCTGTAG ATTTAGCTAT TTTCTCTTTA CATTTAGCAG GAATTTCCTC TATTTTAGGG
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R. lherminieri          GCCTCTGTTG ATCTAGCTAT CTTCTCTCTA CATTTAGCAG GAATTTCATC AATTTTAGGT
B. plinthopyga          GCATCTGTTG ATTTAGCAAT TTTCTCTCTT CACTTAGCTG GAATTTCATC TATTTTAGGA
P. chrysostoma          GCTTCTGTTG ATTTAGCAAT TTTTTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
S. peregrina            GCTTCTGTTG ATTTAGCTAT CTTCTCCCTT CATTTAGCTG GAATTTCATC AATTTTAGGA
S. crassipalpis         GCTTCTGTTG ATTTAGCTAT TTTTTCTCTA CATTTAGCTG GAATTTCTTC AATTTTAGGA
S. cooleyi              GCTTCTGTTG ATTTAGCTAT TTTTTCCCTA CACTTAGCTG GAATTTCTTC AATTTTAGGA
S. bullata              GCTTCTGTTG ATTTAGCCAT TTTTTCCCTA CATTTAGCCG GAATTTCTTC AATTTTAGGA
S. africa               GCTTCTGTTG ATTTAGCTAT TTTTTCTCTC CATTTAGCTG GAATTTCTTC AATTCTAGGA
S. ruficornis           GCTTCTGTTG ATTTAGCTAT TTTTTCTCTT CATTTAGCCG GAATTTCTTC AATTTTAGGA
S. argyrostoma          GCTTCTGTTG ATCTAGCTAT TTTTTCTCTT CACTTAGCTG GAATTTCTTC AATTTTAGGA
C. cadaverina           GCTTCTGTTG ATTTAGCTAT TTTTTCTTTA CACTTAGCAG GAATTTCTTC AATTTTAGGA
E. latifrons            GCTTCTGTTG ATTTAGCAAT TTTTTCTTTA CATTTAGCCG GAATTTCTTC AATTTTAGGA
C. vicina               GCTTCTGTTG ATTTAGCTAT ATGTTCTTTT CACTTAGCAG GAATTTCTTC AATTATAGGA
L. sericata "1"         GCATCTGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
L. cuprina              GCTTCTGTTG ATTTAGCTAT TTTTTCTCTT CATTTAGCAG GAATTTCCTC AATTTTAGGA
L. illustris            GCTTCTGTTG ATTTAGCTAT TTTTTCTCTT CATTTAGCAG GAATTTCATC AATTTTAGGA
L. sericata "41"        GCTTCTGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
L. sericata "17"        GCATCTGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
L. sericata "34"        GCTTCTGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
L. sericata "USA"       GCTTCTGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCAG GAATTTCTTC AATTTTAGGA
P. sialia               GCATCAGTTG ATCTAGCTAT TTTCTCTTTA CATTTAGCAG GAATTTCATC AATTTTAGGA
C. callipes             GCTTCAGTTG ATCTAGCTAT TTTTTCTTTA CATTTAGCTG GAATTTCATC AATTTTAGGA
C. macellaria           GCTTCAGTTG ATCTAGCTAT TTTCTCTCTT CATTTAGCCG GAATTTCATC AATTTTAGGA
P. regina "1"           GCATCTGTTG ATCTAGCTAT TTTCTCTCTT CACTTAGCAG GAATTTCTTC AATTTTAGGA
P. regina "2"           GCATCTGTTG ATCTAGCTAT TTTCTCTCTT CACTTAGCAG GAATTTCTTC AATTTTAGGA
P. terraenovae          GCATCTGTTG ATTTAGCTAT TTTCTCTCTT CACTTGGCCG GAATTTCTTC AATTTTAGGA
C. bezziana             GCATCAGTCG ATTTAGCTAT TTTCTCTTTA CATTTAGCAG GAATTTCCTC AATTCTAGGA
C. megacephala "1"      GCATCAGTTG ATTTAGCTAT TTTCTCTTTA CACTTAGCAG GAATTTCTTC AATTTTAGGA
C. megacephala "2"      GCATCAGTTG ATTTAGCTAT TTTCTCTTTA CACTTAGCAG GAATTTCTTC AATTTTAGGA
C. norrisi              GCTTCAGTTG ATTTAGCTAT TTTTTCTCTT CACTTAGCAG GAATTTCTTC AATTTTAGGA
C. varipes              GCTTCAGTTG ATTTAGCTAT TTTCTCTTTA CATTTAGCAG GAATTTCTTC AATTTTAGGA
C. inclinata            GCATCAGGTG ATTTAGCTAT TTTCTCTTTA CATTTAACTG GGATTTCTTC CATTTTAGGA
C. marginalis           GCATCAGTTG ATTTAGCTAT TTTCTCATTA CATTTAGCTG GAATTTCCTC AATTTTAGGA
C. semimetallica        GCATCAGTTG ATTTAGCTAT TTTCTCTCTT CATTTAGCCG GAATTTCCTC AATTTTAGGA
C. putoria "USA"        GCATCAGTTG ATTTAGCTAT TTTCTCTTTA CATTTAGCCG GAATTTCTTC AATTTTAGGA
C. putoria "SA"         GCATCAGTTG ATTTAGCTAT TTTCTCTTTA CATTTAGCCG GAATTTCTTC AATTTTAGGA
C. chloropyga           GCATCAGTTG ATTTAGCTAT TTTTTCTTTA CATCTAGCCG GAATTTCATC AATTTTAGGA
C. rufifacies           GCATCAGTTG ATTTAGCTAT TTTTTCTTTA CACTTAGCTG GAATTTCATC AATTTTAGGG
C. albiceps "SA"        GCATCAGTTG ATTTAGCTAT TTTTTCTTTA CACTTAGCTG GAATTTCATC AATTTTAGGA
C. albiceps "USA"       GCATCAGTTG ATTTAGCTAT TTTTTCTTTA CACTTAGCTG GAATTTCATC AATTTTAGGA

                                                                            540
M. domestica            GCAGTAAATT TTATTACAAC TGTTATTAAC ATACGATCAA CAGGAATTAC ATTCGATCGA
P. silenus              GCAGTAAACT TTATCACAAC TATTATTAAT ATACGCTCTA CTGGAATCTC ATTAGATCGA
H. lineatum             GCAGTTAATT TTATCACTAC TATCATTAAT ATACGATCAA CAGGAATTTC ACTAGATCGC
H. bovis                GCAGTTAATT TTATCACTAC TATCATTAAT ATACGATCAA CAGGAATTTC ACTAGATCGC
O. ovis                 GCAGTTAATT TTATCACTAC TATCTTTTTT ATACGATCAA CAGGAATTTC ACTAGATCGC
W. vigila               GCTGTAAATT TTATTACAAC TGTTATTAAT ATACGATCAA CAGGAATTAC ATTTGATCGA
G. intestinalis         GCAGTAAATT TCATTACAAC TGTTATTAAT ATACGATCAA CGGGAATTAC CTTTGACCGT
B. devia                GCTGTTAATT TTATTACAAC AGTAATTAAT ATACGTGCAA CAGGAATTTC ATTTGATCGA
R. lherminieri          GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTCGATCGA
B. plinthopyga          GCAGTAAATT TTATTACTAC AGTAATTAAT ATACGATCTA CAGGTATTAC TTTTGATCGA
P. chrysostoma          GCAGTAAATT TTATTACAAC AGTAATTAAT ATACGATCGA CAGGAATTAC CTTTGATCGA
S. peregrina            GCAGTAAATT TTATTACTAC AGTTATTAAT ATACGATCTT CTGGTATTAC ATTTGATCGA
S. crassipalpis         GCAGTAAATT TTATTACTAC AGTAATTAAT ATACGATCTA CAGGAATTAC CTTTGATCGA
S. cooleyi              GCAGTAAATT TTATTACTAC AGTTATTAAT ATACGATCTA CAGGTATTAC ATTTGACCGA
S. bullata              GCAGTAAATT TTATTACTAC AGTTATTAAT ATACGATCTA CAGGTATTAC ATTTGATCGA
S. africa               GCAGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATCAC TTTGGATCGA
S. ruficornis           GCAGTAAATT TTATTACTAC AGTAATTAAT ATACGATCTA CAGGAATTAC CTTTGATCGA
S. argyrostoma          GCAGTAAATT TTATTACTAC AGTAATTAAT ATACGATCTA CAGGTATTAC TTTTGATCGA
C. cadaverina           GCTGTAAATT TTATTACAAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGACCGA
E. latifrons            GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGACCGA
C. vicina               GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAG CAGGAATTAC ATTCGATCGA
L. sericata "1"         GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
L. cuprina              GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
L. illustris            GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGGATTAC TTTTGATCGA
L. sericata "41"        GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
L. sericata "17"        GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
L. sericata "34"        GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
L. sericata "USA"       GCTGTAAATT TTATTACTAC AGTTATTAAT ATACGATCAA CAGGAATTAC TTTTGATCGA
P. sialia               GCTGTAAATT TTATTACAAC CGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. callipes             GCAGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTCGATCGA
C. macellaria           GCAGTAAATT TCATTACAAC TGTAATTAAT ATACGATCAA CAGGAATTAC ATTCGATCGA
P. regina "1"           GCTGTAAATT TCATTACAAC TGTAATTAAT ATACGATCAA CTGGAATTAC ATTTGATCGA
P. regina "2"           GCTGTAAATT TCATTACAAC TGTAATTAAT ATACGATCAA CTGGAATTAC ATTTGATCGA
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P. terraenovae          GCTGTAAATT TCATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGATCGA
C. bezziana             GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGATCGA
C. megacephala "1"      GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGATCGA
C. megacephala "2"      GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTAGATCGA
C. norrisi              GCTGTAAATT TCATTACTAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. varipes              GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. inclinata            GCTGTAAATT TTATTACAAC TGTGAATAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. marginalis           GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGATCGA
C. semimetallica        GCAGTAAATT TTATTACAAC AGTAATTAAT ATACGATCAA CAGGAATTAC ATTTGATCGA
C. putoria "USA"        GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. putoria "SA"         GCTGTAAATT TTATTACAAC TGTAATTAAT ATACGATCTA CAGGAATTAC ATTTGACCGA
C. chloropyga           GCTGTAAATT TTATTACAAC AGTAATTAAT ATACGGTCTA CAGGAATTAC ATTTGACCGA
C. rufifacies           GCCGTAAATT TTATTACAAC TGTTATTAAT ATACGATCTA CAGGAATTAC ATTTGATCGA
C. albiceps "SA"        GCTGTAAATT TTATTACAAC TGTTATTAAT ATACGATCTA CAGGAATCAC ATTTGATCGA
C. albiceps "USA"       GCTGTAAATT TTATTACAAC TGTTATTAAT ATACGATCTA CAGGAATCAC ATTTGATCGA

                                                                          600
M. domestica            ATGCCTTTAT TTGTTTGATC AGTTGTAATT ACTGCATTAT TATTATTATT ATCTCTTCCT
P. silenus              ATACCCTTAT TTGTATGATC AGTAGGAATT ACTGCTTTAT TATTATTATT ATCTTTACCA
H. lineatum             ATACCTTTAT TTGTCTGATC AGTCGGAATT ACAGCTTTAC TATTACTATT ATCTCTTCCT
H. bovis                ATACCTTTAT TTGTCTGATC AGTCGGAATT ACAGCTTTAC TATTACTATT ATCTCTTCCT
O. ovis                 ATACCTTTAT TTGTCTGATC AGTCGGAATT ACCGCTTTAC TATTACTATT ATCTCTTCCT
W. vigila               ATACCTCTAT TTGTTTGATC CGTAGTGATT ACTGCTTTAT TACTTCTTCT ATCCTTACCT
G. intestinalis         ATACCACTAT TTGTTTGATC AGTAGCTATT ACAGCTCTAC TTCTTCTCCT CTCCCTACCA
B. devia                ATACCCCTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TATTACTTTT ATCTCTTCCA
R. lherminieri          ATACCTTTAT TTGTTTGATC AGTAATAATC ACTGCTTTAT TACTTCTTTT ATCATTACCA
B. plinthopyga          ATACCTTTAT TTGTTTGATC TGTAGTAATT ACTGCTTTAT TATTACTTCT TTCTTTACCT
P. chrysostoma          ATACCTTTAT TTGTTTGATC TGTAGTTATT ACAGCCCTAT TATTACTTCT TTCTTTACCA
S. peregrina            ATGCCTTTAT TTGTATGATC AGTAGTAATT ACAGCTTTAC TTTTATTACT TTCTTTACCC
S. crassipalpis         ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCCCTAC TTTTACTTTT ATCTTTACCG
S. cooleyi              ATACCTTTAT TTGTTTGATC TGTAGTAATT ACAGCTTTAC TTTTACTTCT TTCTCTACCT
S. bullata              ATACCTTTAT TTGTTTGATC TGTAGTAATT ACAGCTTTAC TTTTACTTCT TTCCCTACCT
S. africa               ATACCTTTAT TTGTATGATC TGTAGTAATC ACAGCCCTAC TTTTATTACT TTCTTTACCT
S. ruficornis           ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTCTAC TTTTACTTTT ATCTTTACCT
S. argyrostoma          ATACCCCTTT TTGTTTGATC AGTAGTAATT ACCGCTTTAC TTCTCCTTCT ATCCCTACCC
C. cadaverina           ATACCACTAT TCGTTTGATC AGTAGTAATT ACAGCTTTAT TACTTTTACT ATCTTTACCT
E. latifrons            ATACCTCTTT TTGTTTGATC TGTAGTAATT ACAGCTTTAT TACTTTTATT ATCTTTACCA
C. vicina               ATACCATTAT TTGTTTGATC AGTAGTAATT ACAGCTATAG GACTGTTATT CTCTTTACCA
L. sericata "1"         ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TACTGTTATT ATCATTACCA
L. cuprina              ATACCTTTAT TTGTGTGATC AGTAGTAATT ACAGCTTTAT TACTTTTATT ATCATTACCA
L. illustris            ATGCCTTTAT TCGTTTGATC AGTAGTAATT ACAGCTTTAT TACTTTTATT ATCATTACCA
L. sericata "41"        ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TACTTTTATT ATCATTACCA
L. sericata "17"        ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TACTGTTATT ATCATTACCA
L. sericata "34"        ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TACTTTTATT ATCATTACCA
L. sericata "USA"       ATACCTTTAT TTGTTTGATC AGTAGTAATT ACAGCTTTAT TACTTTTATT ATCATTACCA
P. sialia               ATACCATTAT TTGTTTGATC TGTAGCTATT ACTGCTTTAC TACTTTTGTT ATCTCTACCA
C. callipes             ATACCTCTAT TTGTTTGATC AGTAGTAATT ACTGCTTTAT TATTATTATT ATCTTTACCA
C. macellaria           ATGCCTTTAT TCGTATGATC AGTAGTAATT ACTGCTCTTT TACTTTTATT ATCTTTACCA
P. regina "1"           ATACCTTTAT TTGTTTGATC TGTAGTTATT ACTGCTCTAT TACTTTTATT ATCTTTGCCT
P. regina "2"           ATACCTTTAT TTGTTTGATC TGTAGTTATT ACTGCTCTAT TACTTTTATT ATCTTTACCT
P. terraenovae          ATACCTTTAT TTGTTTGATC TGTAGTTATT ACTGCTCTTT TACTTTTATT ATCTTTACCA
C. bezziana             ATACCTTTAT TCGTATGATC TGTAGTTATT ACTGCTTTAT TATTATTATT ATCTTTACCA
C. megacephala "1"      ATACCTTTAT TTGTATGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. megacephala "2"      ATACCTTTAT TCGTATGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. norrisi              ATACCTTTAT TTGTTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. varipes              ATACCTTTAT TCGTTTGATC TGTAGTTATT ACTGCTCTTC TTCTATTATT ATCTTTACCA
C. inclinata            ATACCATTAT TCGCTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. marginalis           ATACCTTTAT TTGTTTGATC TGTAGTAATT ACTGCTTTAT TATTATTGTT ATCTTTACCA
C. semimetallica        ATACCTTTAT TCGTTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCATTACCA
C. putoria "USA"        ATACCATTAT TCGTTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. putoria "SA"         ATACCATTAT TCGTTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. chloropyga           ATACCACTAT TCGTTTGATC TGTAGTTATT ACTGCTCTAT TATTATTATT ATCTTTACCA
C. rufifacies           ATACCTTTAT TTGTATGATC TGTAGTTATT ACTGCTCTTC TTTTATTATT ATCATTACCA
C. albiceps "SA"        ATACCTTTAT TCGTATGATC TGTAGTTATT ACTGCTCTTC TTTTATTATT ATCATTACCA
C. albiceps "USA"       ATACCTTTAT TCGTATGATC TGTAGTTATT ACTGCTCTTC TTTTATTATT ATCATTACCA

                                                                          660
M. domestica            GTTCTTGCTG GAGCTATTAC TATACTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
P. silenus              GTATTAGCAG GAGCTATTAC AATACTGTTA ACCGATCGAA ACTTAAATAC TTCTTTTTTT
H. lineatum             GTATTAACCG GTGCTATCAC AATATTATTA ACAGATCGAA ACTTAAATAC ATCATTTTTT
H. bovis                GTTTTAACCG GTGCTATCAC AATATTATTA ACAGATCGAA ACTTAAATAC ATCATTTTTT
O. ovis                 GTATTAGCCG GTGCTATCAC AATATTATTA ACAGATCGAA ACTTAAATAC ATCATTTTTT
W. vigila               GTATTAGCTG GAGCAATCAC TATACTTTTA ACAGATCGAA ATCTTAATAC TTCCTTTTTT
G. intestinalis         ATCTTGGCGG AAGCAATTAC AATACTTTTA ACAGACCGTA ATTTAAATAC ATCATTCTTT
B. devia                GTTTTAGCAG GAGCAATTAC AATATTATTA ACAGATCGAA ATCTTAATAC TTCATTTTTT
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R. lherminieri        GTTCTTGCTG GAGCTATTAC TATATTATTA ACTGACCGAA ATATTAATAC TTCATTTTTT
B. plinthopyga        GTACTTGCTG GTGCAATTAC TATATTATTA ACTGATCGAA ATATTAATAC TTCATTCTTT
P. chrysostoma        GTACTTGCAG GAGCAATTAC AATATTATTA ACAGATCGAA ATATTAATAC ATCATTCTTT
S. peregrina          GTTCTTGCCG GAGCAATTAC AATATTATTA ACTGATCGAA ATATTAATAC TTCATTTTTT
S. crassipalpis       GTACTTGCAG GAGCTATTAC TATATTATTA ACTGACCGAA ATATTAATAC CTCTTTTTTC
S. cooleyi            GTACTTGCTG GAGCAATTAC TATACTATTA ACTGATCGAA ATATTAATAC TTCATTCTTT
S. bullata            GTACTTGCTG GAGCAATTAC TATACTATTA ACTGATCGAA ATATTAATAC TTCATTCTTT
S. africa             GTACTTGCCG GAGCTATTAC TATATTATTA ACTGATCGAA ATATTAATAC TTCATTTTTT
S. ruficornis         GTACTTGCAG GAGCTATTAC TATATTATTA ACTGACCGAA ATATTAACAC TTCCTTCTTT
S. argyrostoma        GTACTTGCAG GAGCAATTAC TATATTATTA ACTGACCGAA ATATTAATAC TTCATTTTTT
C. cadaverina         GTTCTAGCTG GTGCTATTAC AATATTATTA ACAGACCGAA ACCTTAATAC TTCATTCTTC
E. latifrons          GTATTAGCCG GTGCTATTAC TATACTATTA ACAGATCGAA ATCTTAATAC CTCATTTTTT
C. vicina             GTATTAGCAG GTGCTATTTC TATAGTATTA TCAGATCGAT ATCGTAATGC ATCATTCTAT
L. sericata "1"       GTATTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTTAATAC ATCATTCTTT
L. cuprina            GTATTAGCTG GAGCTATTAC AATACTTTTA ACAGATCGAA ATCTTAATAC ATCATTCTTT
L. illustris          GTATTAGCTG GAGCTATTAC TATACTTTTA ACTGATCGAA ATCTTAATAC TTCATTCTTT
L. sericata "41"      GTATTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTTAATAC ATCATTCTTT
L. sericata "17"      GTATTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTTAATAC ATCATTCTTT
L. sericata "34"      GTATTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTTAATAC ATCATTCTTT
L. sericata "USA"     GTATTAGCAG GAGCTATTAC AATACTTTTA ACAGACCGAA ATCTTAATAC ATCATTCTTT
P. sialia             GTATTAGCAG GTGCTATTAC TATATTATTA ACTGATCGAA ACTTAAATAC TTCATTTTTT
C. callipes           GTTTTAGCTG GAGCTATTAC TATATTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
C. macellaria         GTTTTAGCCG GAGCTATTAC TATACTTTTA ACTGATCGAA ATTTAAACAC TTCATTCTTT
P. regina "1"         GTATTAGCCG GTGCTATTAC TATATTATTA ACTGATCGAA ATTTAAACAC TTCATTCTTT
P. regina "2"         GTATTAGCCG GTGCTATTAC TATATTATTA ACTGATCGAA ATTTAAACAC TTCATTCTTT
P. terraenovae        GTATTAGCAG GTGCTATTAC TATATTATTA ACTGACCGAA ATTTAAATAC TTCATTTTTT
C. bezziana           GTATTAGCTG GAGCTATTAC TATGTTATTA ACTGACCGAA ATTTAAACAC TTCATTCTTT
C. megacephala "1"    GTATTAGCTG GAGCTATTAC TATATTATTA ACTGACCGAA ATCTAAATAC TTCATTCTTT
C. megacephala "2"    GTATTAGCTG GAGCTATTAC TATATTATTA ACTGACCGAA ATCTAAATAC TTCATTCTTT
C. norrisi            GTATTAGCTG GAGCTATTAC TATATTATTG ACTGATCGAA ATTTAAATAC ATCATTCTTT
C. varipes            GTATTAGCAG GAGCTATTAC TATACTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
C. inclinata          GTATTAGCCG GAGCTATTAC TATACTATTA ACAGACCGAA ATCTAAATAC TTCGTTCTTT
C. marginalis         GTATTAGCAG GAGCTATTAC TATACTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
C. semimetallica      GTATTAGCAG GAGCTATTAC TATATTATTA ACTGACCGAA ATCTAAATAC TTCATTCTTC
C. putoria "USA"      GTATTAGCAG GAGCTATTAC TATATTATTA ACTGACCGAA ATTTAAACAC TTCATTCTTT
C. putoria "SA"       GTATTAGCAG GAGCTATTAC TATATTATTA ACTGACCGAA ATTTAAACAC TTCATTCTTT
C. chloropyga         GTATTAGCAG GAGCTATTAC TATATTATTA ACCGACCGAA ATTTAAATAC TTCATTCTTT
C. rufifacies         GTATTAGCAG GTGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
C. albiceps "SA"      GTATTAGCCG GCGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT
C. albiceps "USA"     GTATTAGCCG GTGCAATTAC TATATTATTA ACTGATCGAA ATTTAAATAC TTCATTCTTT

                                                                         720
M. domestica          GACCCAGCTG GAGGAGGTGA TCCAATTCTT TACCAACACT TATTCTGATT CTTTGGACAT
P. silenus            GATCCAGCAG GATGAAGCGA TCCTATTTTA TACCAACACC TATTTTGATT TTTTGGACAT
H. lineatum           GATCCCGCAG GAGGAGGAGA TCCAATTCTC TATCAACATT TATTTTGATT TTTCGGTCAT
H. bovis              GATCCCGCAG GAAGAAGAGA TCCAATTCTC TATCAACATT TATTTTGATT TTTCGGTCAT
O. ovis               GATCCCGCAG GAGGAGGAGA TCCAATTCTC TATCAACATT TATTTTGATT TTTCGGTCAT
W. vigila             GACCCCGCAG GTGGAGGAGA TCCTATTCTT TATCAACATT TATTTTGATT TTTTGGGCAC
G. intestinalis       GACCCCGCAG GAGGTGGAGA TCCAATCTTA TATCAACATT TATTCTGATT TTTTGGTCAT
B. devia              GATCCTGCAG GAGGAGGGGA TCCAATTCTT TACCAACATT TATTTTGATT TTTTGGTCAT
R. lherminieri        GACCCAGCAG GAGGAGGAGA CCCAATTTTA TACCAACATT TATTTTGATT CTTTGGACAC
B. plinthopyga        GACCCTGCAG GAGGAGGAGA TCCAATTCTA TACCAACACT TATTCTGATT CTTTGGACAT
P. chrysostoma        GATCCAGCTG GAGGAGGAGA TCCTATTCTT TATCAACATT TATTCTGATT TTTCGGACAC
S. peregrina          GATCCTGCAG GAGGAGGAGA CCCAATTCTA TACCAACATC TATTTTGATT TTTTGGACAC
S. crassipalpis       GACCCAGCAG GAGGAGGAGA TCCTATTTTA TACCAACACC TATTTTGATT TTTCGGTCAC
S. cooleyi            GACCCTGCAG GAGGAGGAGA CCCAATTTTA TACCAACATT TATTTTGATT CTTTGGGCAC
S. bullata            GACCCTGCAG GAGGGGGAGA TCCAATTCTT TATCAACATT TATTTTGATT CTTTGGACAT
S. africa             GACCCGGCAG GAGGAGGAGA TCCTATTCTA TATCAACATT TATTTTGATT CTTTGGGCAT
S. ruficornis         GACCCAGCAG GAGGAGGAGA CCCTATTTTA TACCAACACT TATTTTGATT TTTTGGTCAC
S. argyrostoma        GATCCAGCAG GAGGAGGAGA TCCAATTCTA TATCAACACT TATTTTGATT TTTTGGTCAT
C. cadaverina         GACCCAGCAG GAGGAGGAGA CCCAATTCTA TACCAACATT TATTTTGATT TTTTGGTCAC
E. latifrons          GACCCTGCAG GAGGAGGAGA CCCTATTCTA TACCAACATT TATTTTGATT CTTTGGTCAT
C. vicina             GACCCAGCAG GAGGAGGAGA TCCAATTCTA ---------- ---------- ----------
L. sericata "1"       GACCCTGCAG GAGGAGGAGA TCCAATTTTA TACCAACATT TAT------- ----------
L. cuprina            GACCCAGCAG GAGGAGGAGA CCCAATTTTA TATCAACATT TATTTTGATT CTTTGGACAT
L. illustris          GACCCAGCAG GAGGAGGAGA CCCAATTTTA TACCAACATT TATTTTGATT CTTTGGTCAT
L. sericata "41"      GACCCTGCAG GAGGAGGAGA TCCAATTTTA TACCAACATT TATTTTGTAC CTTTGGACAC
L. sericata "17"      GACCCTGCAG GAGGAGGAGA TCCAATTTTA TACCAACATT TAT------- ----------
L. sericata "34"      GACCCTGCAG GAGGAGGAGA TCCAATTTTA TACCAACATT TATT------ ----------
L. sericata "USA"     GACCCTGCAG GAGGAGGTGA TCCAATTTTA TACCAACATT TATTTTGATT CTTTGGACAC
P. sialia             GACCCAGCAG GAGGGAGGGA CCCTATTTTA TATCAACATT TATTTTGATT TTTTGGCCAT
C. callipes           GATCCAGCAG GAGGAGGAGA TCCAATTTTA TATCAACATT TATTTTGATT CTTTGGACAT
C. macellaria         GATCCAGCCG GAGGAGGAGA TCCAATTTTA TACCAACACT TATTTTGATT CTTTGGACAC
P. regina "1"         GACCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACACT TATTCTGATT CTTTGGTCAC
P. regina "2"         GACCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACACT TATTCTGATT CTT-------
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P. terraenovae            GATCCAGCTG GAGGAGGAGA CCCTATTTTA TACCAACATT TATTTTGATT CTTCGGTCAC
C. bezziana               GACCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACACT TATTCTGATT CTTCGGACAT
C. megacephala "1"        GATCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACATT TATTTTGATT CTTTGGACAT
C. megacephala "2"        GATCCAGCAG GAGGAGGAGA TCCTATTGTA TATCAATATT TATTTTGATC CTACGAACAT
C. norrisi                GATCCAGCTG GAGGAGGAGA TCCTATTTTA TACCAACACT TATTTTGATT CTTTGGTCAT
C. varipes                GATCCAGCAG GAGGAGGTGA CCCTATTTTA TACCAACATT TATTTTGATT CTTTGGTCAT
C. inclinata              GACCCAGCAG GAGGAGGAGA CCCTTTTTTA TACCAACACT TGTTCTGATT CTTTGGACAT
C. marginalis             GATCCAGCAG GAGGAGGAGA TCCTATTTTA TACCAACA-- --TTTAGATT CTTTGG-CAT
C. semimetallica          GATCCGGCAG GAGGGGGAGA CCCAATTTTA TATCAACATT TATTTTGATT CTTTGGACAT
C. putoria "USA"          GACCCAGCAG GAGGAGGAGA TCCTATTTTA TACCAACACT TATTTTGATT CTTTGGACAT
C. putoria "SA"           GATCCAGCAG GAGGAGGAGA TCCTATTTTA TACCAACACT TATTTTGATT CTTCGGACAT
C. chloropyga             GACCCAGCAG GAGGAGGAGA CCC------- ---------- ---------- ----------
C. rufifacies             GATCCAGCAG GAGGGGGAGA CCCTATTTTA TATCAACACT TATTTTGATT CTTTGGTCAT
C. albiceps "SA"          GATCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACATT TATTTTGATC ATAAGGACAT
C. albiceps "USA"         GATCCAGCAG GAGGAGGAGA TCCTATTTTA TATCAACATT TATTTTGATT CTTTGGACAT

                                                                         780
M. domestica              CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
P. silenus                CCTGAAGTAT ATATTTTAAT TCTACCAGGA TTCGGAATAA TTTCTCATAT CATTAGTCCA
H. lineatum               CCAGAAGTTT ATATCTTAAT TTTACCAGGA TTTGGAATAA TTTCTCATAT TATTAGACAA
H. bovis                  CCAGAAATTT ATATCTTAAT TTTACCAGGA TTTGGAATAA TTTCTCATAT TATTAGACAA
O. ovis                   CCAGAAATTT ATATCTTAAT TTTACCAGGA TTTGGAATAA TTTCTCATAT TATTAGACAA
W. vigila                 CCAGAAGTTT ATATTTTAAT TCTACCTGGA TTTGGTATAA TTTCACACAT TATTAGTCAA
G. intestinalis           CCAGAAGTAT ATATTTTAAT TCTTCCGGGA TTTGGAATAA TTTCTCACAT TATTAGTCAA
B. devia                  CCAGAAGTTT ATATTTTAAT TTTACCAGGA TTTGGATTAG TTTCTCATGT TATTAGTCAA
R. lherminieri            CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTCGGAATAA TCTCTCATAT TATTAGTCAA
B. plinthopyga            CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCCCATAT TATTAGTCAA
P. chrysostoma            CCTGAAGTTT ATATTTTAAT TTTACCGGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
S. peregrina              CCTGAAGTAT ACATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
S. crassipalpis           CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTCGGAATAA TTTCTCACAT TATTAGTCAA
S. cooleyi                CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTTGGAATAA TTTCCCATAT TATTAGTCAA
S. bullata                CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTTGGAATAA TTTCCCATAT TATTAGTCAA
S. africa                 CCTGAAGTTT ACATTTTAAT TTTACCAGGA TTTGGAATAA TTTCTCACAT TATTAGTCAA
S. ruficornis             CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTCGGGATAA TTTCTCATAT TATTAGTCAA
S. argyrostoma            CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
C. cadaverina             CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
E. latifrons              CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTCGGGATAA TTTCCCATAT TATTAGTCAA
C. vicina                 ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"           ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina                CCT------- ---------- ---------- ---------- ---------- ----------
L. illustris              CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
L. sericata "41"          CCTGAAGTTT ATATTTTAAT TTTACCCGGG A--------- ---------- ----------
L. sericata "17"          ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"          ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"         CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
P. sialia                 CCTGAAGTAT ATATTTTAAT TCTACCAGGA TTTGGAATAA TTTCCCATAT TATTAGTCAA
C. callipes               CCTGAAGTAT ATATTTTAAT TTTACCTGGA TTCGGGATAA TTTCTCACAT TATTAGTCAA
C. macellaria             CCTGAAGTAT ATATTTTAAT TCTACCTGGA TTTGGAATAA TTTCACATAT TATTAGTCAA
P. regina "1"             CCTGAAGTTT ATATTTTAAT TCTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
P. regina "2"             ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae            CCTGAAGTAT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCTCATAT TATTAGTCAA
C. bezziana               CCTGAAGTTT ATATTTTAAT TTTACCAGGA TTCGGAATAA TTTCTCATAT TATTAGTCAA
C. megacephala "1"        CCTGAAGTTT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCTCATAT TATTAGTCAA
C. megacephala "2"        CCAGTAGATT ATATTTTCAT TCTACCCG-- ---------- ---------- ----------
C. norrisi                CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
C. varipes                CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCTCATAT TATTAGTCAA
C. inclinata              CCAGAAGATT ATATTTTAAT TTTTACCGGG A--------- ---------- ----------
C. marginalis             CCTGAAGTTT ATA--TTATT TTTACCGGGG G--------- ---------- ----------
C. semimetallica          CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTTGGAATAA TTTCTCATAT TATTAGTCAA
C. putoria "USA"          CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCACATAT TATTAGTCAA
C. putoria "SA"           CCAGAATTTT ATATTTTAAT TCTCCCCGGG GG-------- ---------- ----------
C. chloropyga             ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies             CCAGAAGTAT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCTCATAT CATTAGTCAA
C. albiceps "SA"          CCATAAGTTT ATATTTTCAT CCCACCCG-- ---------- ---------- ----------
C. albiceps "USA"         CCAGAAGTTT ATATTTTAAT TTTACCTGGA TTCGGAATAA TTTCTCATAT TATTAGTCAA

                                                                         840
M. domestica              GAA------- ---------- ---------- ---------- ---------- ----------
P. silenus                GAATCAGGAA AAAAAGAAAC ATTCGGATCT TTAGGAATAA TTTATGCTAT ACTAACTATT
H. lineatum               GAATCAGGAA AAAAGGAAAC TTTCGGATCA TTAGGGATAA TTTATGCAAT ATTAGCTATT
H. bovis                  GAATCAGGAA AAAAGGAAAC TTTCGGATCA TTAGGGATAA TTTATGCAAT ATTAGCTATT
O. ovis                   GAATCCGGAA AAAAGGAAAC TTTCGGATCA TTAGGGATTA ATTATGCAAT ATTAGCTATT
W. vigila                 GAA------- ---------- ---------- ---------- ---------- ----------
G. intestinalis           GAATCTGGAA AGAAGGAAAC TTTTGGAGCC CTAGGAATAA TTTACGCAAT ATTAGCTATT
B. devia                  GAA------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri      GAA------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga      GAA------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma      GAA------- ---------- ---------- ---------- ---------- ----------
S. peregrina        GAA------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis     GAA------- ---------- ---------- ---------- ---------- ----------
S. cooleyi          GAA------- ---------- ---------- ---------- ---------- ----------
S. bullata          GAA------- ---------- ---------- ---------- ---------- ----------
S. africa           GAA------- ---------- ---------- ---------- ---------- ----------
S. ruficornis       GAA------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma      GAA------- ---------- ---------- ---------- ---------- ----------
C. cadaverina       GAATCAGGAA AAAAGGAAAC TTTCGGTTCA CTAGGGATAA TTTATGCTAT ACTAGCTATT
E. latifrons        GAATCAGGAA AGAAGGAAAC TTTCGGTTCA TTAGGAATAA TTTATGCTAT ATTAGCTATT
C. vicina           ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"     ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina          ---------- ---------- ---------- ---------- ---------- ----------
L. illustris        GAATCAGGAA AGAAGGAAAC ATTCGGATCA CTAGGAATAA TTTATGCTAT ATTAGCTATT
L. sericata "41"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"   GAATCAGGTA AAAAGGAAAC ATTCGGTTCA TTAGGGATGA TTTATGCCAT ATTAGCTATT
P. sialia           GAATCAGGAA AAAAAGAAAC TTTTGGTTCA CTAGGAATAA TTTATGCTAT ACTAGCTATT
C. callipes         GAATCAGGAA AGAAGGAAAC TTTCGGATCA TTAGGAATAA TTTATGCTAT ACTAGCTATT
C. macellaria       GAATCAGGAA AGAAGGAAAC ATTCGGATCT TTAGGAATGA TTTATGCTAT ATTAGCTATT
P. regina "1"       GAATCAGGAA AGAAGGAAAC TTTTGGATCA TTAGGAATAA TTTATGCTAT ATTAGCTATT
P. regina "2"       ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae      GAATCAGGAA AGAAGGAAAC TTTCGGATCT TTAGGAATAA TTTACGCTAT ATTAGCTATT
C. bezziana         GAATCAGGAA AAAAGGAAAC TTTYGGATCC TTAGGRATAA TTTATGCTAT ACTAGCTATT
C. megacephala "1"  GAATCAGGAA AAAAGGAAAC TTTCGGATCT TTAGGAATGA TTTATGCTAT ACTAGCTATT
C. megacephala "2"  ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi          GAATCAGGTA AGAAGGAAAC TTTCGGATCT TTAGGAATAA TTTATGCTAT ATTAGCTATT
C. varipes          GAATCAGGTA AGAAGGAAAC TTTTGGATCA TTAGGAATAA TTTATGCTAT ACTAGCTATT
C. inclinata        ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis       ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica    GAATCAGGAA AAAAGGAAAC TTTCGGATCA TTAGGAATAA TTTATGCTAT ATTAGCTATT
C. putoria "USA"    GAATCAGGAA AGAAGGAAAC TTTCGGATCT TTAGGAATAA TTTATGCTAT ATTAGCTATT
C. putoria "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga       ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies       GAATCAGGAA AAAAGGAAAC CTTTGGATCT TTAGGAATAA TTTATGCAAT ATTAGCTATT
C. albiceps "SA"    ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"   GAATCAGGAA AAAAGGAAAC ATTTGGATCT TTAGGAATAA TTTATGCAAT ATTAGCTATT

                                                                    900
M. domestica        ---------- ---------- ---------- ---------- ---------- ----------
P. silenus          GGCTTACTTG GGTTTATTGT ATGAACTCAT CATATATTCA CAGTAGGTAT AGATGTTGAC
H. lineatum         GGATTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CAGTTGGAAT AGACCATGAC
H. bovis            GGATTATTAG GATTTATTGT ATGAACTCAT CATATATTTA CAGTTGGAAT AGACTGTGAC
O. ovis             GGATTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CAGTGGGAAT AGACGTTGAC
W. vigila           ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis     GGATTACTCG GATTCATTGT ATGGGCACAT CACATATTTA CTGTAGGAAT AGATGTAGAT
B. devia            ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri      ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga      ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma      ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina        ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis     ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi          ---------- ---------- ---------- ---------- ---------- ----------
S. bullata          ---------- ---------- ---------- ---------- ---------- ----------
S. africa           ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis       ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma      ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina       GGTTTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CCGTAGGAAT AGACGTTGAT
E. latifrons        GGGCTATTAG GATTCATTGT ATGAGCCCAC CATATATTCA CAGTAGGGAT AGATGTAGAC
C. vicina           ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"     ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina          ---------- ---------- ---------- ---------- ---------- ----------
L. illustris        GGATTATTAG GATTTATTGT ATGAGCACAT CACATATTTA CTGTAGGGAT AGACGTTGAT
L. sericata "41"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"   GGATTATTAG GATTTATTGT TTGAGCTCAT CATATATTTA CAGTAGGAAT AGACGTTGAT
P. sialia           GGTCTATTAG GATTTATTGT ATGAGCCCAT CATATATTTA CTGTAGGAAT AGATGTTGAT
C. callipes         GGTTTATTAG GATTTATTGT TTGAGCTCAT CATATATTTA CTGTTGGAAT AGACGTTGAT
C. macellaria       GGTTTATTAG GATTTATTGT TTGAGCTCAT CACATGTTTA CTGTTGGAAT AGACGTTGAT
P. regina "1"       GGTCTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CTGTTGGAAT AGACGTTGAT
P. regina "2"       ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae      GGATTATTAG GATTTATTGT TTGAGCTCAT CATATATTCA CCGTAGGAAT AGACGTTGAT
C. bezziana         GGTCTATTAG GATTTATTGT ATGAGCTCAT CACATATTCA CAGTTGGAAT AGACGTAGAT
C. megacephala "1"  GGTCTATTAG GATTTATTGT ATGAGCTCAC CACATGTTTA CTGTTGGAAT AGACGTAGAC
C. megacephala "2"  ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi          GGTTTATTAG GATTCATTGT ATGAGCTCAT CATATATTTA CTGTTGGAAT AGACGTAGAT
C. varipes          GGTTTATTAG GATTTATTGT ATGAGCTCAC CATATATTTA CTGTAGGAAT AGACGTAGAT
C. inclinata        ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis       ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica    GGATTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CTGTTGGAAT AGACGTTGAT
C. putoria "USA"    GGTTTATTAG GATTTATTGT ATGAGCTCAT CACATGTTTA CTGTTGGAAT AGACGTAGAT
C. putoria "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga       ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies       GGATTATTAG GATTTATTGT ATGAGCTCAT CATATATTCA CTGTAGGAAT GGATGTAGAT
C. albiceps "SA"    ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"   GGTCTATTAG GATTTATTGT ATGAGCTCAT CATATATTTA CTGTAGGAAT GGATGTAGAT

                                                                  960
M. domestica        ---------- ---------- ---------- ---------- ---------- ----------
P. silenus          ACACGAACAT ATTCCCTTTC AGCCACGATA ATCATTGCTG TCCCAACAGG AATTAAAATT
H. lineatum         ACACGAGCTT ATTTCACTTC AGCCACCATA ATTATTGCTG TACCAACTGG AATTAAAATT
H. bovis            ACACGAGCTT ATTTCACTTC AGCAACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
O. ovis             ACACGAGCTT ATTTCACTTC AGCAACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
W. vigila           ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis     ACTCGAGCTT ATTTCACTTC AGCAACAATA ATTATTGCTG TCCCAACGGG AATTAAAATT
B. devia            ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri      ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga      ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma      ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina        ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis     ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi          ---------- ---------- ---------- ---------- ---------- ----------
S. bullata          ---------- ---------- ---------- ---------- ---------- ----------
S. africa           ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis       ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma      ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina       ACACGAGCTT ATTTTACATC AGCTACTATA ATTATTGCTG TTCCAACAGG AATTAAAATT
E. latifrons        ACTCGAGCTT ATTTCACCTC AGCTACTATA ATTATTGCTG TTCCAACAGG AATTAAAATT
C. vicina           ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"     ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina          ---------- ---------- ---------- ---------- ---------- ----------
L. illustris        ACTCGAGCTT ACTTTACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
L. sericata "41"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"   ACACGAGCTT ACTTTACTTC AGCTACTATA ATTATTGCTG TACCAACTGG AATTAAGATT
P. sialia           ACTCGAGCCT ATTTTACTTC AGCTACAATA ATCATTGCCG TTCCAACTGG AATTAAAATT
C. callipes         ACACGAGCTT ATTTTACTTC AGCTACAATA ATTATTGCTG TTCCAACTGG AATTAAAATT
C. macellaria       ACTCGAGCTT ACTTCACTTC AGCTACAATA ATTATTGCTG TTCCAACTGG AATTAAGATT
P. regina "1"       ACACGAGCTT ACTTTACTTC AGCAACTATA ATTATTGCTG TACCAACTGG AATTAAAATT
P. regina "2"       ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae      ACTCGAGCTT ACTTTACTTC AGCCACAATA ATTATTGCTG TTCCAACTGG AATTAAAATT
C. bezziana         ACTCGAGCTT ACTTCACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
C. megacephala "1"  ACACGAGCTT ATTTCACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAGATT
C. megacephala "2"  ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi          ACACGAGCTT ACTTTACATC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
C. varipes          ACTCGAGCTT ACTTCACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
C. inclinata        ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis       ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica    ACTCGAGCTT ATTTTACTTC AGCTACAATA ATTATTGCCG TACCTACTGG AATTAAAATT
C. putoria "USA"    ACTCGAGCTT ACTTCACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAGATT
C. putoria "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga       ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies       ACTCGAGCAT ATTTCACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT
C. albiceps "SA"    ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"   ACTCGAGCAT ATTTTACTTC AGCTACAATA ATTATTGCTG TACCAACTGG AATTAAAATT

                                                                  1020
M. domestica        ---------- ---------- ---------- ---------- ---------- ----------
P. silenus          TTTAGATGAT TAGCAATTCT TCACGGAACA CAATTCAATT ATTCGCCAGC TACTTTATGA
H. lineatum         TTCAGAGGAT TAGCAACTTT ACATGGAACA CAATTCAACT ATTCACCCGC AACTTTATGA
H. bovis            TTCAGATGAT TAGCAACTTT ACATGGAACA CAATTCAACT ACTCTCCTGC AACTTTATGA
O. ovis             TTCAGATGAT TAGCAACTTT ACATGGAACA CAATTCAACT ACTCTCCTGC AACTTTATGA
W. vigila           ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis     TTTAGTTGAC TAGCAACATT ACACGGAACC CAGTTAAGCT ATTCCCCATC AATTCTGCGA
B. devia            ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           TTTAGTTGAT TAGCAACTCT TTATGGTACC CAATTAAATT CTTCCCCAGC TACTTTATGA
E. latifrons            TTCAGTTGAC TAGCAACTCT TTATGGTACT CAATTAAACT CTTCTCCAGC TACATTATGA
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            TTTAGTTGAT TAGCAACTCT TTATGGAACT CAATTAAATT ATTCTCCTGC TACTTTATGA
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       TTTAGTTGAT TAGCAACTCT TTATGGAACT CAATTAAACT ATTCCCCTGC TACTTTATGA
P. sialia               TTTAGATGAT TAGCAACCCT ATACGGAACT CAATTAAATT ATTCCCCATC TACTTTATGA
C. callipes             TTCAGTTGAT TAGCCACTCT TTACGGAACT CAATTAAACT ATTCACCAGC TACATTATGA
C. macellaria           TTCAGTTGAT TAGCTACTCT TTATGGAACT CAATTAAATT ATTCTCCAGC TACACTATGA
P. regina "1"           TTCAGTTGAC TAGCAACTCT TTATGGAACT CAATTAAATT ACTCTCCAGC AACTCTATGA
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          TTTAGTTGAC TAGCAACTCT TTATGGAACT CAATTAAATT ACTCCCCAGC TACTTTATGA
C. bezziana             TTTAGCTGAT TAGCAACTCT TTACGGAACT CAATTAAATT ATTCTCCAGC TACTTTATGA
C. megacephala "1"      TTCAGTTGAT TAGCAACTCT TTACGGAACA CAATTAAATT ATTCTCCAGC TACTTTATGA
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TTTAGTTGAT TAGCAACTCT TTACGGAACA CAATTAAATT ATTCTCCAGC TACTCTATGA
C. varipes              TTTAGTTGAT TAGCTACTCT TTATGGAACT CAATTAAATT ATTCACCAGC TACATTATGA
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TTTAGTTGAT TAGCAACTCT TTATGGAACT CAATTAAACT ATTCCCCAGC TACTTTATGA
C. putoria "USA"        TTCAGTTGAT TAGCTACTCT TTATGGTACT CAATTAAACT ATTCTCCAGC TACTTTATGA
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           TTTAGTTGAT TAGCAACTCT TTATGGAACT CAATTAAATT ATTCTCCAGC TACTTTATGA
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       TTTAGTTGAT TAGCAACTCT TTATGGAACA CAATTAAATT ACTCCCCAGC TACCTTATGA

                                                                      1080
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              TCTTTAGGAT TGGTTTTCCT ATTTACAGTA GGAGTTCTAA CAGGCGTTAT TTTAGCTAAT
H. lineatum             TCTTTAGGGT TTGTTTTTTT ATTTACAGTT GGAGGATTAA CTGGAGTAAT TTTAGCTAAT
H. bovis                TCTTTAGGGT TTGTTTTTTT ATTTACAGTT GGAGGATTAA GTGGAGTAAT TTTAGCTAAT
O. ovis                 TCTTTAGGGT TTGTTTTTTT ATTTACAGTT GGAGGATTAA CTGGAGTAAT TTTAGCTAAT
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         GCTCTAGGAT TTGTATTTTT ATTTACAGTT GGAGGATTAA CCGGAGTAGT GTTGGCTAAC
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           GCCTTAGGAT TTGTATTCTT ATTTACAGTA GGAGGGTTAA CTGGAGTTAT TCTAGCTAAT
E. latifrons            GCATTAGGAT TTGTATTCTT ATTTACAGTA GGAGGATTAA CTGGAGTTAT TTTAGCTAAT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            GCTTTAGGGT TTGTATTTTT ATTCACAGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAT
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       GCTTTAGGAT TTGTATTTTT ATTCACTGTA GGAGGTTTAA CTGGAGTTGT TTTAGCTAAC
P. sialia               GCATTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CAGGTGTTGT TTTAGCTAAT
C. callipes             GCATTAGGAT TCGTCTTTTT ATTCACTGTA GGGGGATTAA CTGGTGTTGT ATTAGCTAAT
C. macellaria           GCTTTAGGGT TCGTATTCTT ATTCACAGTA GGAGGATTAA CTGGTGTTGT TTTAGCTAAC
P. regina "1"           GCTTTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CTGGTGTTGT TTTAGCTAAT
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae          GCTTTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CTGGAGTTGT TCTAGCTAAC
C. bezziana             GCTTTAGGAT TCGTATTCTT ATTTACTGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAC
C. megacephala "1"      GCTTTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              GCTTTAGGAT TCGTATTTTT ATTTACTGTA GGAGGATTAA CAGGAGTTGT TTTAGCTAAT
C. varipes              GCTCTAGGAT TTGTATTTTT ATTTACTGTA GGAGGACTAA CAGGAGTTGT TTTAGCAAAC
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        GCTTTAGGAT TTGTATTTTT ATTTACAGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAT
C. putoria "USA"        GCTTTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAT
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           GCCTTAGGAT TTGTATTCTT ATTTACTGTA GGAGGATTAA CTGGAGTAGT TTTAGCTAAT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       GCTTTAGGAT TTGTATTTTT ATTTACTGTA GGAGGATTAA CTGGAGTTGT TTTAGCTAAT

                                                                     1140
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              TCTTCAATTG ACATCATCCT ACACGATACA TACTATGTGG TAGCCCATTT CCATTATGTA
H. lineatum             TCATCTATTG ATATTATTTT ACATGATACA TATTATGTAG TAGCTCACTT CCATTATGTT
H. bovis                TCATCTATGG ATATTATTTT ACATGATACA TATTATGTAG TAGCTCACTT CCATTATGTT
O. ovis                 TCATCTATTG ATATTATTTT ACATGATACA TATTATGTAG TAGCTCACTT CCATTATGTT
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         TCATCTATTG ATATTGTTCT TCATGACACT TACTATGTAG TAGCTCACTT CCATTATGTT
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           TCTTCAGTAG ATATTATTCT TCATGATACA TACTATGTAG TTGCCCATTT CCATTATGTC
E. latifrons            TCTTCAGTAG ATATTATTCT TCATGACACA TATTATGTAG TTGCTCATTT TCACTACGTT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            TCTTCAGTTG ATATTATTTT ACATGATACA TATTATGTAG TAGCTCATTT TCACTATGTA
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       TCTTCAGTTG ATATTATTTT ACATGATACA TACTATGTAG TAGCTCACTT CCATTATGTT
P. sialia               TCTTCCCTTG ATATTATACT TCATGACACA TATTATGTAG TAGCTCACTT CCATTATGTT
C. callipes             TCATCTATTG ATATTATTTT ACATGATACT TATTATGTAG TAGCCCACTT CCACTATGTA
C. macellaria           TCATCAATTG ATATTATCTT ACATGATACT TATTATGTAG TAGCTCACTT CCACTATGTA
P. regina "1"           TCATCAATCG ATATTATTCT TCATGATACA TATTATGTAG TAGCTCATTT CCACTATGTA
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          TCTTCTATTG ATATTATTTT ACACGATACA TATTATGTAG TAGCTCATTT CCACTATGTA
C. bezziana             TCATCAGTTG ATATTATTTT ACATGATACA TATTATGTAG TTGCCCATTT CCATTATGTA
C. megacephala "1"      TCATCAATTG ACATTATTTT ACATGATACA TATTATGTAG TAGCTCACTT CCATTATGTT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TCTTCTATTG ATATTATTTT ACATGATACT TACTATGTAG TAGCTCACTT CCACTATGTA
C. varipes              TCTTCTATTG ATATTATTTT ACATGATACA TATTATGTAG TAGCCCACTT CCATTATGTT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TCTTCTGTTG ATATTATTCT ACATGACACA TATTATGTAG TTGCCCACTT CCATTATGTA
C. putoria "USA"        TCTTCAGTTG ATATTATTCT TCATGACACA TATTATGTAG TAGCTCATTT CCACTATGTA
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           TCATCTATTG ATATTATTTT ACATGACACA TACTATGTAG TAGCTCACTT CCATTATGTT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       TCATCTATTG ATATTATTTT ACATGATACA TATTATGTAG TAGCTCACTT CCATTATGTT

                                                                     1200
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              CTATCTATAG GAGCTGTATT CGCTATCATA GCTGGATTTA TTCATTGATT TCCATTATTC
H. lineatum             TTATCTATAG GAGCTGTATT TGCCATTATA GCTGGATTCA TTCATTGATT TCCATTATTT
H. bovis                TTATCTATAG GAGCTGTATT TGCCATTATA GCTGGATTCA TTCATTGATT TCCATTATTT
O. ovis                 TTATCTATAG GAGCTGTATT TGCCATTATA GCTGGATTCA TTCATTGATT TCCATTATTT
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         CTATCAATAG GAGCTGTATT TGCTATTATA GCCGGTTTTA TTCATTGATT CCCACTATTT
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           CTATCTATAG GAGCTGTATT CGCTATTATA GCAGGATTTG TACACTGATA CCCTTTATTT
E. latifrons            CTATCTATAG GAGCAGTATT TGCAATTATA GCAGGATTTG TTCACTGATA CCCTTTATTT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            TTATCAATAG GAGCTGTATT TGCTATTATA GCAGGATTCG TTCATTGATA TCCTCTATTT
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       TTATCAATGG GAGCTGTATT TGCTATTATA GCAGGATTTG TTCACTGATA TCCTTTATTT
P. sialia               TTATCTATAG GAGCTGTATT TGCAATTATA GCAGGGTTTG TACATTGATT CCCATTATTT
C. callipes             TTATCAATAG GAGCAGTATT TGCTATTATA GCAGGATTTG TTCACTGATA CCCTTTATTT
C. macellaria           TTATCTATAG GAGCAGTATT TGCTATTATA GCAGGATTTG TACACTGATA CCCTCTATTT
P. regina "1"           TTATCAATAG GAGCTGTATT TGCTATTATA GCTGGATTTG TCCACTGATT CCCTTTATTT
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          TTATCAATAG GAGCTGTCTT TGCTATTATA GCTGGATTTG TTCATTGATT CCCTTTATTT
C. bezziana             TTATCAATAG GAGCTGTATT CGCTATTATA GCAGGATTTG TTCATTGATT CCCTTTATTT
C. megacephala "1"      CTATCAATGG GAGCTGTATT TGCTATTATA GCAGGATTTG TTCATTGATT CCCTCTATTT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TTATCAATAG GAGCTGTATT TGCTATTATA GCTGGATTTG TTCACTGATT CCCTTTATTT
C. varipes              CTTTCTATAG GAGCTGTATT TGTTATTATA GCTGGTTTCG TTCATTGATT CCCTTTATTT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TTATCAATAG GAGCTGTATT TGCTATTATA GCAGGATTTG TTCATTGATT TCCTTTATTT
C. putoria "USA"        TTATCAATAG GAGCTGTATT CGCTATTATA GCAGGGTTTG TTCATTGATT CCCTCTATTT
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           CTTTCAATAG GAGCTGTATT TGCTATTATA GCAGGATTTG TACATTGATT CCCATTATTT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       CTTTCAATAG GAGCTGTATT CGCTATTATA GCAGGATTCG TTCATTGATT CCCATTATTT

                                                                    1260
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              TCTGGATTAA CGTTAAACGA AAAGTTATTA AAAAATCAAT TTATTATTAT ATTCTTAGGA
H. lineatum             ACAGGATTAA CATTAAATGT TAAATTATTA AAAAGCCAAT TTGTCATTAT ATTTTTAGGA
H. bovis                ACAGGATTAA CATTAAATGT TAAACTATTA AAAAGCCAAT TTGTCATTAT ATTTTTAGGA
O. ovis                 ACAGGATTAA CATTAAATGT TAAATTATTA AAAAGCCAAT TTGTCATTAT ATTTTTAGGA
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ACAGGATTAA CCTTAAACAA TAAATGGTTA AAAAGTCAAT TTATTACAAT ATTTATTGGA
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           ACAGGATTAA CTTTAAATGG TAAAATGTTA AAAAGTCAAT TTACTATTAT ATTTATTGGA
E. latifrons            ACAGGATTAA CTTTAAATGG AAAAATACTA AAAAGTCAAT TTACTATTAT ATTTATTGGA
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            ACAGGACTAA CTTTAAATGC AAAGATGTTA AAGAGTCAAT TTACTATTAT ATTTATTGGA
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       ACAGGATTAA CTTTAAATAC TAAGATATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGG
P. sialia               ACCGGATTAA CTCTTAATAA TAAATTATTA AAAAGTCAAT TTACCATTAT ATTTATTGGA
C. callipes             ACTGGATTAA CTTTAAATAG AAAGTTATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA
C. macellaria           ACAGGATTAA CTTTAAATAG AAAGTTATTA AAGAGTCAAT TTGCTATTAT ATTTATTGGG
P. regina "1"           ACTGGATTAA CATTAAATAA TAAGTTATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGG
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae          ACTGGACTAA CATTAAATAA CAAGTTATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGT
C. bezziana             ACTGGATTGA CTTTAAACGG TAAATTATTA AAAAGTCAAT TTACTATTAT ATTTATTGGA
C. megacephala "1"      ACTGGATTAA CTTTAAATAG CAAGTTATTA AAGAGTCAAT TTGCTATTAT ATTTATCGGA
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              ACTGGATTAA CTTTAAATAG TAAGTTACTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA
C. varipes              ACTGGATTAA CTCTAAATAG TAAGTTATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        ACTGGATTAA CTTTAAACGG TAAGATATTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA
C. putoria "USA"        ACTGGATTAA CTCTAAATGG TAAGATATTA AAGAGTCAAT TTGCTATTAT ATTTATTGGA
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           ACTGGATTAA CCTTAAATAA TAAAATACTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       ACTGGATTAA CTCTAAATAA TAAAATACTA AAAAGTCAAT TTGCTATTAT ATTTATTGGA

                                                                     1320
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              GTAAACCTAA CCTTTTTTCT TCAACACTTC ATAGGTCTGG CTGGGATACC ACGACGTTAT
H. lineatum             GTAAATTTAA CCTTTTTCCT TCAACACTTT TTAGGGTTAG CTGGCATACC TCGACGTTAT
H. bovis                GTAAATTTAA CCTTTTTCCT TCAACACTTT TTAGGGTTAG CTGGCATACT TCGACGTTAT
O. ovis                 GTAAATTTAA CCTTTTTCCT TCAACACTTT TTAGGGTTAG CTGGCATACC TCGACGTTAT
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         GTAAACATAA CTTTCTTCCC CCAGCATTTC CTTGGGTTAG CTGGAATACT TCGACGATAC
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           GTTAATATTA CATTCTTCCC TCAACATTTC TTAGGGTTAG CAGGAATACC TCGACGATAC
E. latifrons            GTTAATATTA CATTTTTCCC CCAACATTTC TTAGGATTAG CGGGAATACC ACGACGATAT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            GTAAATTTAA CTTTCTTCCC TCAACATTTT TTAGGACTAG CAGGAATACC GCGACGATAC
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGATTAG CAGGAATACC ACGACGATAT
P. sialia               GTAAATTTAA CATTTTTCCC TCAACACTTC TTAGGGCTAG CTGGAATACC ACGACGATAC
C. callipes             GTAAATTTAA CTTTCTTTCC TCAACATTTC TTAGGATTAG CTGGAATACC TCGACGATAT
C. macellaria           GTAAATTTAA CTTTCTTCCC TCAACACTTC TTAGGATTAG CAGGTATACC TCGACGATAC
P. regina "1"           GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGATTAG CTGGAATGCC TCGACGATAC
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          GTAAATTTAA CCTTCTTCCC TCAACATTTC TTAGGATTAG CTGGAATACC TCGACGATAC
C. bezziana             GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGATTAG CAGGAATACC TCGACGATAC
C. megacephala "1"      GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGATTAG CAGGTATACC TCGACGATAC
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGGTTAG CTGGTATACC TCGACGATAT
C. varipes              GTAAATTTAA CATTCTTCCC ACAACACTTC CTAGGATTAG CTGGAATACC TCGACGATAT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGACTAG CAGGTATACC TCGACGATAC
C. putoria "USA"        GTAAATTTAA CATTCTTCCC TCAACATTTC TTAGGATTAG CAGGAATACC TCGACGATAT
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           GTAAATTTAA CATTCTTCCC TCAACATTTT TTAGGACTAG CTGGTATACC TCGACGATAC
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       GTAAATTTAA CATTCTTTCC TCAACATTTT TTAGGATTAG CTGGTATACC TCGACGATAC

                                                                     1380
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              TCAGATTATC CTGATGCATA CACAACATGA AATGTAATCT CAACTATAGG TTCATCAATC
H. lineatum             TCTGATTATC CAGATGCATA TACCACATGA AATGTAATTT CAACTATTGG ATCATCAATT
H. bovis                TCTGATTATC CAGATGCTTA TACCACATGA AATGTAATTT CAACTATTGG ATCATCAATT
O. ovis                 TCTGATTATC CAGATGCTTA TACCACATGA AATGTAATTT CAACTATTGG ATCATCAATT
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         TCTGATTATC CAGATGCATA CACTACTTGA AACGTAATTT CATCTATCGG ATCAACAATT
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri        ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga        ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma        ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina          ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis       ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi            ---------- ---------- ---------- ---------- ---------- ----------
S. bullata            ---------- ---------- ---------- ---------- ---------- ----------
S. africa             ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis         ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma        ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina         TCAGATTACC CTGATGCTTA CACTACTTGA AATGTAATTT CTACTATTGG GTCAACAATC
E. latifrons          TCAGATTACC CTGATGCTTA CACAACTTGA AATGTAGTTT CTACTATTGG GTCAACAATC
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          TCAGATTACC CAGATGCTTA CACAACTTGA AATGTAATTT CTACAATTGG GTCAACAATT
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     TCAGACTACC CAGATGCTTA CACAACTTGA AATGTAATTT CTACAATTGG GTCAACAATT
P. sialia             TCAGATTATC CAGATGCTTA TACAACTTGA AATGTAATTT CAACTATTGG TTCAACAATT
C. callipes           TCAGATTATC CAGATGCTTA TACAACTTGA AATGTAATCT CTACAATTGG TTCAACAATT
C. macellaria         TCAGATTACC CAGATGCTTA TACTACTTGA AATGTAATCT CTACAATTGG TTCAACAATT
P. regina "1"         TCAGATTACC CAGATGCTTA CACGGCTTGA AACGTAATCT CTACAATTGG TTCAACAATC
P. regina "2"         ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae        TCTGACTATC CAGATGCTTA CACAGCTTGA AATGTAATTT CTACTATTGG TTCAACAATT
C. bezziana           TCAGATTATC CAGATGCTTA CACAACTTGA AATGTAATCT CTACAATTGG TTCAACAATT
C. megacephala "1"    TCAGACTATC CAGACGCTTA CACAGCTTGA AATGTAATTT CTACAATTGG TTCAACAATT
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            TCTGATTATC CAGATGCTTA CACAGCTTGA AATGTAATTT CTACAATTGG TTCAACAATT
C. varipes            TCAGATTATC CAGATGCTTA CACAGCTTGA AATGTAATTT CTACAATTGG ATCAACAATT
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      TCAGATTATC CAGATGCTTA TACAGCTTGA AATGTAATTT CTACAATTGG TTCAACAATT
C. putoria "USA"      TCAGACTATC CAGATGCTTA CACAGCTTGA AATGTAATTT CTACAATTGG TTCAACAATT
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         TCAGACTATC CAGATGCTTA CACAACATGA AATGTTATTT CAACAATTGG ATCAACAATT
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     TCAGATTACC CAGATGCTTA TACAGCATGA AATGTTATCT CAACAATTGG GTCAACAATT

                                                                     1440
M. domestica          ---------- ---------- ---------- ---------- ---------- ----------
P. silenus            TCTTTATTAA GAATTGTATT ATTTCTATTC ATTATCTGGG AAAGATTAGT ATCCCAACGT
H. lineatum           TCTCTTTTAA GTATTTTATT ATTTCTATTT ATTATTTGAG AAAGATTACT ATCACAACGA
H. bovis              TCTCTTTTAA GTATTTTATT ATTTCTATTT ATTATTTGAG AAAGATTACT ATCACAACGA
O. ovis               TCTCTTTTAA GTATTTTATT ATTTCTATTT ATTATTTGAG AAAGATTACT ATCACAACGA
W. vigila             ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis       TCGCTAATAA GAATTATATT CTTCCTATTC ATCATTTGAG AAGGATTTAT TACTCAACGC
B. devia              ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri        ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga        ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma        ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina          ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis       ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi            ---------- ---------- ---------- ---------- ---------- ----------
S. bullata            ---------- ---------- ---------- ---------- ---------- ----------
S. africa             ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis         ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma        ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina         TCATTACTAG GAATTTTATT TTTCTTTTTC ATTATCTGAG AAAGTTTAGT TTCACAACGT
E. latifrons          TCATTATTAG GAATTCTATT TTTCTTTTTT ATTATTTGAG AAAGTTTAGT TTCACAACGT
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          TCCCTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTCTTGT AACTCAACGT
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     TCTTTATTAG GAATTTTATT CTTCTTCTTT ATTATTTGAG AAAGTCTTGT ATCTCAACGT
P. sialia             TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATCTGAG AAAGTCTAAT ATCTCAACGT
C. callipes           TCATTACTAG GAATTTTATT CTTCTTTTTC ATTATTTGAG AAAGTTTAGT AACTCAACGA
C. macellaria         TCATTACTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT TACTCAACGA
P. regina "1"         TCATTATTAG GAATTTTATT TTTCTTTTTT ATTATTTGAG AAAGTTTAGT ATCTCAACGT
P. regina "2"         ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae        TCATTACTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCACAACGT
C. bezziana           TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA
C. megacephala "1"    TCATTATTAG GAATTTTATT CTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT TTCTCAACGA
C. varipes            TCATTACTAG GAATTTTATT TTTCTTCTTC ATTATTTGAG AAAGTTTAGT TTCTCAACGA
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA
C. putoria "USA"      TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     TCATTATTAG GAATTTTATT TTTCTTTTTC ATTATTTGAG AAAGTTTAGT ATCTCAACGA

                                                                  1500
M. domestica          ---------- ---------- ---------- ---------- ---------- ----------
P. silenus            TACGTATTAT TCTCTATTCA ATTAAATTCT TCAATTGAAT GATTACAAAA CACTCCACCA
H. lineatum           CAAGTATTAT TTCCTATCCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACACCACCC
H. bovis              CAAGTATTAT TTCCTATCCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACACCACCC
O. ovis               CAAGTATTAT TTCCTATCCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACACCGCCC
W. vigila             ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis       CAAGTAATCT TCCCTATCCA ACTTAGTTCA TCAATCGAAT GGTTACAAAA TACACCCCCT
B. devia              ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri        ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga        ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma        ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina          ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis       ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi            ---------- ---------- ---------- ---------- ---------- ----------
S. bullata            ---------- ---------- ---------- ---------- ---------- ----------
S. africa             ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis         ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma        ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina         CAAGTTCTAT ACCCTGTTCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACTCCGCCA
E. latifrons          CAAGTTTTAT ACCCTGTTCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACCCCACCA
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          CAAGTTTTAT TCCCTGTTCA ATTAAATTCA TCAATTGAAT GACTACAAAA TACTCCACCA
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     CAAGTTTTAT TCCCTGTTCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACTCCACCA
P. sialia             CAAGTATTAT TCCCTATACA ATTAAATTCA TCAATTGAAT GACTACAAAA TACCCCACCA
C. callipes           CAAGTATTAT TCCCTGTTCA ATTAAATTCA TCAATTGAAT GACTACAAAA TACTCCACCA
C. macellaria         CAAGTATTAT TCCCAGTTCA ATTAAATTCA TCAATTGAAT GACTACAAAA TACTCCACCA
P. regina "1"         CAAGTTTTAT TCCCTGTACA ATTAAATTCA TCTATTGAAT GATTACAAAA TACTCCACCA
P. regina "2"         ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae        CAAGTTTTAT TCCCTGTCCA ATTAAATTCA TCAATTGAAT GATTACAAAA TACTCCACCA
C. bezziana           CGAGTTTTAT TTCCTGTTCA ACTAAATTCA TCAATTGAAT GGTTACAAAA TACTCCACCT
C. megacephala "1"    CGAGTTTTAT TCCCTGTTCA ACTAAATTCA TCAATTGAAT GATTACAAAA TACTCCACCA
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            CGAGTTTTAT TCCCTGTTCA ATTAAATTCA TCAATTGAAT GACTACAAAA TACTCCACCA
C. varipes            CGAGTTTTAT TCCCTGTTCA ACTAAATTCA TCAATTGAAT GACTACAAAA TACTCCACCA
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      CGAGTTTTAT TCCCTGTTCA ACTAAATTCT TCAATTGAAT GATTACAAAA TACTCCACCA
C. putoria "USA"      CGAGTTTTAT TCCCTGTACA ACTAAATTCA TCAATTGAAT GATTACAAAA TACTCCACCA
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         CAAGTTCTAT TTCCTATTCA ATTAAATTCA TCAATTGAAT GACTTCAAAA TACTCCTCCA
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     CAAGTTTTAT TTCCTATTCA ATTAAATTCA TCAATTGAAT GACTTCAAAA TACTCCTCCA

                                                                  1560
M. domestica          ---------- ---------- ---------- ---------- ---------- ----------
P. silenus            TCTGAACATT CATACTCTGA ACTCCCTTTA TTAACTAATT TCTAA----- ----------
H. lineatum           TCTGAACACT CTTATTCTGA ACTCCCACTA TTAACTAATT TCTAA----- ----------
H. bovis              TCTGAACACT CTTATTCTGA ACTCCCACTA TTAACTAATT TCTAA----- ----------
O. ovis               TCTGAACACT CTTATTCTGA ACTCCCATTA TTAACTAATT TCTAA----- ----------
W. vigila             ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis       TCCGAACACT GCTATTCTGA ACTTCCTTTA CTAACTAACT TCTAA----- ----------
B. devia              ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri      ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga      ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma      ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina        ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis     ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi          ---------- ---------- ---------- ---------- ---------- ----------
S. bullata          ---------- ---------- ---------- ---------- ---------- ----------
S. africa           ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis       ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma      ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina       GCTGAACATA GTTATTCTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
E. latifrons        GCCGAACATA GTTATTCTGA ATTGCCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. vicina           ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"     ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina          ---------- ---------- ---------- ---------- ---------- ----------
L. illustris        GCTGAACATA GTTATTCTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
L. sericata "41"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"   GCTGAACATA GTTATTCTGA ATTGCCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
P. sialia           TCTGAACACA GTTATAATGA ATTGCCCTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. callipes         GCTGAACACA GTTATAGTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
C. macellaria       GCTGAACACA GTTATAGTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
P. regina "1"       GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
P. regina "2"       ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae      GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. bezziana         GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. megacephala "1"  GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. megacephala "2"  ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi          GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. varipes          GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. inclinata        ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis       ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica    GCTGAACACA GTTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. putoria "USA"    GCTGAACACA GCTATAGTGA ATTACCTTTA TTAACTAACT TCTAATATGG CAGATTAGTG
C. putoria "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga       ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies       GCTGAACATA GTTATAGTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG
C. albiceps "SA"    ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"   GCTGAACATA GTTATAGTGA ATTACCTTTA TTAACTAATT TCTAATATGG CAGATTAGTG

                                                                      1620
M. domestica        ---------- ---------- ---------- ---------- ---------- ----------
P. silenus          ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum         ---------- ---------- ---------- ---------- ---------- ----------
H. bovis            ---------- ---------- ---------- ---------- ---------- ----------
O. ovis             ---------- ---------- ---------- ---------- ---------- ----------
W. vigila           ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis     ---------- ---------- ---------- ---------- ---------- ----------
B. devia            ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri      ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga      ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma      ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina        ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis     ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi          ---------- ---------- ---------- ---------- ---------- ----------
S. bullata          ---------- ---------- ---------- ---------- ---------- ----------
S. africa           ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis       ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma      ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina       CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
E. latifrons        CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTG TTAGAAA--- ----------
C. vicina           ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"     ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina          ---------- ---------- ---------- ---------- ---------- ----------
L. illustris        CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAT--- ----------
L. sericata "41"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"    ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"   CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAT--- ----------
P. sialia           CAATGGATTT AAGCTTCATA TATAAAGTAT TTTACTTTTA TTAGAAATAA ATTTTATATT
C. callipes         CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. macellaria       CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAA---- ----------
P. regina "1"       CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
P. regina "2"       ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae          CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. bezziana             CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. megacephala "1"      CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. varipes              CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAT--- ----------
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. putoria "USA"        CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAA--- ----------
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAT--- ----------
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       CAATGGATTT AAGCTCCATA TATAAAGTAT TTTACTTTTA TTAGAAT--- ----------

                                                                     1680
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           ----ATAAAT GTCAACATGA TCAAATTTAG GTTTACAAGA TAGTTCTTCC CCTTTGATAG
E. latifrons            ----ATAAAT GTCAACATGA TCAAATTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            -TAAATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       ---AATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
P. sialia               ATAAATAAAT GGCAACATGA GCAAATTTAA GCTTACAAGA CAGTTCTTCT CCTCTAATAG
C. callipes             ????ACTAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
C. macellaria           ????ACTAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCC CCTTTAATAG
P. regina "1"           ----ATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          ----ATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCT CCTTTAATAG
C. bezziana             ----ATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA CAGTTCTTCT CCATTAATAG
C. megacephala "1"      ----ATAAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCT CCATTAATAG
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              -----TAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCC CCATTAATAG
C. varipes              --AAATAAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCT CCATTAATAG
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        ----ATAAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCT CCATTAATAG
C. putoria "USA"        ---AATAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCT CCATTAATAG
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           ----ACAAAT GTCAACATGA GCAAGTTTAG GTTTACAAGA TAGTTCTTCA CCATTAATAG
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       ----ACAAAT GTCAACATGA GCAAATTTAG GTTTACAAGA TAGTTCTTCA CCATTAATAG

                                                                     1740
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri       ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga       ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma       ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina         ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis      ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi           ---------- ---------- ---------- ---------- ---------- ----------
S. bullata           ---------- ---------- ---------- ---------- ---------- ----------
S. africa            ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis        ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma       ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina        AACAATTAGT CTTTTTTCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTATTCTAG
E. latifrons         AACAATTAGT CTTTTTTCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. vicina            ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"      ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina           ---------- ---------- ---------- ---------- ---------- ----------
L. illustris         AACAATTAAT CTTTTTCCAC GATCACGCAC TTTTAATTTT AGTAATAATT ACTGTTCTTG
L. sericata "41"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"    AACAATTAAT CTTTTTCCAT GATCACGCAC TTTTAATTTT AGTAATAATT ACTGTACTTG
P. sialia            AACAACTAAT CTTTTTTCAT GATCATGCAC TTTTAATTTT ATTAATAATT ACTATTTTAG
C. callipes          AACAATTAAT CTTCTTTCAC GATCATGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. macellaria        AACAATTAAT TTTCTTTCAC GATCATGCCC TTTTAATTTT AGTAATAATT ACTGTCCTAG
P. regina "1"        AACAATTAAT TTTCTTCCAT GACCATGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
P. regina "2"        ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae       AACAATTAAT CTTTTTTCAT GACCATGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. bezziana          AACAATTAAT CTTTTTTCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. megacephala "1"   AACAATTAAT CTTTTTCCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. megacephala "2"   ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi           AACAATTAAT CTTTTTCCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTTTTAG
C. varipes           AACAATTAAT CTTTTTTCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTATTAG
C. inclinata         ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis        ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica     AACAATTAAT CTTCTTCCAC GACCACGCAC TTTTAATTTT AGTTATAATT ACTGTTTTAG
C. putoria "USA"     AACAATTAAT CTTTTTCCAT GACCATGCAC TTTTAATTTT AGTAATAATT ACTGTTCTAG
C. putoria "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga        ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies        AACAATTAAT CTTTTTTCAT GATCACGCAC TTTTAATTTT AGTAATAATT ACTGTTTTAG
C. albiceps "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"    AACAATTAAT CTTTTTCCAT GACCACGCAC TTTTAATTTT AGTAATAATT ACTGTACTAG

                                                                      1800
M. domestica         ---------- ---------- ---------- ---------- ---------- ----------
P. silenus           ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum          ---------- ---------- ---------- ---------- ---------- ----------
H. bovis             ---------- ---------- ---------- ---------- ---------- ----------
O. ovis              ---------- ---------- ---------- ---------- ---------- ----------
W. vigila            ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis      ---------- ---------- ---------- ---------- ---------- ----------
B. devia             ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri       ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga       ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma       ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina         ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis      ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi           ---------- ---------- ---------- ---------- ---------- ----------
S. bullata           ---------- ---------- ---------- ---------- ---------- ----------
S. africa            ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis        ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma       ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina        TAGGGTACTT AATATTTATA TTATTTTTTA ACAAATATGT AAATCGATAC CTACTCCATG
E. latifrons         TAGGGTACTT AATATTTATA CTATTTTTTA ATAAGTATGT AAATCGATAC TTACTACATG
C. vicina            ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"      ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina           ---------- ---------- ---------- ---------- ---------- ----------
L. illustris         TAGGATATTT AATATTTATA TTATTTTTTA ACAAATATGT AAATCGATAT TTATTACATG
L. sericata "41"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"    TAGGATACTT AATGTTTATA TTATTTTTTA ACAAATATGT AAATCGATAT TTATTACACG
P. sialia            TAGGATACCT AATATTTATG TTATTTTTTA ATAAATATAT CAATCGATAT CTATTACATG
C. callipes          TAGGTTATTT AATATTTATA TTATTTTTTA ATAAGTATGT AAATCGTTAT TTACTACACG
C. macellaria        TAGGTTATTT AATATTTATA TTATTTTTTA ATAAGTATGT AAATCGTTAT TTATTACATG
P. regina "1"        TAGGTTATTT AATATTTATA TTATTCTTTA ATAAATATGT AAATCGATAT CTACTCCATG
P. regina "2"        ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae          TAGGTTATTT AATATTTATA CTATTTTTCA ACAAATATGT AAATCGATAC TTACTTCATG
C. bezziana             TAGGTTATTT AATATTTATA CTATTTTTTA ACAAATATGT AAATCGATAT TTACTTCACG
C. megacephala "1"      TAGGTTATTT AATATTTATG TTATTTTTTA ATAAGTACGT AAATCGATAT TTACTTCACG
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TAGGTTATTT AATATTTATG TTATTTTTTA ATAAATACGT AAATCGATAT TTACTTCACG
C. varipes              TAGGTTATTT AATATTTATA CTATTTTTTA ACAAATATGT AAATCGATAC TTACTACACG
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TAGGTTATTT AATATTTATA TTATTTTTTA ATAAGTATGT AAATCGATAT CTACTTCACG
C. putoria "USA"        TAGGTTACTT AATATTTATA TTATTTTTTA ACAAATATGT AAATCGATAC TTACTTCATG
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           TAGGTTATCT AATATTCATA TTATTTTTTA ATAAATACGT AAATCGATAT TTACTTCACG
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       TAGGTTATCT AATATTTATA TTATTTTTTA ATAAATATGT AAATCGATAT TTACTTCACG

                                                                      1860
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           GACAAACTAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTATTA TTTATTGCAT
E. latifrons            GACAAACTAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTACTA TTTATTGCAT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            GACAAACTAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTATTA TTTATTGCTT
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
P. sialia               GACAAACTAT CGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATCGCTT
C. callipes             GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
C. macellaria           GACAAACTAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTATTA TTTATTGCTT
P. regina "1"           GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTGTTA TTTATTGCTT
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
C. bezziana             GACAAACTAT TGAAATTATT TGAACAATTC TACCTGCAAT TATTTTATTA TTTATTGCTT
C. megacephala "1"      GACAAACTAT TGAAATTATT TGAACAATTC TACCTGCAAT TATTTTATTA TTTATTGCTT
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
C. varipes              GTCAAACTAT TGAAATTATT TGAACAATTC TACCAGCAAT TATTTTATTA TTTATTGCTT
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        GACAAACTAT TGAAATTATT TGAACTATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
C. putoria "USA"        GACAAACTAT TGAAATTATT TGAACAATTT TACCTGCAAT TATTTTATTA TTTATTGCTT
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           GACAAACTAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTATTA TTTATTGCTT
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       GACAAACCAT TGAAATTATT TGAACAATTT TACCAGCAAT TATTTTATTA TTTATTGCTT

                                                                      1920
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           TCCCTTCCCT TCGACTTTTA TACTTATTAG ATGAAATTAA TGAACCTTCT ATTACATTAA
E. latifrons            TCCCCTCTCT CCGACTTTTA TATTTATTAG ATGAAATTAA TGAACCTTCT ATTACTTTAA
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            TTCCTTCTCT TCGATTATTA TACTTACTTG ATGAAATTAA TGAACCTTCA ATTACTTTAA
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       TCCCTTCTCT TCGACTTTTA TATTTACTTG ATGAAATTAA TGAACCTTCA ATTACTTTAA
P. sialia               TACCTTCATT ACGACTTTTA TATTTATTAG ACGAAATTAA TGAACCTTCT ATTACTCTTA
C. callipes             TTCCTTCTTT ACGACTTTTA TATTTATTAG ATGAAATTAA TGAACCTTCT ATTACTTTAA
C. macellaria           TCCCTTCTTT ACGACTTTTA TATTTATTAG ATGAAATTAA TGAACCTTCA ATTACTTTAA
P. regina "1"           TTCCTTCTTT ACGACTTCTT TACTTACTAG ATGAAATTAA TGAACCTTCA ATTACTTTAA
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          TTCCTTCTCT ACGACTTTTA TACTTATTAG ATGAAATTAA TGAACCTTCA ATTACCTTAA
C. bezziana             TTCCTTCTTT ACGACTTTTA TACTTATTAG ATGAAATTAA TGAACCTTCA ATTACTTTAA
C. megacephala "1"      TTCCTTCTTT ACGACTTTTA TACTTATTAG ATGAAATCAA TGAACCTTCA ATTACTTTAA
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              TCCCTTCTTT ACGACTTTTA TACTTATTAG ATGAAATTAA TGAACCTTCA ATTACTTTAA
C. varipes              TCCCTTCTTT ACGACTTTTA TATTTATTAG ATGAAATTAA TGAACCTTCA ATTACTTTAA
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        TCCCTTCCTT ACGACTTTTA TATTTACTTG ATGAAATTAA TGAACCCTCA ATTACTTTAA
C. putoria "USA"        TCCCTTCTCT TCGACTTTTA TATTTATTAG ACGAAATTAA TGAACCTTCA ATTACTTTAA
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           TTCCTTCTTT ACGATTATTA TATCTATTAG ATGAAATTAA CGAACCCTCA ATTACTTTAA
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       TTCCTTCTTT ACGATTATTA TATTTATTAG ATGAAATTAA TGAACCTTCT ATTACTTTAA

                                                                          1980
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           AGGCAATTGG ACATC----- ---------- ---------- ---------- ----------
E. latifrons            AGGCAATCGG ACACCAATGA TATTGAAGTT ACGAATATTC AGATTTTGCA AATGTTGAAT
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            AGGCAATTGG ACATCAATGA TACTGAAGTT ATGAATATTC AGACTTTACA AATATTGAAT
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       AGGCAATTGG TCATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCA AATATTGAAT
P. sialia               AAGCAATTGG ACATCAATGA TATTGAAGCT ACGAATATTC AGACTTTACA AATATTGAAT
C. callipes             AGGCAATTGG TCATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AACATTGAAT
C. macellaria           AGGCTATTGG TCATCAATGA TATTGAAGTT ATGAATATTC TGATTTTGCA AATATTGAAT
P. regina "1"           AGGCAATTGG ACATCAATGA TATTGAAGTT ATGAATATTC TGATTTTGCA AACATTGAGT
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae        AGGCAATTGG ACACCAATGA TACTGAAGTT ATGAATATTC AGATTTTGCA AATATTGAAT
C. bezziana           AGGCTATTGG TCATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AACATTGAAT
C. megacephala "1"    AGGCTATTGG ACATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AATATTGAAT
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            AGGTTATTGG ACACCAATGA TATTGAAGTT ATGAATATTC AGATTTCGCA AATATTGAAT
C. varipes            AGGCTATTGG ACACCAATGA TACTGAAGTT ATGAATATTC AGATTTCGCA AATATTGAAT
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      AGGCTATTGG TCATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCA AATATTGAAT
C. putoria "USA"      AGGCAATTGG TCACCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AATATTGAAT
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         AGGCAATTGG ACATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AATATTGAAT
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     AGGCAATTGG ACATCAATGA TATTGAAGTT ATGAATATTC AGATTTTGCT AATATTGAAT

                                                                       2040
M. domestica          ---------- ---------- ---------- ---------- ---------- ----------
P. silenus            ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum           ---------- ---------- ---------- ---------- ---------- ----------
H. bovis              ---------- ---------- ---------- ---------- ---------- ----------
O. ovis               ---------- ---------- ---------- ---------- ---------- ----------
W. vigila             ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis       ---------- ---------- ---------- ---------- ---------- ----------
B. devia              ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri        ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga        ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma        ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina          ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis       ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi            ---------- ---------- ---------- ---------- ---------- ----------
S. bullata            ---------- ---------- ---------- ---------- ---------- ----------
S. africa             ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis         ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma        ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina         ---------- ---------- ---------- ---------- ---------- ----------
E. latifrons          TTGACTCTTA TATAATTCCC ACTAATGAAT TATCAATTGA TAGTTTTCGT TTATTAGATG
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          TTGATTCATA CATAATTCCT ACAAATGAAT TATCAATTGA TAGATTCCGT TTATTAGACG
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     TCGATTCATA TATAATTCCT ACTAACGAAT TATCAATTGA TAGCTTTCGT TTATTAGATG
P. sialia             TTGACTCTTA TATAATTCCT TCAAATGAAT TATTAACAGA TAGTTTCCGT CTATTAGACG
C. callipes           TTGATTCTTA TATAATTCCT ACAAATGAAC TATCAATTGA TAGCTTCCGT TTATTAGATG
C. macellaria         TTGATTCTTA TATAATTCCT ACAAATGAAT TATCAGTTGA TAGTTTCCGT TTACTAGATG
P. regina "1"         TTGATTCTTA TATAATTCCT ACAAATGAAT TATCAATTGA TAGTTTTCGT TTATTAGACG
P. regina "2"         ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae        TTGATTCTTA TATAATTCCT ACAAATGAAT TATCAATTGA TAGTTTCCGT TTATTAGACG
C. bezziana           TTGATTCATA TATAATCCCT ACTAACGAAT TATCAATTGA CAGCTTCCGT CTATTAGATG
C. megacephala "1"    TTGATTCATA CATAATTCCT ACTAACGAAC TATCAATTGA TAGCTTCCGT CTACTAGACG
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            TTGACTCTTA TATAATCCCT ACTAATGAAT TAGCAGTTGA TGGATTTCGT TTATTAGATG
C. varipes            TTGATTCTTA TATAATTCCT ACTAATGAAT TAGCAGTTGA TGGATTTCGT TTATTAGACG
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      TTGATTCTTA CATAATTCCT ACTAATGAAT TATCAATTGA TAACTTCCGT TTATTAGACG
C. putoria "USA"      TTGATTCTTA CATAATTCCT ACTAATGAAC TATCAACTGA TGGTTTTCGT CTTTTAGATG
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         TTGATTCTTA TATAATTCCT ACAAATGAAT TATCAATTGA TAGTTTTCGT TTATTAGATG
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     TTGATTCATA CATAATTCCT ACAAACGAAT TATCAATTGA TAGATTCCGT TTATTAGATG

                                                                       2100
M. domestica          ---------- ---------- ---------- ---------- ---------- ----------
P. silenus            ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum           ---------- ---------- ---------- ---------- ---------- ----------
H. bovis              ---------- ---------- ---------- ---------- ---------- ----------
O. ovis               ---------- ---------- ---------- ---------- ---------- ----------
W. vigila             ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis       ---------- ---------- ---------- ---------- ---------- ----------
B. devia              ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri       ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga       ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma       ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina         ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis      ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi           ---------- ---------- ---------- ---------- ---------- ----------
S. bullata           ---------- ---------- ---------- ---------- ---------- ----------
S. africa            ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis        ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma       ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina        ---------- ---------- ---------- ---------- ---------- ----------
E. latifrons         TTGATAACCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTG ACTGCCGCAG
C. vicina            ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"      ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina           ---------- ---------- ---------- ---------- ---------- ----------
L. illustris         TTGATAACCG AGTAGTTTTA CCAATAAATT CTCAAATTCG AATTTTAGTA ACTGCCGCAG
L. sericata "41"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"    TAGATAATCG AGTAGTTTTA CCAATAAATT CTCAAGTTCG AATTTTAGTA ACTGCTGCTG
P. sialia            TTGATAACCG AGTAATTCTT CCAATAAACT CGCAAATTCG AATTTTAGTC ACAGCAGCTG
C. callipes          TAGATAACCG AGTAGTTTTA CCAATAAATT CACAAATTCG AATTTTAGTA ACAGCTGCTG
C. macellaria        TTGATAATCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTA ACAGCAGCTG
P. regina "1"        TTGATAATCG AGTAGTTTTA CCAATAAATT CACAAATTCG AATTTTAGTG ACAGCAGCCG
P. regina "2"        ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae       TTGATAATCG AGTAGTTTTA CCAATAAATT CACAAATTCG AATTTTAGTA ACAGCAGCTG
C. bezziana          TTGATAATCG AGTAGTATTA CCAATAAATT CACAAATTCG AATCTTAGTA ACAGCAGCTG
C. megacephala "1"   TTGATAATCG AGTAGTATTA CCAATAAATT CACAAATTCG AATTTTAGTA ACAGCAGCTG
C. megacephala "2"   ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi           TTGATAACCG AGTAGTTTTA CCTATAAATT CTCAAATTCG AATTTTAGTA ACAGCAGCTG
C. varipes           TTGATAATCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTA ACAGCAGCTG
C. inclinata         ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis        ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica     TTGATAATCG AGTAATTTTA CCTATAAATT CACAAATCCG AATTTTAGTA ACAGCAGCTG
C. putoria "USA"     TTGATAATCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTT ACAGCAGCTG
C. putoria "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga        ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies        TTGATAATCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTA ACAGCCGCTG
C. albiceps "SA"     ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"    TTGATAATCG AGTAGTTTTA CCTATAAATT CACAAATTCG AATTTTAGTG ACAGCAGCTG

                                                                      2160
M. domestica         ---------- ---------- ---------- ---------- ---------- ----------
P. silenus           ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum          ---------- ---------- ---------- ---------- ---------- ----------
H. bovis             ---------- ---------- ---------- ---------- ---------- ----------
O. ovis              ---------- ---------- ---------- ---------- ---------- ----------
W. vigila            ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis      ---------- ---------- ---------- ---------- ---------- ----------
B. devia             ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri       ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga       ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma       ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina         ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis      ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi           ---------- ---------- ---------- ---------- ---------- ----------
S. bullata           ---------- ---------- ---------- ---------- ---------- ----------
S. africa            ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis        ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma       ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina        ---------- ---------- ---------- ---------- ---------- ----------
E. latifrons         ATGTAATTCA TTCTTGAACT ATCCCAGCTC TAGGGGTAAA GGTAGATGGA ACTCCAGGTC
C. vicina            ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"      ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina           ---------- ---------- ---------- ---------- ---------- ----------
L. illustris         ATGTAATTCA TTCTTGAACT ATCCCAGCAT TAGGAGTAAA GGTAGATGGT ACTCCTGGTC
L. sericata "41"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"     ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"    ATGTAATTCA TTCTTGAACT ATCCCAGCAC TAGGAGTAAA GGTAGATGGA ACTCCTGGTC
P. sialia            ATGTAATTCA TTCATGAACT ATTCCAGCAT TAGGAGTTAA AGTTGATGGT ACTCCGGGTC
C. callipes          ATGTAATTCA TTCATGAACT ATTCCAGCAT TAGGAGTTAA GGTAGATGGT ACACCTGGTC
C. macellaria        ACGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTAGATGGT ACACCTGGTC
P. regina "1"        ATGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTTGATGGT ACACCTGGAC
P. regina "2"        ---------- ---------- ---------- ---------- ---------- ----------
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P. terraenovae          ATGTAATTCA TTCATGAACT ATCCCAGCTT TAGGAGTTAA GGTAGATGGT ACACCCGGAC
C. bezziana             ATGTAATTCA TTCATGAACT ATTCCAGCTT TAGGGGTTAA GGTAGATGGT ACACCAGGTC
C. megacephala "1"      ACGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTAGATGGT ACACCAGGAC
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              ACGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTAAA GGTAGATGGA ACACCTGGAC
C. varipes              ATGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTAAA GGTAGATGGA ACACCAGGAC
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        ATGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTAGACGGT ACCCCAGGAC
C. putoria "USA"        ACGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTAGATGGT ACACCTGGTC
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           ACGTAATTCA TTCATGAACT ATTCCAGCTT TAGGAGTTAA GGTAGATGGT ACTCCAGGTC
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       ACGTAATTCA TTCATGAACT ATCCCAGCTT TAGGAGTTAA GGTAGATGGT ACTCCAGGAC

                                                                        2220
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
R. lherminieri          ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga          ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma          ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina            ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis         ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi              ---------- ---------- ---------- ---------- ---------- ----------
S. bullata              ---------- ---------- ---------- ---------- ---------- ----------
S. africa               ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis           ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma          ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina           ---------- ---------- ---------- ---------- ---------- ----------
E. latifrons            GATTAAATCA AACAAACTTT TTAATTAACC GCCCTGGCTT ATTTTACGGA CAATGTTCAG
C. vicina               ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"         ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina              ---------- ---------- ---------- ---------- ---------- ----------
L. illustris            GACTAAACCA AACAAATTTT TTAATTAATC GACCTGGTTT ATTTTACGGA CAATGTTCAG
L. sericata "41"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"        ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"       GACTAAATCA AACAAATTTT TTAATTAACC GACCAGGTTT ATTTTACGGA CAATGTTCAG
P. sialia               GATTAAACCA AACTAATTTT TTAATTAATC GACCTGGTTT ATTTTATGGA CAATGTTCAG
C. callipes             GACTAAATCA AACTAATTTT TTAATCAATC GACCCGGTTT ATTTTATGGA CAATGTTCAG
C. macellaria           GATTAAATCA AACTAATTTC TTAATTAATC GACCTGGTTT ATTTTATGGA CAATGTTCAG
P. regina "1"           GACTAAATCA AACTAATTTC TTAATTAATC GACCAGGTTT ATTTTATGGA CAATGTTCAG
P. regina "2"           ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae          GATTAAACCA AACAAACTTT TTAATTAATC GACCAGGTTT ATTTTATGGA CAATGTTCAG
C. bezziana             GATTAAATCA AACTAATTTT TTAATTAATC GACCTGGTTT ATTTTATGGA CAATGCTCAG
C. megacephala "1"      GATTAAATCA AACTAATTTC TTAATTAATC GACCTGGTTT ATTTTATGGA CAATGTTCAG
C. megacephala "2"      ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi              GATTAAACCA AACTAATTTT TTAATTAATC GACCTGGTTT ATTTTATGGA CAATGTTCAG
C. varipes              GATTAAACCA AACTAATTTC TTAATTAATC GACCTGGTTT ATTTTACGGA CAATGTTCAG
C. inclinata            ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis           ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica        GACTAAATCA AACTAATTTT TTAATTAATC GACCTGGTTT ATTTTATGGG CAATGTTCAG
C. putoria "USA"        GATTAAATCA AACTAATTTT TTAATTAATC GTCCTGGTTT ATTTTATGGA CAATGTTCAG
C. putoria "SA"         ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga           ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies           GATTAAACCA AACTAATTTT TTAATTAACC GACCTGGATT ATTTTATGGA CAATGTTCAG
C. albiceps "SA"        ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"       GATTAAACCA AACTAATTTT TTAATTAACC GACCTGGATT ATTTTATGGA CAATGTTCAG

                                                                        2280
M. domestica            ---------- ---------- ---------- ---------- ---------- ----------
P. silenus              ---------- ---------- ---------- ---------- ---------- ----------
H. lineatum             ---------- ---------- ---------- ---------- ---------- ----------
H. bovis                ---------- ---------- ---------- ---------- ---------- ----------
O. ovis                 ---------- ---------- ---------- ---------- ---------- ----------
W. vigila               ---------- ---------- ---------- ---------- ---------- ----------
G. intestinalis         ---------- ---------- ---------- ---------- ---------- ----------
B. devia                ---------- ---------- ---------- ---------- ---------- ----------
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R. lherminieri        ---------- ---------- ---------- ---------- ---------- ----------
B. plinthopyga        ---------- ---------- ---------- ---------- ---------- ----------
P. chrysostoma        ---------- ---------- ---------- ---------- ---------- ----------
S. peregrina          ---------- ---------- ---------- ---------- ---------- ----------
S. crassipalpis       ---------- ---------- ---------- ---------- ---------- ----------
S. cooleyi            ---------- ---------- ---------- ---------- ---------- ----------
S. bullata            ---------- ---------- ---------- ---------- ---------- ----------
S. africa             ---------- ---------- ---------- ---------- ---------- ----------
S. ruficornis         ---------- ---------- ---------- ---------- ---------- ----------
S. argyrostoma        ---------- ---------- ---------- ---------- ---------- ----------
C. cadaverina         ---------- ---------- ---------- ---------- ---------- ----------
E. latifrons          AAATTTGTGG GGCTAATCAT AGTTTTATGC CAATTGTAAT CGAAAGAATC CCAGTAAACT
C. vicina             ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "1"       ---------- ---------- ---------- ---------- ---------- ----------
L. cuprina            ---------- ---------- ---------- ---------- ---------- ----------
L. illustris          AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGAATT CCAGTAAATT
L. sericata "41"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "17"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "34"      ---------- ---------- ---------- ---------- ---------- ----------
L. sericata "USA"     AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGAATT CCAGTAAATT
P. sialia             AGATTTGCGG TGCTAACCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. callipes           AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCTGTAAATT
C. macellaria         AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
P. regina "1"         AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT CGAAAGAATC CCAGTAAATT
P. regina "2"         ---------- ---------- ---------- ---------- ---------- ----------
P. terraenovae        AAATTTGTGG AGCTAATCAT AGTTTTATGC CAATTGTAAT TGAAAGAATT CCAGTAAATT
C. bezziana           AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. megacephala "1"    AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. megacephala "2"    ---------- ---------- ---------- ---------- ---------- ----------
C. norrisi            AAATTTGTGG AGCTAACCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. varipes            AAATTTGTGG AGCTAACCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. inclinata          ---------- ---------- ---------- ---------- ---------- ----------
C. marginalis         ---------- ---------- ---------- ---------- ---------- ----------
C. semimetallica      AAATTTGTGG AGCAAATCAT AGTTTTATAC CAATTGTAAT TGAAAGTATT CCAGTAAATT
C. putoria "USA"      AAATTTGTGG AGCTAATCAT AGTTTTATAC CAATCGTAAT TGAAAGTATT CCAGTAAATT
C. putoria "SA"       ---------- ---------- ---------- ---------- ---------- ----------
C. chloropyga         ---------- ---------- ---------- ---------- ---------- ----------
C. rufifacies         AAATTTGTGG AGCTAATCAC AGTTTTATAC CAATTGTAAT TGAAAGAATT CCAGTAAATT
C. albiceps "SA"      ---------- ---------- ---------- ---------- ---------- ----------
C. albiceps "USA"     AAATTTGTGG AGCTAATCAC AGTTTTATAC CAATTGTAAT TGAAAGAATT CCAGTAAATT


M. domestica          ---------- ---------- ---------- ---------- --
P. silenus            ---------- ---------- ---------- ---------- --
H. lineatum           ---------- ---------- ---------- ---------- --
H. bovis              ---------- ---------- ---------- ---------- --
O. ovis               ---------- ---------- ---------- ---------- --
W. vigila             ---------- ---------- ---------- ---------- --
G. intestinalis       ---------- ---------- ---------- ---------- --
B. devia              ---------- ---------- ---------- ---------- --
R. lherminieri        ---------- ---------- ---------- ---------- --
B. plinthopyga        ---------- ---------- ---------- ---------- --
P. chrysostoma        ---------- ---------- ---------- ---------- --
S. peregrina          ---------- ---------- ---------- ---------- --
S. crassipalpis       ---------- ---------- ---------- ---------- --
S. cooleyi            ---------- ---------- ---------- ---------- --
S. bullata            ---------- ---------- ---------- ---------- --
S. africa             ---------- ---------- ---------- ---------- --
S. ruficornis         ---------- ---------- ---------- ---------- --
S. argyrostoma        ---------- ---------- ---------- ---------- --
C. cadaverina         ---------- ---------- ---------- ---------- --
E. latifrons          ATTTTATCAA ATGAATTTC? ??TAGTAT?? AAACTCTTCA TT
C. vicina             ---------- ---------- ---------- ---------- --
L. sericata "1"       ---------- ---------- ---------- ---------- --
L. cuprina            ---------- ---------- ---------- ---------- --
L. illustris          ACTTTATTAA GTGAATTTCT AATAATAT?? AAATTCTTCA TT
L. sericata "41"      ---------- ---------- ---------- ---------- --
L. sericata "17"      ---------- ---------- ---------- ---------- --
L. sericata "34"      ---------- ---------- ---------- ---------- --
L. sericata "USA"     ACTTTATTAA GTGAATTTCT AATAATAT?? AAATTCTTCA TT
P. sialia             ATTTTATTAA ATGAATCTCT AATAATATAT AAATTATTCA TT
C. callipes           ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. macellaria         ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
P. regina "1"         ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
P. regina "2"         ---------- ---------- ---------- ---------- --
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P. terraenovae          ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. bezziana             ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. megacephala "1"      ACTTTATCAA GTGAATTTCT AATAATGT?? AAACTCTTCA TT
C. megacephala "2"      ---------- ---------- ---------- ---------- --
C. norrisi              ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. varipes              ACTTTATTAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. inclinata            ---------- ---------- ---------- ---------- --
C. marginalis           ---------- ---------- ---------- ---------- --
C. semimetallica        ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. putoria "USA"        ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. putoria "SA"         ---------- ---------- ---------- ---------- --
C. chloropyga           ---------- ---------- ---------- ---------- --
C. rufifacies           ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
C. albiceps "SA"        ---------- ---------- ---------- ---------- --
C. albiceps "USA"       ACTTTATCAA ATGAATTTCT AATAATGT?? AAACTCTTCA TT
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Appendix C

A Standardised Larval Rearing Protocol For Forensic Studies

To date, there has been little standardisation of rearing techniques, despite publications drawing attention to the influence of maggot density on development rates and final size (e.g. Smit, 1931; Goodbrod & Goff, 1990; Gaudry *et al.*, 2001), effects of disturbance on pupariation behaviour (Denlinger & Zdarek, 1990) and fluctuating temperatures affecting day degree estimates (e.g. Introna *et al.*, 1989; Davies & Ratcliffe, 1994). This lack of standardisation has resulted in an inability to compare many studies of the same or congeneric species.

i) Colony maintenance

Fly colonies should be initiated from wild populations, and it is preferable to augment captive populations with wild-caught individuals every few generations to prevent inbreeding. Although little has been published on the effects of inbreeding on fly development, there is evidence that extended periods of inbreeding can impact on the duration of larval development (e.g. Laurence, 1988).

Flies should be provided with a subsistence diet; for example, a 50:50 mixture of full cream milk powder and sugar is effective (pers. obs.). Female flies should also be provided with a protein meal (e.g. blood or fresh meat) some days prior to an oviposition medium being introduced, in order that egg maturation can occur. Although milk powder contains protein, it was found that females would not oviposit if they did not have access to a high protein diet (pers. obs.).

ii) Oviposition medium

Flies of different species have preferred oviposition behaviours and substrates (e.g. Zumpt & Patterson, 1952; Prins, 1982; Meskin, 1986; Byrd & Butler, 1994, 1996, 1998; Byrd & Allen, 2001b; chapter 4, this study). Although larvae apparently develop normally on a variety of substrates, females may oviposit normally only if they are provided with a medium under which they can crawl (e.g. *Chrysomya megacephala*, this study) and may avoid very moist substrates such as chicken liver (e.g. *Chrysomya marginalis*, this study). Thus, researchers should provide female flies with a variety of oviposition media to determine their preferences. Eggs may be removed later and reared on a standard medium.

iii) Egg collection

For many experiments, rearing of siblings may be preferable to rearing a mixed cohort of larvae, primarily because all the eggs deposited by a single female tend to be laid within a period of 5 minutes. However, most calliphorid species lay communal egg batches, so ensuring that all eggs in a group are from the same female may be difficult. One option is to keep gravid females isolated. This procedure works well for *Lucilia sericata, Chrysomya putoria* and *C. chloropyga*, but isolated females of *Chrysomya marginalis* will not usually oviposit (pers. obs.). Another option is to assume that eggs in parallel rows were deposited by the same female, because flies tend to remain relatively stationary during oviposition and the resulting eggs therefore have a similar orientation. Occasionally, however, female flies do alter their positions whilst ovipositing, especially if they are disturbed (pers. obs.), so the egg collection may be conservative. This method also assumes that two females did not oviposit parallel to each other. As a rule of thumb, small batches (<150 eggs) consisting of one or two egg layers arranged in parallel can be safely assumed to be siblings.

Collected eggs can be transferred directly to the medium on which the larvae are to be reared. Eggs from the same batch form a cohesive bond, and separating the eggs manually generally results in high mortality, so it is recommended that newly hatched larvae (instead of eggs), are separated into treatments if required. Eggs tend to desiccate rapidly, especially at high temperatures and low humidity, but excess moisture is also detrimental to egg development (Hobson, 1932a). Usually, if the eggs were placed on chicken liver in a closed Petri dish that was lined with damp (not wet) cotton wool, egg mortality was low (chapter 4, this study). If egg development is slow, spraying the inside of the lid of the Petri dish with fresh water helps to prevent egg desiccation. Care must be taken, however, to prevent the lid of the Petri dish from adhering to the base and preventing the free circulation of air.

iv) Larval development

Larvae should be maintained in a maximum density of 1 larva per 2g of substrate to prevent the accumulation of metabolic heat by the substrate (Goodbrod & Goff, 1990). However, larvae tend to form dense aggregations and localised "hotspots" may still develop in the rearing medium irrespective of overall maggot density (pers. obs.). Generally, if fewer than

50 maggots were reared together such heat production was minimised (pers. obs.), but further study is required to determine threshold levels. Additionally, at low larval densities (e.g. isolated maggots), development may be slower than at median larval densities where maggot generated heat is not measurable (pers. obs.), and further research is therefore needed. It is also recommended that frequent (or constant) records of temperature in the substrate are kept, especially since substrate and air temperatures may differ (Catts, 1992).

Many researchers measure maggots (length or mass) during their development to provide data that can be used to age maggots collected by forensic investigators. As illustrated in chapter 4, there is often a large variation in size of same-age individuals, so the ranges of length and mass should be recorded. Physical measurements are not always a good indicator of physiological age (see Gaudry *et al.,* 2001), as dwarf or abnormally large individuals are often produced.

A more reliable method for determining development rates is to record the time taken to reach developmental landmarks. It is recommended that frequent (e.g. 6-hourly) observations are made, or that observation times are staggered between batches of maggots. Infrequent observations will result in overestimation of stadial durations, will decrease the resolution of the useable information, and will introduce error. To improve the resolution of data, the duration of incubation (I), first instar (L1), second instar (L2), feeding third instar (L3f), larval wandering (L3w) and pupariation (P) should be recorded. It is especially important to separate the L3w stage from both the L3f and the P stages, because at temperature extremes (pers. obs.), high larval density (pers. obs) or if disturbance occurs (pers. obs.; Denlinger & Zdarek, 1994), L3w duration is very plastic.

v) Pupariation

At the onset of larval exodus, larvae should have access to a pupariation medium. Most authors (e.g. Byrd & Butler, 1996, 1997, 1998; Byrd & Allen, 2001b) provide vermiculite, but clean river sand is equally effective (pers. obs.). During wandering, larvae tend to aggregate in dark or shaded areas, or bury themselves. Physical disturbance, either by other maggots "jostling", or by researchers, can delay pupariation (Denlinger & Zdarek, 1994; pers. obs.), so care should be taken to limit larval densities in the pupariation medium and human disturbance should cease at larval exodus. It is possible to isolate larvae as they leave the feeding medium so that accurate determinations of the onset of pupariation can be made, but such a method is time consuming.

Tanned puparia may be removed from the pupariation medium until eclosion, but it is not recommended unless the humidity balance is controllable, because puparia are prone to desiccation. When large numbers of puparia were transferred to Petri dishes, late-eclosing adults were often malformed as a result of being walked over by other flies during emergence (pers. obs.). Therefore, puparia should be kept in low densities, and sufficient space must be made available to eclosing adults.