Effects of temperature on the development, behaviour and geography of blowflies in a forensic context

This thesis is submitted in fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Of

Rhodes University

By

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December 2007

This thesis is dedicated to my parents, my sister and to Kyla Orr, for all their love and support over my university career

Abstract

The development of immature insects is commonly employed in forensic investigations to estimate time of death, or postmortem interval (PMI), of a corpse on which they are feeding. The bulk of this thesis focuses on factors influencing the accuracy of developmental data, and exploring how and why developmental data differ between studies involving the same species, and between different species. Because carrion-feeding insects are ectotherms, temperature may be expected to significantly influence their behaviour, development and distribution, and the remainder of the thesis therefore focuses on the thermal biology and geographical distribution of seven forensically important blowflies. The species include *Chrysomya albiceps*, *C. putoria*, *C. chloropyga*, *C. megacephala*, *C. marginalis*, *C. inclinata* and *Calliphora croceipalpis*.

A robust experimental design for estimating developmental models is outlined and tested. It is recommended that forensic entomologists should involve at least six constant temperatures, starting at about 7°C above the relevant developmental zero (D_0) and going to about 10°C above the upper critical temperature, and a temporal sampling interval with a relative precision of about 10%.

Using this design, focused experiments consistently provided the most reliable developmental data, while data pooled from different studies yielded inconsistent results. Similarly, developmental data from closely related species differed significantly, and surprisingly so did developmental data from different populations of the same species. Possible explanations for the latter lay in the different methods of data collection but only temporal sampling resolution had a direct influence on the accuracy of developmental data. Consequently, disparities in such data were primarily ascribed to genetic differences and phenotypic plasticity.

Comparisons between numerous thermal thresholds of larvae, pupae and adults support this conclusion and suggest a phylogenetic component to the thermal biology of blowflies. Further comparisons were made between these temperature thresholds and the

distributions of blowfly species present on two rhinoceros carcasses. These comparisons suggest that blowfly larvae with high upper lethal temperature thresholds dominate in interspecific competition in favorable thermal environments by raising maggot mass temperature above the thresholds of other carrion-feeding blowflies, through maggot-generated heat.

Bioclimatic modeling using maximum entropy analysis provided a successful means of predicting whether a species is likely to occur in an area, and whether it would therefore be expected in a local carcass community. It also showed that temperature was less important than moisture in shaping the geographical distribution of African carrion blowflies.

Based on these results, several recommendations are made for the practice of forensic entomology.

Declaration

This thesis has not been submitted under any other university outside of Rhodes University (Grahamstown, South Africa) for any other degree. The work presented in this thesis is that of the authors, unless otherwise stated.

Publications

Accepted Manuscripts

Richards CS, Paterson IH, Villet MH. (2007) Estimating the age of immature *Chrysomya albiceps* (Diptera: Calliphoridae), correcting for temperature and geographic latitude. Int J Legal Med. On-line Early doi http://dx.doi.org/10.1007/s00414-007-0201-7

Manuscripts under correction

Richards CS, Villet MH (2008) Factors affecting accuracy and precision of thermal summation models of insect development used to estimate postmortem intervals. Int J Legal Med. In press

Submitted Manuscripts

Richards CS, Villet MH (Submitted on 10 August 2007) Data quality in thermal summation models of development of forensically important blowflies. Med Vet Entomol.

Richards CS, Crous K, Villet MH (Submitted on 19 September 2007) Models of development for the blowflies *Chrysomya chloropyga* and *C. putoria* (Diptera: Calliphoridae). Med Vet Entomol.

Richards CS, Price BW, Villet MH (Submitted on 19 February 2008) Thermal eco-physiology of seven carrion-feeding blowflies (Diptera: Calliphoridae) in South Africa. Entomol Exp Appl.

Richards CS, Williams KA, Villet MH (Submitted on 3 March 2008) Predicting geographic distribution of seven blowfly species (Diptera: Calliphoridae) in South Africa. Afr Entomol.

Conference Proceedings

Richards CS, Price BW, Villet MH (2005) Physical and behavioural thermoregulation of blowflies (Diptera: Calliphoridae) and the implications for PMI estimates. Proceedings of the 15th Congress of the ESSA. 2005. Rhodes University. Grahamstown, South Africa

Richards CS, Villet MH (2007) Latitude-related variation in the development rates of the green blowfly *Chrysomya albiceps* (Diptera: Calliphoridae). 5th Meeting of the European Association for Forensic Entomology, 2nd – 5th May 2007. Brussels, Belgium

Richards, C.S., K.L. Crous, M.H. Villet (2007) Larval development of two forensically important blowflies, *Chrysomya chloropyga* Weidemann and *C. putoria* Weidemann (Diptera: Calliphoridae), in relation to their distribution in South Africa. The 33rd bi-annual conference of the Zoological Society of Southern Africa. 8-11 July 2007. North-West University, Potchefstroom, South Africa

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Acknowledgments

I am sincerely grateful to my supervisor, Prof. Martin Villet, for all his advice and guidance through this thesis. Despite all other responsibilities, I always felt like a priority to him. I have thoroughly enjoyed this experience, thank you.

Thank you to Kyla Orr for her love and support over the past few years. This PhD was a huge undertaking that was made easier by her encouragement. Thank you to my parents and my sister for allowing me to become the person I want to be; my digs mates and close friends, for relieving pressure during stressful times.

Thank you to the South African National Research Foundation (NRF) and Rhodes University for financing this project. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation.

Additional acknowledgements are listed at the end of each chapter, detailing individuals involved in the work of particular chapters.

I

General Introduction

Preface

This chapter introduces the primary focus of this thesis, forensic entomology, and highlights problem areas within this field that will be addressed.

Post-mortem interval (PMI) is the time between the death and discovery of a corpse (Adams and Hall 2003). Several natural processes are associated with decomposition, including rigor mortis, livor mortis, dehydration, algor mortis, autolysis, autodigestion and putrefaction (Campobasso et al. 2001). But many of these processes are limited to the first 72 hours after death, and can only be used in short PMI estimates (Henssge et al. 1995; Al-Alousi 2002; Bourel et al. 2003). Other complications arise in calculating short PMI from these processes as they are reciprocal functions and become vague very quickly (Henssge et al. 1995; Bourel et al. 2003). There are several algorithms based on core body temperature (Nelson 2000) and brain, liver and rectum temperature (Al-Alousi 2002) which are used to calculate short PMI, but many of them have high error due to mathematical and biological difficulties and cannot be used in law enforcement (Nelson 2000; Querido & Phillips 2001).

Entomological decomposition in and on the corpse, is an on-going process that can be measured easily and can be used to estimate both short and long PMIs (Henssge et al.

1995; Amendt et al. 2007). Short PMIs are based on the oldest developing individuals on the body while long PMIs are on arthropod successional patterns (Amendt et al. 2007). But insects only colonize a body after death, therefore forensic entomology can only provide a minimum PMI, rather than the actual time of death. Because blowflies (Diptera: Calliphoridae) are the first to colonize a body (Turner & Howard 1992; Bourel 2003), the focus of estimates of short minimum PMIs is often on them when using entomological evidence, and for this reason, this thesis deals with short minimum PMIs only. The assumption behind these estimates is that by calculating the age of developing blowflies from the crime scene, it is possible to estimate an accurate PMI (Greenberg 1985; Catts 1992).

Because small insects, and therefore blowflies, are ectotherms, their rate of development is heavily influenced by the environmental temperatures they experience (Sharpe and de Michelle 1977; Adams & Hall 2003; Lefebvre & Pasqueraul 2004). This relationship has huge implications for accurate estimates of PMI (Greenberg 1991). Therefore, to calculate an accurate PMI from developing insects, two separate sets of thermal information are required. The first set is the environmental temperatures experienced by the developing insects on the body (Greenberg 1985; Catts 1992). To obtain this, a record of ambient temperatures is gathered, usually from a weather station nearest to the body discovery site (Smith 1986; Archer 2004; Amendt et al. 2007). Unfortunately, there may be significant difference between the ambient temperatures of the weather station and the site (Anderson and Cervenka 2002; Archer 2004) because they might be at different altitudes to one another, or experience different exposure to identical weather conditions (Goff 1991; Archer 2004). For this reason, ambient temperature is recorded on site for several days after the body is discovered (Anderson 1995), and a regression relationship is then derived between these temperatures and simultaneously recorded weather station temperatures (Haskell et al. 2001). This derived equation is then used to correct the weather station temperature records to ambient site temperature for the duration that the body was thought to be *in situ* (Byrd and Castner 2001; Haskell et al. 2001; Archer 2004).

Because maggots feed gregariously and are significantly exothermic in the 2nd and 3rd larval instars, they may not develop at the ambient temperature of the site, but rather at higher temperatures (Deonier 1940; Payne 1965; Greenberg 1990, 1991; Turner & Howard 1992, Slone and Gruner 2007). Unfortunately, there is no published model that describes this relationship successfully. The most recent publication in this area (Slone and Gruner 2007) noted a relationship between the volume and temperature of maggot masses, but had insufficient information to build a model of the thermal environment of gregarious maggot mass. Despite the evident need for focused research in this area, this thesis is not directed at overcoming this problem and therefore this topic is not discussed further.

Once maggot mass temperature has been estimated for the duration that the body was *in situ*, an estimate of PMI can be made using this information and development data obtained in the laboratory.

Development data are simply data pertaining to the duration of development of immature stages recorded at different constant temperatures, which are then summarized in one or more of three developmental models, namely isomorphen diagrams (Grassberger and Reiter 2001), isomegalen diagrams (Reiter 1984) and thermal summation models (Higley and Haskell 2001), for the purpose of estimating PMI. In South Africa there is only one published study reporting isomorphen and isomegalen diagrams, for *Chrysomya albiceps* (Wiedemann) only (Richards et al. 2007). Outside of South Africa, there are only two published studies reporting isomegalen diagrams on blowflies, for *Calliphora vicina* (Meigen) (Reiter 1984) and *Lucilia sericata* (Meigen) (Grassberger and Reiter 2001), and three published studies reporting isomorphen diagrams on blowflies (Grassberger and Reiter 2001, 2002; Grassberger et al. 2003) for *L. sericata*, *Protophormia terraenovae* (Robineau-Desvoidy), and *C. albiceps* respectively. The evident lack of available published isomorphen and isomegalen diagrams may suggest that these models are not a preferred method for calculating PMI. Nevertheless, these two models are the only known approach to calculating PMI from samples of dead maggots and diagrams for a wider range of species are needed, particularly in South Africa.

Similarly, there is only one published study reporting thermal summation constants for one species of South African Calliphoridae (Richards et al. 2007), alongside the few anecdotal publications reporting raw development data at one or two temperatures (Prins 1982, Laurence 1988; Leipoldt and van der Linde 1993; Stadler and van der Linde 1995). Outside South Africa, only a few publications provide calculated thermal summation constants, for a handful of forensically important blowfly species. By far the majority of published studies in this field report development data in their raw form (commonly the minimum, mean and/or maximum values of the developmental data summarised in tables). For an accurate PMI to be estimated from these data, the reader is often left to calculate thermal summation constants from an already reduced data set. For estimating PMI, it may be more beneficial to present thermal summation constants and/or isomorphen and/or isomegalen diagrams as opposed to a table of raw development data.

It is commonly thought that thermal summation models are the preferred approach to calculate PMI (Higley and Haskell 2001), but the limited thermal summation constants available in the literature suggest that either 1) forensic entomologists use unpublished data to calculate PMI; 2) the limited thermal summation constants on the few forensically important blowfly species that is published is sufficient in all other cases; or 3) that entomologists derive thermal summation constants from the published summarized raw data. PMI estimates should not be calculated from unpublished data because the credibility of data that is not peer-reviewed can be challenged and is unlikely to stand in court (Byrd and Castner 2001). Although there may be only a handful of carrion-breeding Calliphoridae species that are common in forensic cases, it is highly unlikely that they alone will satisfy all needs for PMI estimates accurately, and that other less common species will be important in some cases. The most likely explanation for the limited number of published thermal summation constants lies with the third alternative. If this is the case, then it will become evident in this thesis that more published thermal summation constants are needed.

Despite 77 publications involving development data for blowflies in some form or another, there are currently no recommended guidelines that recognize a standard method for obtaining raw development data of adequately high quality, specifically for the purpose of estimating PMIs (from any of the three available models). This is an area of concern that needs to be addressed and one that is not limited to South Africa.

There is a recognized statistical method for analyzing developmental data for thermal summation models (Higley and Haskell 2001; Greenberg and Kunich 2002). Traditionally, it has been used to calculate development thresholds for the purpose of biological control (Réaumur 1735; Pedigo 1996) and has been modified for forensic entomology (Higley and Haskell 2001; Greenberg and Kunich 2002). This method is commonly referred to as the thermal summation model, or temperature/rate graph, where the x-intercept and the inverse of the slope of a linear regression are used as the thermal summation constants to calculate PMI. Because the true relationship is sigmoidal and not linear (Ikemoto and Takai 2000; Higley and Haskell 2001), problems arise when using this method to identify the points nearest the extremes of the linear approximation that deviate significantly from it (Ikemoto and Takai 2000) and because of this, it has been suggested that it is not the most accurate approach to describing the best-fitting relationship (Ikemoto and Takai 2000). Despite this, no published forensic studies other than that of Villet et al. (2006) and Richards et al. (2007) use this analytical method.

Besides the lack of development data on forensically important South African blowflies, there is also a lack of information in other areas of their general thermal biology. Lunt (2002) and Williams (2002) are the only authors to systematically investigate other areas of South African blowfly thermal biology. Lunt (2002) studies studied the phylogenetic prediction of developmental constants, and Williams (2002) studied the lower temperature thresholds of nervous activity and coordinated movement, and the upper lethal temperature thresholds of the adult stage of four forensically important blowfly species. Knowing the thermal biology of these flies is particularly important because temperature significantly influences the biology of ectotherms like blowflies (Sharpe and de Michele 1977). Therefore, an understanding of blowfly thermal biology may assist the

forensic interpretation of the behaviour and distribution of larvae and pupae in the carrion environment, and interactions between larvae of different species.

Similarly, blowfly thermal biology may help to explain the geographic distribution of these flies in South Africa. Temperature is widely cited as the primary climatic variable limiting geographic distribution of numerous insect species (Leather et al. 1993; James and Partridge 1998; Klok and Chown 2003), but this has never been reported for blowflies. Along with this, understanding the geographic distribution of blowflies in South Africa is important to better interpreting their forensic importance and useful to focus control measures against vector species. Currently, there is limited information on the geographic distribution of forensically important blowflies in South Africa and what is available is either limited (Williams and Villet 2006) or out of date (Smit and du Plessis 1926; Ullyett 1950; Zumpt 1956, 1965; Pont 1980; Prins 1982).

Scope

This thesis focuses on establishing the role of temperature in the biology of blowflies, in terms of their development, physiological thresholds and ecology at small and large spatial scales, with the intention of developing techniques for Forensic Entomology using South African flies as models.

Aims

This thesis addresses the following hypotheses:

1. Pooling developmental data from several published studies is effective at increasing the quality of data used to make PMI estimates.
2. Aspects of data collection (including sampling size, temporal sampling resolution, and summary statistics) do not influence the estimation of the duration of development of immature stages.
3. Development data from different geographic populations of the same species do not differ significantly from one another.

4. Development data are not transferable between species.
5. Common trends exist between the temperature thresholds of larvae, pupae and adults of different species; the temperature thresholds of larvae play of role in interspecific competition and distribution in the carrion feeding environment; and these thresholds are linked to phylogeny.
6. Temperature significantly influences the geographical distribution of forensically important blowfly species in South Africa.

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II

Data quality in thermal summation models of development of forensically important blowflies

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Preface

This chapter adopts a new regression model for the analysis of developmental data, and identifies minimum requirements for a regression analysis to achieve reliable thermal summation constants. It progresses to the question of combining more than one published data set to achieve these minimum requirements. Finally, this chapter identifies three possible areas that influence data quality and the compatibility of different data sets. This chapter has been submitted to *Medical and Veterinary Entomology*.

Abstract

To highlight some issues regarding data quality that are significant in estimating postmortem intervals from maggots, the developmental constants of thermal summation models for development of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) were calculated from incidental data gathered from twelve published studies, and from data generated specifically for the purpose in a single experiment, using the improved analytical method described by Ikemoto and Takai (2000). The focused experiment involved measuring the timing of five developmental landmarks at nine constant temperatures with a sampling resolution of 6-12 hr, which is characteristic of other published studies. Combining different studies' data yielded inconsistent results, which is ascribed to statistical noise introduced by (at least) disparities in temporal precision,

descriptive statistics, geographical location and rearing diets. A robust experimental design to estimate a developmental model should involve at least six constant temperatures, starting at about 7°C above the relevant developmental zero (D_0) and going almost to the upper critical temperature, and a temporal sampling interval with a relative precision of about 10%, which translates into sampling about every 2hr until hatching, about every 3hr until first ecdysis, and about every 6hr until second ecdysis.

Introduction

A major focus of medico-criminal forensic entomology is to calculate an accurate and precise estimate of the time between death and discovery of a corpse, otherwise known as the minimum post mortem interval (PMI) (Greenberg 1991; Catts 1992). Because blowflies are often the first insects to arrive on a corpse, the focus of these estimates is on them (Catts 1992).

The most common method used to calculate minimum PMI is the thermal summation model, a linear regression model based on the temperature-dependent rate of development of immature insects. It works on the assumption of a linear relationship between rate of development and the temperatures experienced by the growing insects (Sharpe and DeMichele 1977; Byrd and Allen 2001; Higley and Haskell 2001; Greenberg and Kunish 2002). The age of an immature insect can be assessed from developmental landmarks and temperature-specific development rates, and this age is used to estimate the PMI. Because blowflies often find a corpse promptly and lay eggs on it almost immediately (Turner and Howard 1992), they are ideal candidates for estimating minimum PMIs.

The precision of the estimate of age from temperature is affected by the number of constant temperatures used in the regression analysis (Ikemoto and Takai 2000), and good regression models are based on no fewer than six points along the linear section of the relationship (Ikemoto and Takai 2000; Sokal and Rohlf 2005). However, because the relationship between developmental rate and temperature is generally sigmoidal (Sharpe

and DeMichele 1977; Ikemoto and Takai 2000; Byrd and Allen 2001; Higley and Haskell 2001), more points are needed in practice to identify the limits of its (approximately) linear mid-section, so that at least eight constant temperatures are needed to characterize the model comprehensively. Few developmental studies on blowfly species achieve this goal: a comprehensive paper by Grassberger and Reiter (2001) used ten temperatures from 15˚C-34˚C; O' Flynn (1983) used eight; Hanski (1976) and Reiter (1984) used seven; and Byrd and Allen (2001) and Williams and Richardson (1984) used six. One possible solution to this problem is to pool data from separate studies. A case study is presented to examine the implications of this approach for data quality and the interpretation of forensic entomological evidence.

Chrysomya megacephala (F.) is a blowfly that has relevance to forensics, medical entomology and public health in Africa, Asia, Australia and South America (Prins 1982; Pont 1985; Wells 1991; Wells and Kurahashi 1994; von Zuben et al. 2001). Numerous authors (Table 2.1) have published rates of development of *C. megacephala*, but Nishida (1984; Nishida et al. 1986) is the only study to present data for more than one constant temperature. Since no thermal summation model for the development of this forensically important fly is currently available, I explored the usefulness of pooling data from the published studies to meet the minimum data requirement of a reliable regression model, and compare the results with those of a single independent experiment designed to provide a precise developmental zero (D_0) and thermal constant (K) for *C. megacephala*.

Materials and Methods

Meta-analysis of published data

Developmental times for six developmental landmarks for *C. megacephala* at various constant temperatures were collected from twelve papers (Table 2.1). The regression constants representing K and D_0 for various developmental landmarks were calculated from the aggregated data using the improved method described by Ikemoto and Takai (2000). This involved plotting the duration of development (D) to a developmental landmark at a constant temperature (T) against the product of the duration and the

temperature (DT). This revised approach balances the weights of upper and lower temperature ranges (Ikemoto and Takai 2000), which allows for a more accurate calculation of *K* and its confidence interval than the traditional approach of plotting 1/D against T. It also helps to identify the upper and lower critical temperatures of the linear section of the developmental curve. In the plot of D against DT, the points form an inverted N-shaped line, where the linear section is represented by the crossbar, and the critical temperatures lie at the vertices (Ikemoto and Takai 2000). When making predictions using linear regression, the largest errors occur at the extremes of the relationship (Sharpe and DeMichele 1977; Higley and Haskell 2001; Sokal and Rohlf 2005), emphasizing the importance of the data in the mid-section of the regression. The data outside the upper and lower critical temperatures are excluded from the analysis as they do not fit on the linear part of the relationship (Sharpe and DeMichele 1977; Ikemoto and Takai 2000). If sufficient temperatures are included, a straight line is fitted to the remaining data using reduced major axis regression (Ikemoto and Takai 2000). The slope of the regression line is D_0, and the intercept is *K*.

New data

About a hundred adult *C. megacephala* specimens were collected with Red Top™ fly traps (Miller Methods, Pretoria) in Grahamstown (33˚19'S 26˚32'E) to found a laboratory fly culture. The culture was maintained at approximately 22˚C under a lighting cycle of 12:12 hours (light:dark). Flies were feed milk powder, sugar and water *ad libitum* for 1 week and then provided with a 200g pork chop as an oviposition medium. After one day numerous eggs were found under the chop and these were monitored frequently until they hatched.

Newly hatched larvae (approximately 1 hour old) were placed ten to a cup in tapered, plastic 250ml cups, each containing 20g of fresh chicken liver. This low density of maggots prevented measurable temperature increases from maggot-generated heat (Goodbrod and Goff 1990) that might have stimulated growth (Higley and Haskell 2001) and avoided stunted growth associated with isolation (Davis and Ratcliffe 1994). Each

cup was steadied in river sand 3.0 - 3.5cm deep in a plastic tub (8 x 8 x 5cm). This provided sufficient substrate for pupariation once the wandering phase began. Cups were placed in Labcon 3104U incubators set at constant temperatures of 17.5˚C, 20˚C, 22.5˚C, 25˚C, 27.5˚C, 30˚C, 32.5˚C, 37.5˚C, and 42.5˚C. Additional chicken liver was added when necessary (e.g. when it dried out or was consumed rapidly at high temperatures) to avoid stunted growth (dos Reis et al 1999).

Twice a day, 5 random maggots were sampled, each from a different cup, at each temperature and placed directly into 70% alcohol. This sampling interval matched the coarsest resolution of published studies, thus avoiding biasing comparisons in favour of the focused experiment. In other comprehensive studies of maggot development, Grassberger et al. (2003) and O'Flynn (1983) measured maggots every 12 hours; Lefebvre and Pasquerault (2004) measured maggots every 8 – 12 hours; Nishida (1984) measured maggots every 6 hours; and Grassberger and Reiter (2001, 2002) measured maggots every 4 hours. Samples were placed directly into alcohol and not killed in hot water because this is standard procedure in South Africa when collecting entomological evidence from a crime scene. Larvae took between 1 and 2 hours to die after which the length of each maggot was measured to 0.1mm using a gauge (Villet 2007), and its instar was determined by inspecting its posterior spiracles (Prins 1982). The onset of wandering was determined graphically as the time when lengths began to shorten. The onset of pupariation and eclosion were recorded and the median time of occurrence for each developmental landmark was calculated from hatching with a precision of six hours. Hatching was used as the starting time because eggs may be fertilized and start developing well before they are laid (Smith 1986; Wells and King 2001).

The results were analyzed using the method (Ikemoto and Takai 2000) outlined above with the computer software programme "Statistica 7". In addition, a t-test was used to test for significant differences between pooled data and new data.

Results

Meta-analysis of published data

The published data for *Chrysomya megacephala* originated from twelve locations in eight countries (Table 2.1). Two other papers were excluded from the analysis: one (Jenson and Miller 2001) because the temperatures were insufficiently constant, and the other (Nishida 1984) because the raw data that it summarises were presented by Nishida et al. (1986). The temperatures ranged from 23.5˚C to 29.4˚C but Lunt (2002) tested as low as 20˚C (Table 2.1). The laboratory diets of the studies were mostly liver of pigs, cows or chickens, but included other diets such as beef, horse meat and pet mince (Table 2.1). Half of the papers reported mean developmental times; others presented minimum and maximum developmental times or raw data (Table 2.1). The temporal precision varied from 6hr (Nishida 1986) to 24hrs (O'Flynn 1983; Wells and Kurahashi 1994; Gabre et al. 2005).

A majority of the data was discarded from the analysis (Table 2.1) because it apparently lay outside the critical temperatures of the linear section of the developmental curve (Fig. 2.1). The resulting R^2 values ranged between 0.89 and 1.00 (mean = 0.98; std. dev. = 0.04) (Table 2.1). The average D_0 value for all developmental events is 19.50˚C (std. err. = 2.09˚C), but there are several statistically significant differences in D_0 between particular events (Fig. 2.2).

New data

The eggs hatched 19 to 21 hours after oviposition. Flies failed to eclose at 42.5˚C. The upper critical temperature was about 35˚C, since the analytical graphs turned back on themselves above 32.5˚C (Fig. 2.3). Very few pupae eclosed at 17.5˚C. The lower critical temperature was probably just below this temperature (Fig. 2.3). Regression analysis was therefore restricted to the temperature range of 17.5-32.5˚C.

K and D_0 values were calculated for 1st ecdysis, 2nd ecdysis, onset of wandering, onset of pupariation and eclosion (Table 2.2). The majority of the data were included in the analysis of all developmental events, and no data were excluded for 2nd ecdysis or eclosion (Fig. 2.3). All R^2–values lay between 0.91 and 0.99 (mean = 0.94; std. dev. = 0.03). The average D_0–value for 2nd ecdysis, onset of wandering, onset of pupariation and eclosion is 10.44˚C (std. err. = 0.12), and none of the D_0 values for particular events differed significantly from one another (Fig. 2.2).

The new data are significantly ($t = -5.32$; d.f. = 8; $p < 0.001$) different to those results found by pooling published data. The values estimated for *K* and D_0 from the pooled data differ significantly from the new data in almost every comparison (Fig. 2.2). The later in development an estimate is made, the more the result from the pooled data become like those of the experimental data. This is attributed to the smaller relative error of measurements made towards the end of development.

Discussion

Meta-analysis of published data

Despite the numerous published sources used in this analysis, only eclosion was represented by more than six analytically usable points, while only two usable points were obtained for 1st and 2nd ecdysis (Table 2.2). The data for wandering and pupariation offer more to analyze but the resulting graphs are erratic (Fig. 2.1). The low *K* values for all of the developmental landmarks except hatching (Fig. 2.2) and the large variation in D_0 values (Fig. 2.2) reduce confidence in these findings.

The disparities in the D_0 values calculated from the pooled data, and the differences between these models and those from the focused experiment, suggest that it is not viable to combine more than one author's work to obtain accurate *K* and D_0 values. Reasons for this could be ascribed to three factors. First, the populations used in the pooled analysis came from eight different countries. It is well documented that different populations of many insect species differ genetically, physiologically, developmentally and

behaviourally (Bohart and Gressit 1951; Zumpt 1965; Honêk 1996; Alstad 1998; Boecklen and Mopper 1998; Hanks and Denno 1998; Richards et al. 2007). Wells and Lamotte (2001) counter-argued that genetic variation between blowfly populations is unlikely as blowflies are extremely mobile, making it easier for populations to mix, and evidence of such mixing has been found in genetic studies that included *C. megacephala* (Harvey et al. 2003). Even if population differences are not genetic, they may show a behavioural response to different environments (Wells and Lamotte 2001; Ayrinhac et al. 2004). This point is discussed in more detail in Chapter 4.

Second, about half of the studies reported means while others used either raw data or minima and maxima, or did not indicate what their data represented. Minima and maxima can be used to estimate a midpoint, but if the underlying distribution is skewed (which is likely: see e.g. Nishida et al. 1986) this will differ from the mean and the median (discussed in Chapter 3). These problems are compounded by not knowing the precision of many of the data. This would lead to a blurring of the pattern and greater variation, but one might still hope to get satisfactory estimates of K and D_0. However, this was not the case (Fig. 2.2), which implies that further factors are involved.

Third, no two studies use the same rearing media (Table 2.1), and diet influences the rate of blowfly development (Goodbrod and Goff 1990; Kaneshraja and Turner 2004; Clark et al. 2006; Ireland and Turner 2006). *Lucilia sericata* developed significantly slower when reared on liver than on heart or lung (Clark et al. 2006). Significant differences in development and size also occurred between maggots reared on liver of different mammals (Clark et al. 2006). Although four of the twelve studies used liver as a growth medium, all were from different animals (Table 2.1). Other media (Table 2.1) might also affect development rates at different life stages (discussed in Chapter 4).

These three factors help to explain why the majority of the data points could not be used in the analysis, and why the rest gave inconsistent results.

Recommendations for experimentally estimating K and D_0

Choosing useful temperatures for developmental studies must be considered an important part of the experimental design, and one should use as wide a range of temperatures as possible. Of the published studies of *C. megacephala* that used more than one temperature (Table 2.2), Nishida et al. (1986) had the greatest range (11˚C) but tested only above 23˚C. Even though maggots were cultivated at nine constant temperatures, some of them lay above the upper critical temperature, and therefore made no contribution to the regression model (Fig. 2.3). Nishida (1984) reported pupariation without eclosion at 35˚C for *C. megacephala*. Although eclosion was recorded at 37.5˚C, the low pupal survival rate (6.98%; n = 43) suggested the temperature was not ideal, whereas the 87.85% (n = 41) pupal survival rate at 32.5% suggests a near-optimal temperature. All developmental estimates except for 1st ecdysis included 32.5˚C (Fig. 2.2), and this seems a reasonable estimate of the upper critical temperature for *C. megacephala*. None of the temperatures were below the lower critical temperature (Fig. 2.3), but the incipient curvature at 17.5°C in the graphs for wandering, pupariation and eclosion indicated that this threshold was near that temperature. Thus, estimates of the lower and upper critical development temperatures for this study's population of *C. megacephala* are 17˚C and 33˚C. These limits lie approximately 7˚C from D_0 (about 10.5˚C) and the upper lethal temperature (about 40˚C), respectively. By identifying upper and lower critical limits to the linear response to temperature, it is possible to fit a reasonably accurate regression line and calculate subsequent *K* and D_0 values using a bare minimum of points, although this will compromise the precision of the estimates.

The consistent D_0 value for four of the five developmental landmarks in this new data is to be expected because the kinetics of metabolism is unlikely to vary between developmental stages in insects (Sharpe and DeMichele 1977). The D_0 value for 1st ecdysis was 2.5˚C higher than for the other developmental events, and although it does not differ significantly for the other estimates, its accuracy is questionable because samples of first instar larvae were taken with less relative temporal precision (i.e. a larger temporal relative error) than the other developmental events due to the brevity of the first instar, particularly at higher temperatures. Most published studies sampled at fixed

intervals that ranged from 4-12hr (e.g. O'Flynn 1983; Nishida et al. 1986; Grassberger and Reiter 2001; 2002; Grassberger et al. 2003; Lefebvre and Pasquerault 2004). Bearing the current results in mind, future studies should aim for a relative temporal precision of about 10%, which translates into a sampling interval of about 2 hr before hatching, about 3 hr before first ecdysis, and about 6 hr before second ecdysis.

Acknowledgements

I thank Iain Patterson and Kierryn Gendall for their assistance in sampling; Koji Chiba for translations of references published in Japanese.

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Table 2.1 Details of twelve studies of development in *C. megacephala*. H = hatching, E1 = 1st ecdysis, E2 = 2nd ecdysis, W = onset of wandering, P = onset of pupariation, A = eclosion.

Source	Diet	Locality	Analysis	Temperatures (°C)	Developmental Events
Bohart and Gressitt 1951	c-ration stew	Guam	Minimum	29.4	H, W, P, A
Gabre et al. 2005	raw beef	Egypt	Raw data	26	H, W, P, A
Goodbrod and Goff 1990	cow liver	Hawaii	Mean	23.5	E1, E2, W, P, A
Khole 1979	unknown	India	Raw data	27	P, A
Levot et al. 1979	ox liver	Australia	Mean	27.5	P
Lunt 2002	chicken liver	South Africa	Mean	20, 25	E1, E2, W, P, A
Nishida 1984	horse meat	Japan	Mean	24, 25, 30, 35	E1, E2, W, P, A
O'Flynn, 1983	pet mince	Australia	Approximation, minimum and maximum	28	W, P, A
Prins 1982	unknown	South Africa	Mean, minimum and maximum	26	H, E1, E2, W, P, A
Subramanian and Mohan 1980	"moistened meat"	India	Minimum and maximum	25.6	H, E1, E2, W, P, A
Wells and Kurahashi 1994	pig liver	India	Mean	27	H, E1, E2, W, P, A
Wijesundara 1957	unknown	Sri Lanka	Unknown	26	A

Table 2.2 Development Zero (D_0) and Thermal Summation (K) constants for six developmental events for *C. megacephala* using the method described by Ikemoto and Takai (2000). Hatching is reported in hr°C. Pairs of constants in bold are statistically significantly different at $\alpha = 0.05$.

	Developmental Zero		Thermal Summation Constant		R^2	N used by analysis	N rejected by analysis
	°C	Std. Err.	d°C	Std. Err.			
Hatching							
Pooled data	12.26	1.23	195.83 (hr°C)	20.34	0.99	3	3
1st ecdysis							
Pooled data	16.75	1.56	4.86	0.65	0.99	4	5
This study	12.49	0.98	14.70	1.96	0.99	4	3
2nd ecdysis							
Pooled data	**16.49**	1.46	**11.67**	0.97	0.97	4	5
This study	**10.57**	1.12	**40.16**	3.83	0.94	7	0
Wandering							
Pooled data	**19.32**	1.07	**27.66**	4.16	0.99	4	9
This study	**10.68**	1.25	**96.05**	10.49	0.93	7	3
Pupariation							
Pooled data	**21.67**	0.44	**23.63**	2.77	1.00	4	7
This study	**10.12**	1.67	**113.45**	16.59	0.92	6	2
Eclosion							
Pooled data	14.68	2.22	**104.75**	24.05	0.89	8	0
This study	10.40	1.60	**207.94**	27.96	0.91	6	0

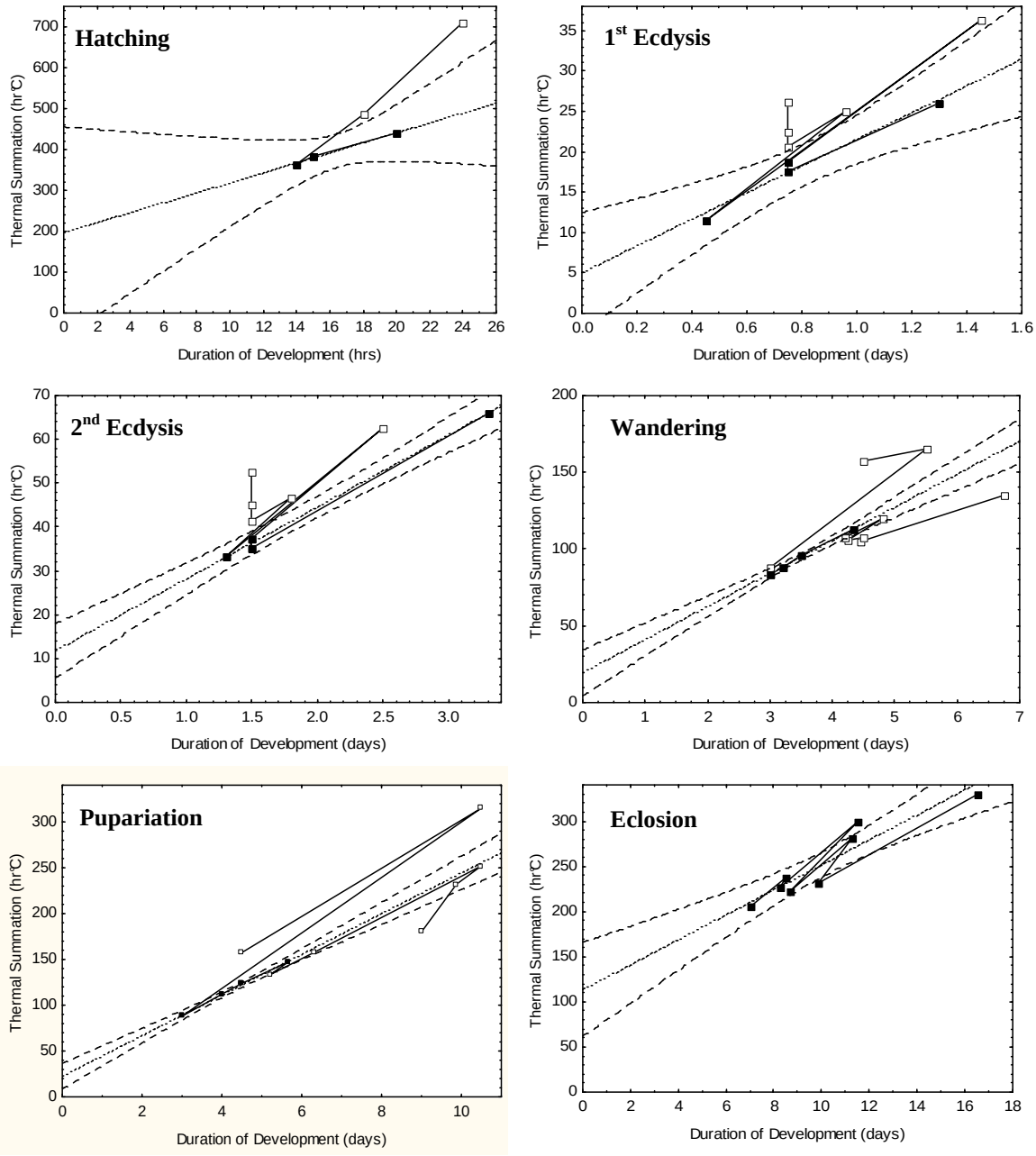

Figure 2.1 Relationships between temperature and development time of *C. megacephala* based on published data (Table 2.1), and major axis regression lines used to determine *K*– and D_0-values for five developmental events. Developmental time (days) for 1st ecdysis – eclosion excludes developmental time (hours) for hatching. Closed symbols mark points used in the regression calculations; open symbols mark points not used in the calculations because they lie beyond the upper and lower critical temperatures of the linear section of the developmental curve (Ikemoto and Takai 2000).

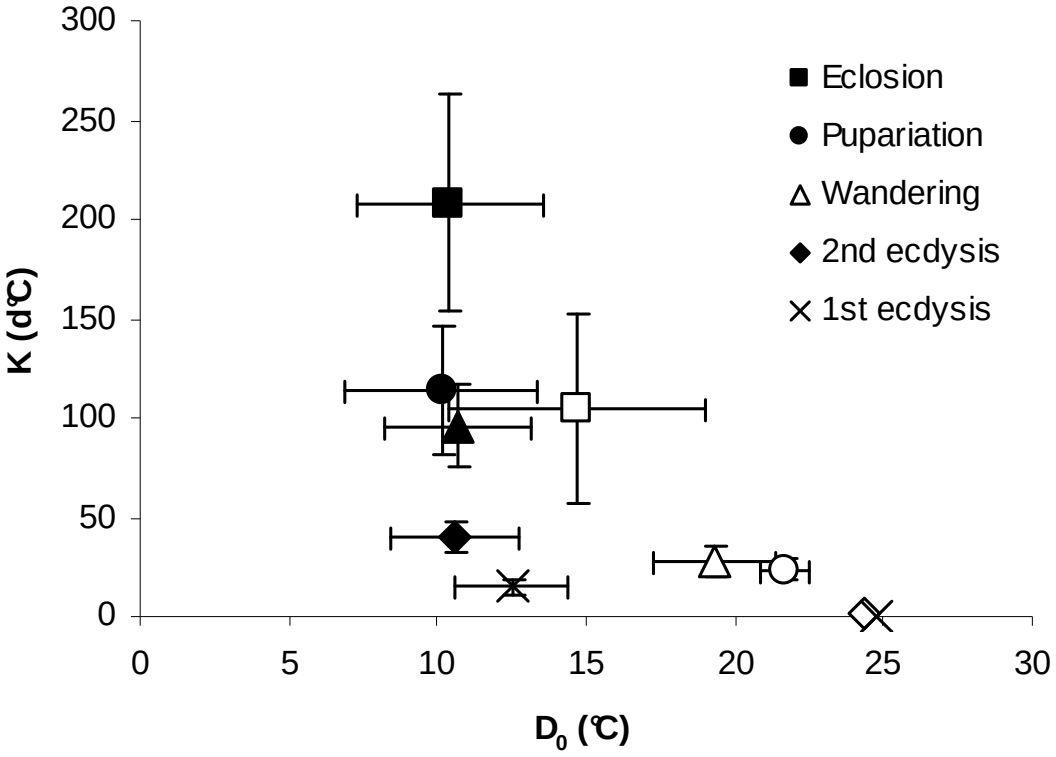

Figure 2.2 *K*– and D_0–values for various developmental events in *C. megacephala*, estimated from data from published studies (open symbols) and data from the current study (filled symbols), with 95% confidence intervals. Where the confidence intervals do not overlap, the estimates differ at $\alpha = 0.05$.

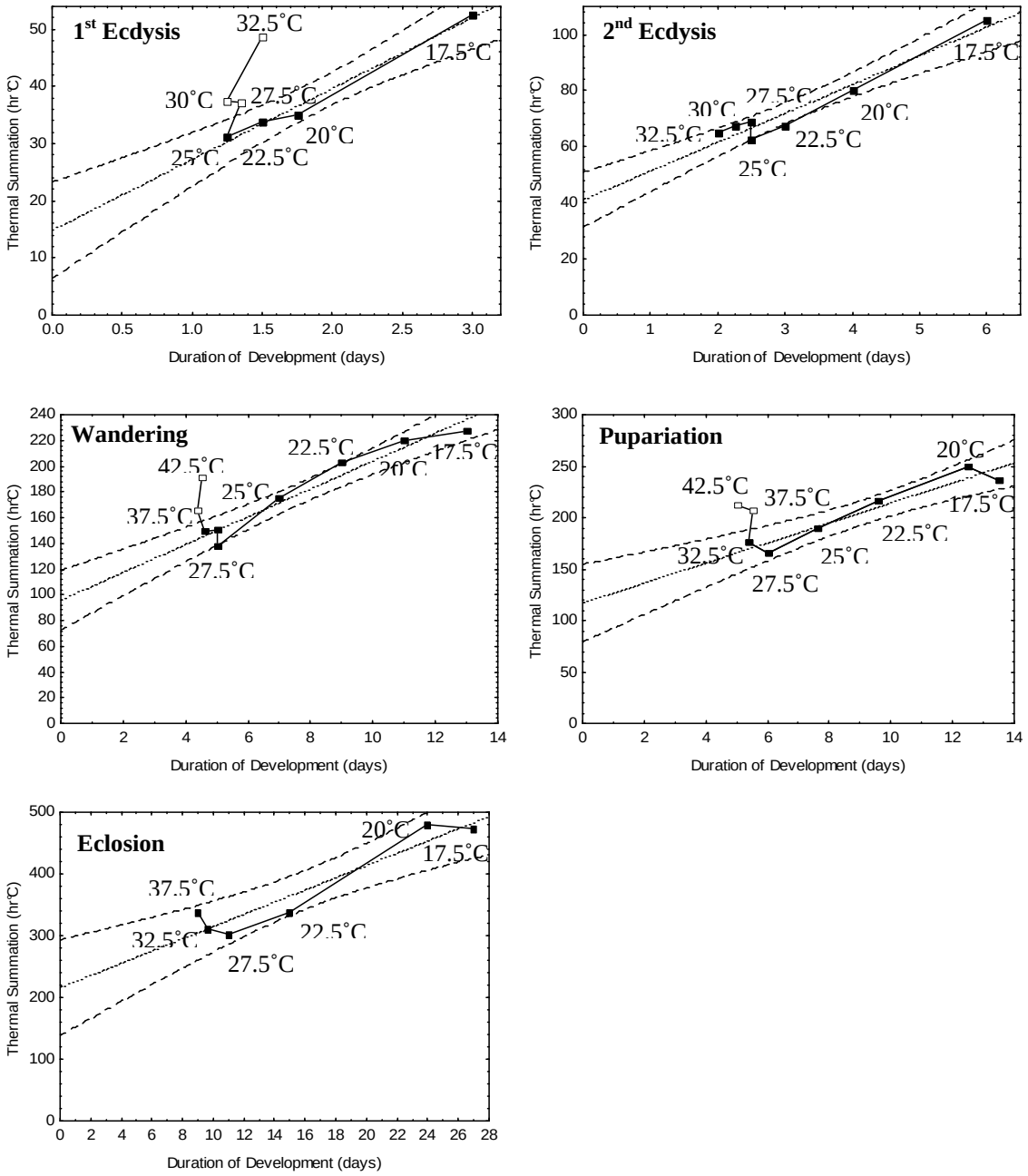

Figure 2.3 Relationships between temperature and development time of *C. megacephala* at nine constant temperatures, and major axis regression lines used to determine *K*– and D_0–values for five developmental events. Closed symbols mark points used in the regression calculations; open symbols mark points not used in the calculations because they lie beyond the upper and lower critical temperatures of the linear section of the developmental curve (Ikemoto and Takai 2000).

III

Factors affecting accuracy and precision of thermal summation models of insect development used to estimate postmortem intervals

Preface

This chapter critically analyses methods of collecting developmental data to establish a single, reliable approach that yields the most accurate and precise thermal summation constants. It attempts to resolve one of the areas that influence data quality and the compatibility of different data sets that was described in Chapter 2. This chapter has been submitted to *International Journal of Legal Medicine*.

Abstract

This chapter investigates the effects that different summary statistics (minimum, median, mean or maximum), temporal sampling resolutions (duration between sampling events), and sample sizes (number of individuals sampled per sampling event) had on the accuracy and precision of the regression coefficients of a typical thermal summation model used to calculate minimum post mortem interval (PMI). No significant differences were found in the values of the developmental constants calculated from different summary statistics of the duration of development. Sample size was found to affect the precision of measurement of the duration of development but had little overall influence on thermal summation constant (K) and developmental threshold (D_0) calculations (and therefore, subsequent PMI estimates), but temporal sampling resolution had a direct

influence on the accuracy of K and D_0 calculations. These data suggest that when numbers of experimental maggots are limited, it is more important to sample more frequently using smaller sample sizes than to sample less frequently with large sample sizes. Furthermore, I suggest that the median is the most representative summary measure of the duration of development and should be used preferentially.

Introduction

The process of obtaining minimum PMI estimates is summarized in Fig. 3.1. These estimates are commonly made (Fig. 3.1: Analysis 4) using a thermal summation model like that described in Chapter 2. If larval lengths are available, the isomorphen diagram estimate can be refined using an isomegalen diagram (Reiter 1984). The thermal summation method relies on the analysis of development data from a minimum of six constant temperatures in the near-linear section of the temperature-growth response curve to make accurate estimates of the minimum PMI (Ikemoto and Takai 2000). Few authors have published development data for more than five constant temperatures, and then only for a handful of forensically important blowfly species (Chapter 2).

Furthermore, the inter-sample interval of published studies has ranged between 5 minutes (Bourel et al. 2003) and 3 hours (Wall et al. 1992) for egg development, and between 2 hours (Ames and Turner 2003) and 24 hours (Davies and Ratcliffe 1994) for larval development. This represents a range of precisions in temporal sampling resolution. A related issue is the effect of changing relative error in temporal precision as the insects age: a 1 hr error in measuring the duration of development a day after hatching is far more serious than a 1 hr error after two weeks of development. The numbers of larvae or pupae in each sample also varies between studies, with Byrd and Butler (1996, 1997) sampling as few as 2 individuals per subsample, per sampling event, and Queiroz (1996) sampling as many as 50 individuals per sampling event. This represents another aspect of precision and data quality that needs attention.

Other problems may lie in the different analytical approaches taken to characterise and summarize the duration of development, namely representing the durations of life stages using minima (Dasgupta and Roy 1969; Greenberg 1991; Davies and Ratcliffe 1994), medians (Ash and Greenberg 1975; Bourel et al. 2003), modes (Kamal 1958), means (Dallwitz 1984; Al-Misned 2001; Ames and Turner 2003) or maxima (Ullyett 1950; So and Dudgeon 1989; Anderson 2000). These summary measures differ in the accuracy with which they represent the true trend in development.

Amendt et al. (2007) provide a detailed account of standard procedures for collecting and processing entomological evidence. This follows numerous other publications detailing the same objective (Catts and Haskell 1990; Greenberg 1991; Bryd and Castner 2001; Greenberg and Kunich 2002), but little attention has focused on best methods for experimental data collection. This paper investigates how the collection and summary of experimental data affects the accuracy and precision of developmental models for *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae), a blowfly found in most parts of Africa (Zumpt 1965), where it feeds prolifically on carrion, giving it forensic importance (Ullyett 1950; Baumgartner and Greenberg 1984; Meskin 1986).

Materials and Methods

Data collection

Development data for *C. chloropyga* (Wied.) were collected as described in Chapter 2, at eight constant temperatures of 15˚C, 17.5˚C, 20˚C, 22.5˚C, 25˚C, 27.5˚C, 30˚C, and 32.5˚C. Every four hours for the first 48 hours, and every eight hours from then to pupariation, one maggot was removed from each of five random cups at each temperature and its instar was recorded.

Analytical methods

First, to compare the effects of using different summary statistics (Fig. 3.1: Analysis 1), all of the raw data were used to calculate the minimum, median, mean and maximum

durations between hatching and either 1st ecdysis, 2nd ecdysis, wandering, pupariation and eclosion at each temperature. The mode was not calculated because the samples were capped at predetermined numbers of maggots and therefore could not represent the distribution of development times.

Next, to gauge the effects of precision and relative error of temporal sampling, only the first sample of maggots of each day was used to calculate the same summary measures. This maintained the sample size at 5 larvae per event but decreased the temporal precision from four or eight hours to 24 hours.

Finally, to examine the effect of sample size, all of the larvae sampled within a day were pooled as though they were all collected at the end of the day, and the summary statistics were recalculated. This increased the sample size to 30 larvae per sampling event in the first two days and to 15 larvae per sampling event thereafter, but decreased the temporal precision of sampling from four or eight hours, respectively, to 24 hours.

Using reduced major axis regression (Ikemoto and Takai 2000), the developmental threshold (D_0) (slope) and thermal summation constant (K) (y intercept) of each developmental event (and the associated 95% confidence intervals) were estimated for each summary statistic for each sampling design.

Results

The mean coefficient of variation (R^2) for the regressions (Fig. 3.2) did not vary significantly between the four summary statistics (Main effects ANOVA, $F_{3, 50} = 2.53$, $p = 0.067$), the two temporal precisions ($F_{1, 50} = 1.17$, $p = 0.285$) or the two sample sizes ($F_{1, 50} = 0.75$, $p = 0.390$), but the mean R^2 for first ecdysis (0.926) was significantly lower than those of the other developmental events ($F_{4, 50} = 16.77$, $p = 0.000$), which all lay between 0.98 and 0.99. This last effect was due to the large relative error in measuring the timing of the first ecdysis. Thus, the overall quality of the models was generally excellent, with little to choose between most of them.

Effects of different summary statistics

Estimates of D_0 of each developmental event did not differ significantly between any of the four measures of duration within each sampling design, since all of their 95% confidence intervals overlapped (i.e. within graphs in Fig. 3.2), but showed wider variation between measures when the relative error in duration was large, i.e. for early developmental events. The minima always produced the lowest estimates of *K*, and the maxima the highest, but this pattern was not seen in the estimates of D_0 (Fig. 3.2).

K differed significantly at 1st ecdysis between maximum data and minimum data (Fig. 3.2c); at wandering phase between maximum data and minimum, median and mean data (Fig. 3.2i), and between mean data and minimum, median and maximum data (Fig. 3.2i); and at pupariation between maximum data and minimum data (Fig. 3.2j). *K* did not differ significantly within any of the durations of development between any of the four measures of duration for the 24-hour precision samples with the smaller sample size (within the middle row in Fig. 3.2).

Effects of temporal precision

With a few minor exceptions that did not form a consistent pattern, the estimates of *K* and D_0 also did not differ significantly between each sampling design i.e. between the top and middle graphs in the same column of Fig. 3.2.

However, *K* increased and D_0 decreased between developmental events (i.e. between graphs within rows in Fig. 3.2), and the associated 95% confidence intervals were consistently narrower, for both *K* and D_0, when the relative error of the sampling was larger i.e. for earlier developmental events, namely 1st ecdysis, 2nd ecdysis and wandering. This is most easily seen by comparing the estimates for 1st ecdysis (with relative errors of up to 24 hr in a day, i.e. 100%) with those for eclosion (with relative errors never more than 24 hr in six days, i.e. 17%).

Effects of sample size

Without focusing on the measures of duration, increased sample size failed to improve the precision of the K and D_0 calculations as both sample sizes produced equally large 95% confidence intervals.

Discussion

Effects of different summary statistics

No significant differences were found in the coefficients of determination or the values of the D_0 calculated from different summary measures of the duration of development (Fig. 3.2). This could be attributed to a Type II error, a failure to detect actual differences through lack of statistical power because sample sizes were too small. Overcoming this would require using more temperatures in the regression models. In the case of estimating D_0, a larger sample is unlikely to have much effect because there was no consistent pattern of variation in the estimates made from the various summary measures (Fig. 3.2), and this source of inaccuracy in the estimation of minimum PMIs is of little concern.

On the other hand, minima and maxima always produced the lowest and highest estimates of K, respectively (Fig. 3.2), which is a logical relationship that might indicate a Type II error. For that reason, it also indicates that some discrimination needs to be shown in choosing a summary measure of duration of development, especially if the number of temperatures used in a particular regression model does provide adequate statistical power.

Any particular investigation will need to estimate a window for minimum PMI (Amendt et al. 2007), and perhaps for this reason, authors have published their results using various summary measures of duration (listed in the introduction). This means that there is currently no single standard measure of tendency accepted in the forensic entomology literature. Accuracy is defined as “the closeness of a measured or computed value to its true value” (Sokal and Rohlf 2005) and, unfortunately, the measure of tendency chosen to represent each sampling population can have a significant effect on the accuracy of the K-

value (e.g. Fig. 3.2c, f, g, i), to represent the true duration of development and thus the accuracy of the minimum PMI estimate. For this reason it is important to choose the most representative measure of tendency of the true duration of development.

It is commonly recommended to estimate minimum PMIs using a large sample (i.e. at least 30 individuals) of the oldest immature blowflies from a case (Amendt et al. 2007), as they are considered to be the most representative indicator of the first oviposition event. These would include the largest feeding larvae, first larvae to enter wandering phase, first puparia to form, etc.

Unfortunately these recommendations have been practiced during collection of experimental data, and several studies have sampled the largest feeding blowfly larvae as the most representative sample for that population (Byrd and Butler 1996, 1997, 1998; Grassberger and Reiter 2001; Greenberg and Kunich 2002). Such samples would represent maxima. Minimum and maximum data represent outlying values (Sokal and Rohlf 2005) of the lower and upper extremes of the duration of development Because the largest immature blowfly is an outlier, it is not the most representative measure of the duration of development and should not be used be used to calculate minimum PMI from thermal summation models.,

For these same reasons, PMI should not be estimated using minimum and/or maximum data, especially if any window of precision (e.g. a standard error) is incorporated into the estimate. An alternative measure of tendency (discussed below), from a large sample size (i.e. at least 30 maggots where possible) of the oldest immature blowflies from a case should be used to estimate the minimum PMI (Amendt et al. 2007), and that the appropriate lower and upper bounds (e.g. 95% confidence intervals) of the regression equation should be used to calculate the relevant window for PMI (Fig. 3.1).

A mean developmental duration represents the average time at which individuals are observed to experience developmental change (Hampton 2003), i.e. the average duration of pupariation or the average duration of wandering. Sokal and Rohlf (2005) advocate the

use of the mean in biological statistics as it is easy to calculate, and it has a smaller standard error (i.e. higher precision) than other measures of tendency. However, means are sensitive to outlying minimum and maximum values because of the way means are calculated. When the data are symmetrical, the mean gives an accurate summary of the duration of development (Fig. 3.3a) but if the data are skewed the mean is affected by the outliers and becomes a particularly skewed estimate of the general trend of development (Fig. 3.3b) (Sokal and Rohlf 2005). Nishida et al. (1986) showed that such skewing is not unusual in blowfly development. For this reason, mean developmental durations are not ideal for calculating K and D_0.

The mode is a measure of tendency that is statistically robust to the effects of outlying values (Sokal and Rohlf 2005, Flower et al. 1998). It refers to the most common duration of development within the experimental population (Hampton 2003), i.e. the most common duration to pupariation or the most common duration to wandering. But, to obtain a reliable mode for K and D_0, the sample size at each sampling event must be at least 30 individuals if it is to represent the underlying frequency distribution (Sokal and Rohlf 2005). This radically increases the total number of individuals needed for an experiment, which is often not feasible when conducting development studies at more than two temperatures. Another problem when using the mode is that it is not uncommon to have more than one mode (bimodal or multimodal) at any experimental event, possibly due to sexual dimorphism or circadian rhythms, resulting in two different PMI estimates with identical statistical support (Sokal and Rohlf 2005). For these reason, modal developmental data are not ideal for the routine calculation of K and D_0.

The median is a measure of tendency that is also robust to the effects of outlying values (Hampton 2003). It refers to the time at which 50% of all individuals experience developmental change (Hampton 2003), i.e. the time taken for 50% of the population to reach 1st ecdysis or pupariation. Although medians have a larger standard error (i.e. less precision) than means for purely mathematical reasons, this has only minor implications for calculating thermal summation models. For this reason it is proposed that the median is the most representative measure of the duration of development when analyzing both

symmetrical and skewed data, and would provide the most reliable routine summary data for estimating K and D_0.

Effects of temporal and sampling precision

Precision is defined as "the closeness of repeated measurements of the same quantity to each other" (Sokal and Rohlf 2005), and can be represented by measures such as standard errors. There are three sampling strategies for developmental studies that affect the precision of the duration data and the precision of K and D_0 calculations, namely, sample size (number of individuals sampled per sampling event), temporal sampling resolution (duration between sampling events), and the number of constant temperatures used to calculate K and D_0 (Ikemoto and Takai 2000). If experimental sampling methods rely on killing maggots to measure them, and sample size and temporal sampling resolution are constrained by the number of eggs laid by females, then scientists are faced with an experimental trade-off between more sampling events and smaller sample size, or larger sample sizes and fewer sampling events.

The K and D_0 values of the four summary measures were more similar for smaller sample sizes than those calculated from the greater sample size (compare the middle and bottom graphs in the same column of Fig. 3.2), particularly when the relative error of the sampling was larger i.e. for earlier developmental events. Furthermore, 95% confidence intervals of K and D_0 were generally narrowest when the temporal sampling resolution was high (in the top row of Fig. 3.2). Therefore, an increase in the sample size affects the precision of measurement of the duration of development, but because it is not itself a direct measure of time, it will not influence the K and D_0 values (and subsequent estimates of PMI) as much as temporal sampling resolution. Therefore, to increase the precision of the K and D_0 values, and subsequent PMI estimates, it is more important to sample more frequently using fewer samples than to sample less frequently using more samples. This is empirically evident from comparing the top and middle rows of each column of Fig. 3.2.

It has been shown elsewhere that the precision of the K and D_0 values can be further increased by increasing the number of temperatures along the regression line used to calculate them (Ikemoto and Takai 2000). Both their precision and their accuracy can be improved by covering as much as possible of the range of temperatures on the linear section of the temperature-growth response curve (Villet et al. 2006).

For the most accurate K and D_0 estimates, sampling frequencies should be substantially shorter than the duration of each developmental event to counteract the effects of relative error. Any remaining uncertainty in K and D_0 is then inherent biological variation that is outside the control of experimental design. A relative error of about 10% in the resolution of temporal sampling of the total development time for each event is an optimal trade-off between effort and accuracy (Richards et al. 2007). This translates to about 2-6 hours for 1st ecdysis, 4-8 hours for 2nd ecdysis, 8-12 hours for wandering and 12-24 hours for pupariation and eclosion, depending on temperature.

Suggestions made in this chapter may be viewed as standards for collecting development data in forensic entomology and complement those standards and guidelines put forth in Amendt et al. (2007), but I recommend other authors test the validity of these findings with other blowfly species/populations

Acknowledgements

I thank Kendall Crous for assistance in data collection.

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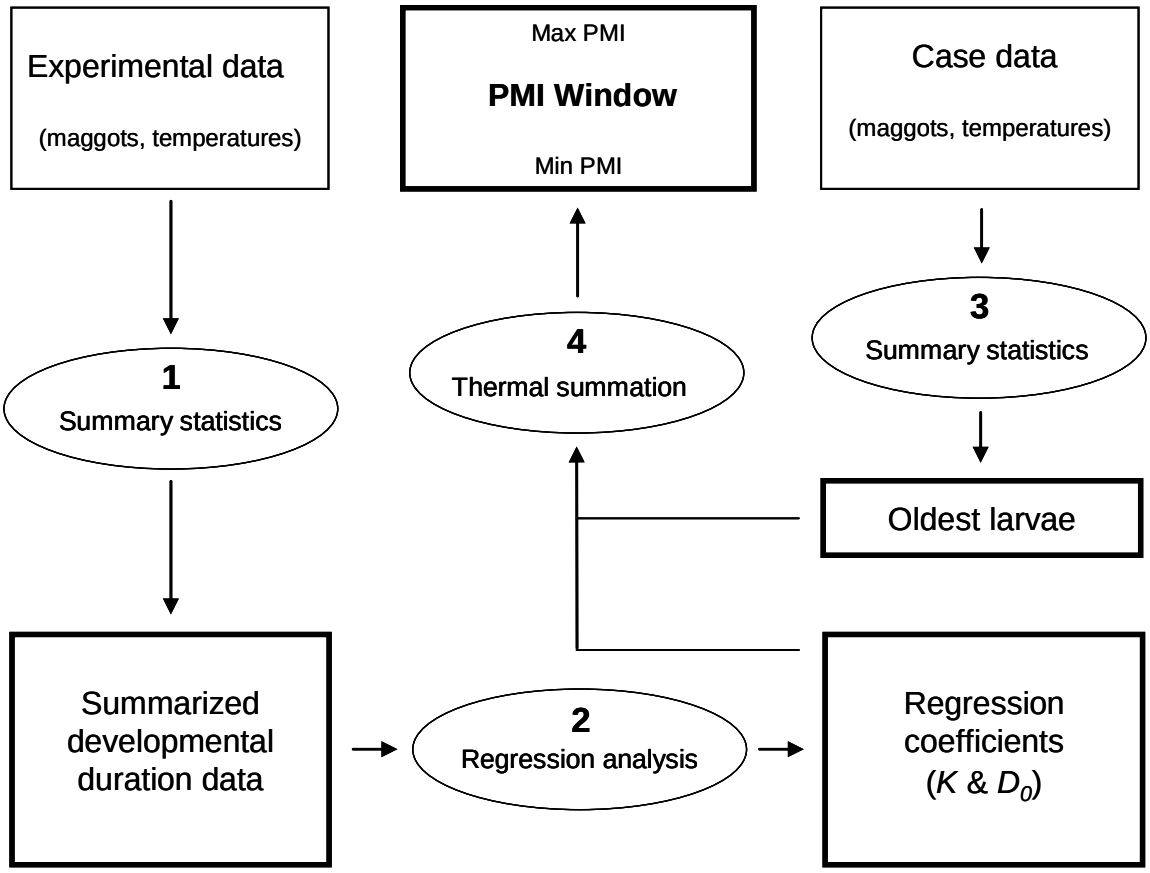

Figure 3.1 Flow diagram summarizing the data sources and analytical process involved in estimating postmortem intervals using a thermal summation model. Rectangles with light outlines represent raw data, rectangles with heavy outlines represent processed data, and ellipses represent analytical steps.

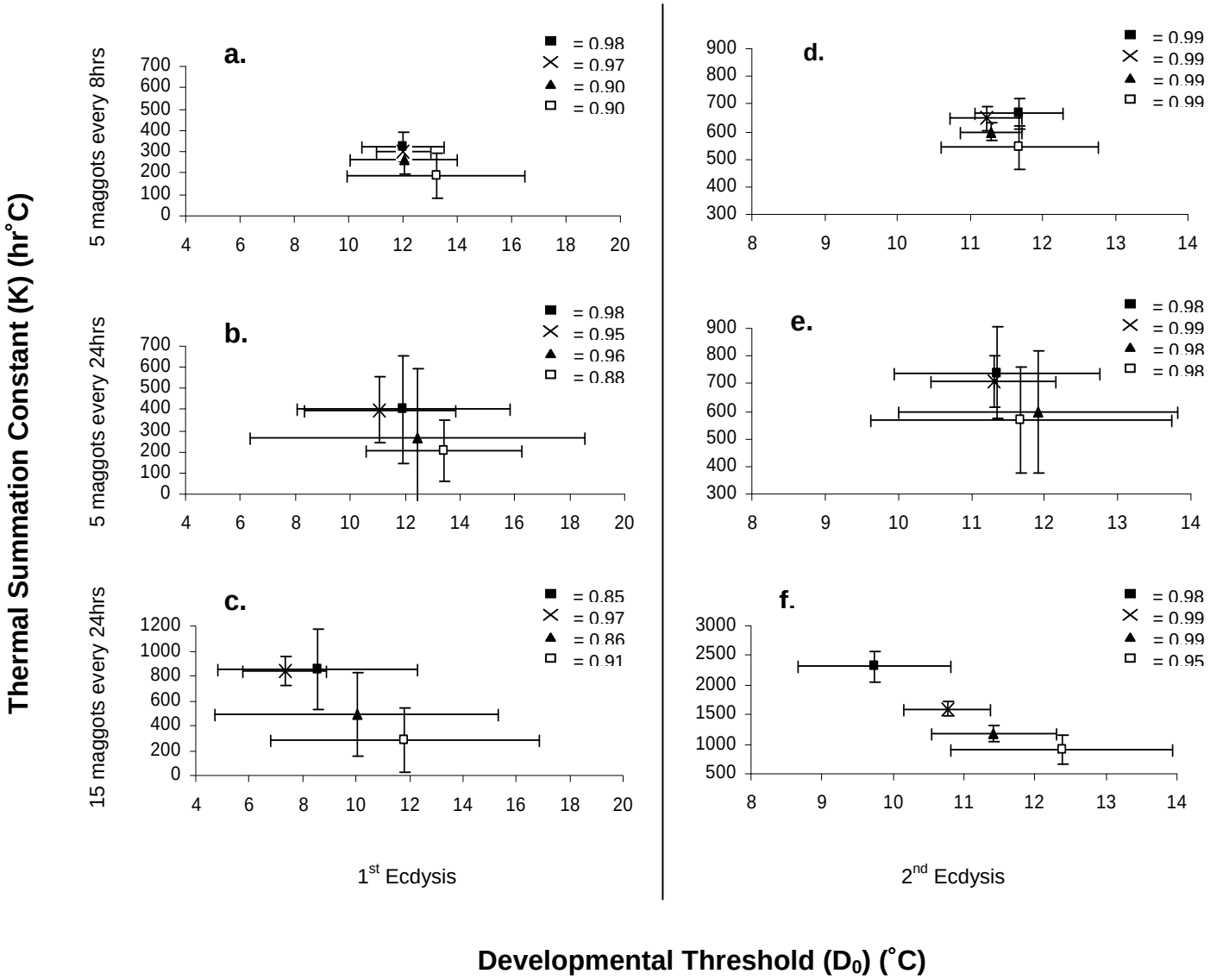

Figure 3.2 continued

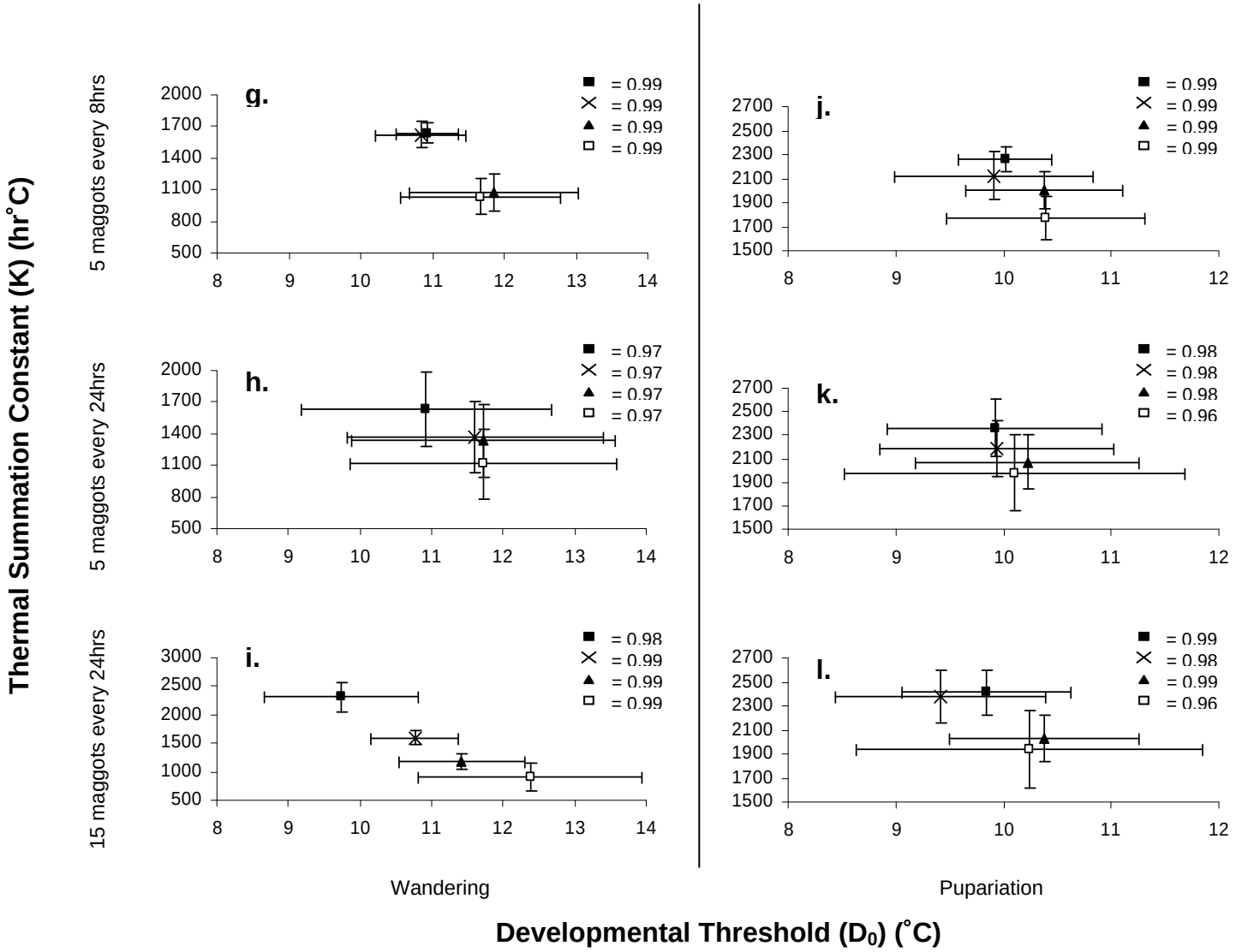

Figure 3.2 continued

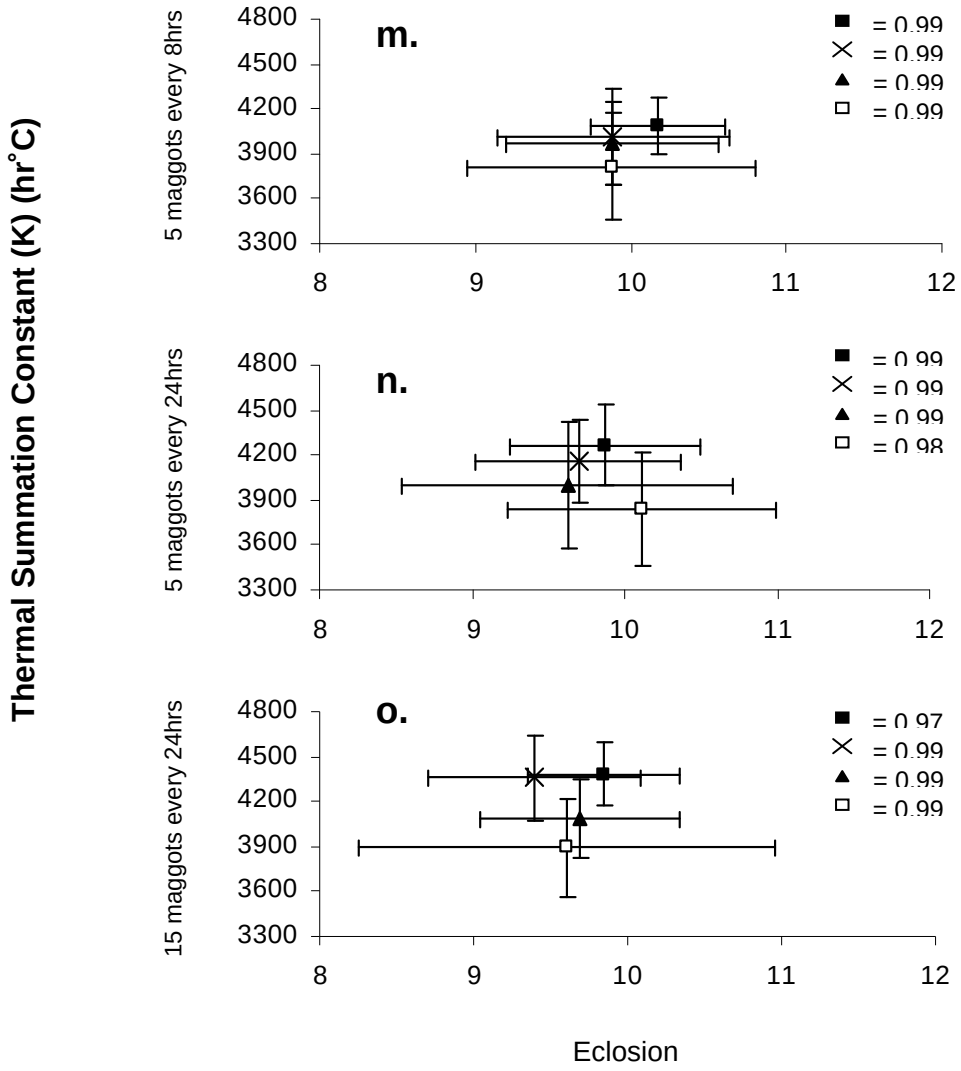

Figure 3.2 Scatterplots of D_0 and K estimated for 1st ecdysis, 2nd ecdysis, onset of wandering, pupariation, and eclosion, from the minimum (open square), median (closed triangle), mean (cross) and maximum (closed square) time taken to reach those developmental events, using data of differing temporal precision and sampling density. Error bars represent 95% confidence intervals. (Note: the y-axis for 'c', 'f' and 'i' are at different scales to those of the graphs above in the same column). Coefficients of determination (R^2) of the regressions used to calculate D_0 and K values for each measure of tendency are given in each figure.

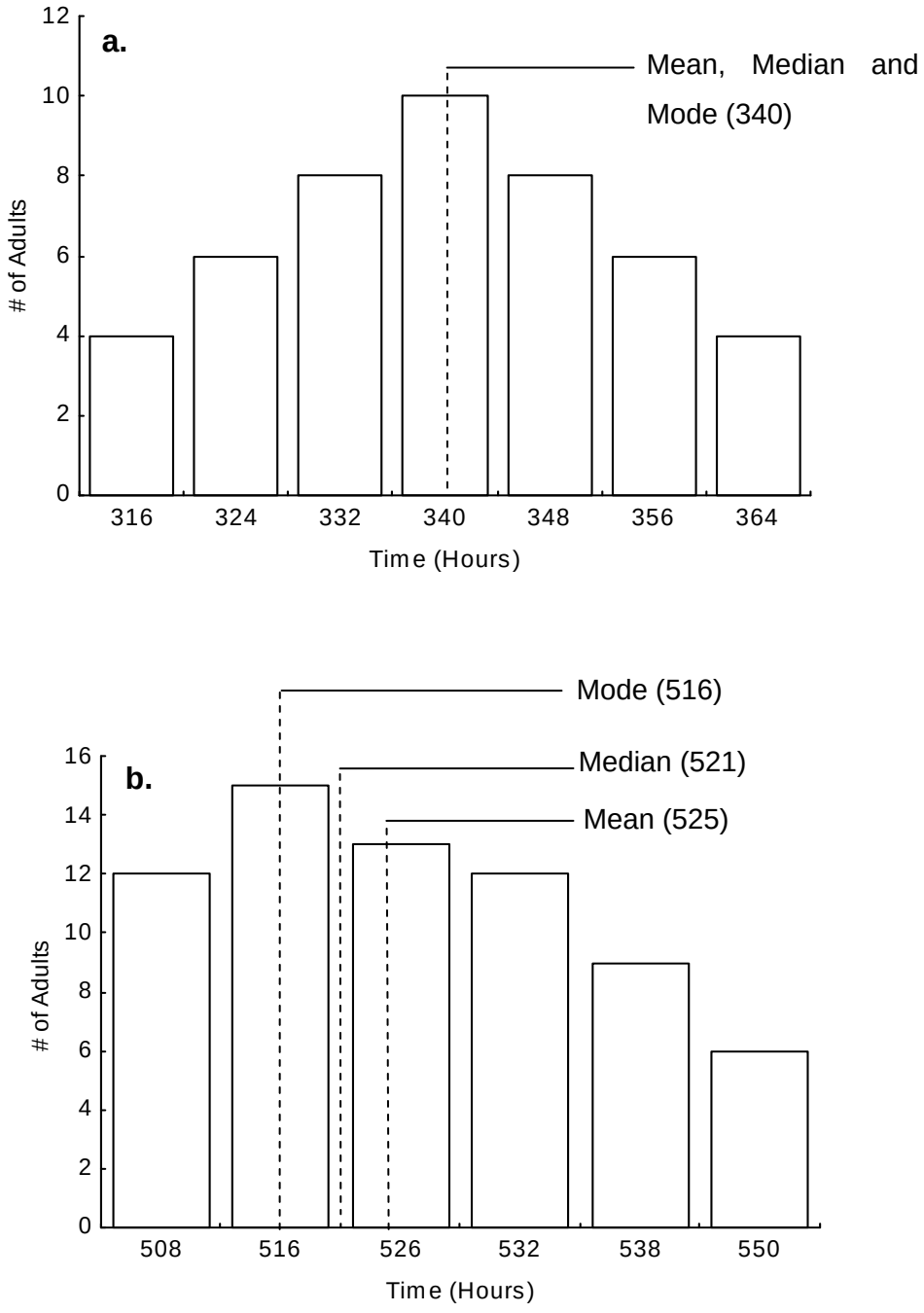

Figure 3.3 Relative positions of the mean, median and mode of hypothetical samples with distributions that are a) symmetrical. b) positively skewed.

IV

Estimating the age of immature Chrysomya albiceps (Diptera: Calliphoridae), correcting for temperature and geographical latitude

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Preface

Chapter 4 provides one explanation for why development data of a single species differ between studies, and attempts to resolve the remaining two areas that influence the compatibility of different data sets that is described in Chapter 2. This chapter was presented at the 5th Meeting of the European Association for Forensic Entomology and published in *International Journal of Legal Medicine* (doi http://dx.doi.org/10.1007/s00414-007-0201-7).

Abstract

Developmental curves for *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) were established at 13 different constant temperatures using developmental landmarks and length as measures of age. The thermal summation constants (K) and developmental zeros (D_0) were calculated for five developmental landmarks using the method described by Ikemoto & Takai (2000). Comparison with the K and D_0 values of these findings to those of three previously published studies of *C. albiceps* suggests that K is directly proportional to geographic latitude, and D_0 is inversely proportional to both K and geographic latitude. Body size and developmental landmarks have a complex relationship because of trade-offs between mortality risk and female fecundity (as measured by body size) at non-optimal temperatures. This relationship can be summarised using

superimposed isomorphen and isomegalen diagrams, which can then be used to make forensic estimates of postmortem intervals from larval body lengths. Finally, future studies providing data for precise forensic estimates of postmortem intervals should use a relative temporal precision of about 10% of the total duration being measured. For many blowflies this translates into a sampling interval of approximately every 2h before hatching, 3h before 1st ecdysis, and 6h before 2nd ecdysis.

Introduction

Chrysomya albiceps (Weid.) is widely distributed throughout Africa, South America and parts of Europe and Asia (Zumpt 1965; Laurence 1981; Povolný 2002; Grassberger et al. 2003; Verves 2004). It causes myiasis in humans and livestock, feeds on carrion and human faeces and breeds prolifically in carrion (Zumpt 1965; Grassberger et al. 2003), making it a medically, veterinarily, sanitationarily and forensically important fly. It is also ecologically significant, being a predator of other dipteran larvae, and its maggots are frequently found around the periphery of corpses (Ullyett 1950; Braack and Retief 1986).

Despite the importance of an understanding of the rate of development in relation to temperature, few authors have published on this topic using *C. albiceps* (Table 4.1). Queiroz (1996) timed development at four different constant temperatures, Grassberger et al. (2003) timed it at five constant temperatures and Marchenko (1988, 2001) presented calculations for 18 different temperatures from an unpublished data set. Ullyett (1950) and Prins (1982) published anecdotal data at single temperatures. No author recorded developmental rates for more than three developmental events, and the results of the studies are apparently not in agreement.

This chapter presents development rates for *C. albiceps* at 13 different constant temperatures for 5 developmental events, and the associated thermal summation constant (measure of thermal time taken to reach each developmental event, K) and developmental zero (temperature below which development ceases, D_0) values. In addition to this, the K

and D_0 of this study are compared to those of published data to explore the disparities between the studies.

Materials and methods

Data collection

The methods for collecting development data are described in Chapter 2.

Length versus developmental event as a measurement of age

Isomorphen and isomegalen diagrams (Grassberger and Reiter 2001) were constructed using the computer programs "Microsoft Excel 2003" and "Statistica 7" and then overlaid (Fig. 4.1) to compare length and developmental events as estimates of age.

Thermal summation constant (K) and development zero (D_0)

By plotting the cumulative proportion of each phase of development represented in each sample, the median durations of all phases were calculated to a precision of hours. Reduced major axis regression was used to calculate K and D_0 from the median durations (Ikemoto & Takai 2000) using the computer program "Statistica 7". In addition, K and D_0 values were calculated in the same way for three published studies (Marchenko 1988, 2001; Queiroz 1996; Grassberger et al. 2003).

Results

Development rates for both larval and puparial stages increased steadily with increasing temperatures (Fig. 4.1). Larvae reached second instar as late as 7.5 days after hatching at 15˚C and as early as half a day at 40˚C and 42˚C. The longest minimum larval development period (duration to the onset of pupariation) and minimum pre-imaginal development periods (duration from hatching to the onset of eclosion) were 22.5 days and 28 days at 17.5˚C and 20˚C, respectively, and the shortest were 5.5 days and 9 days at 30˚C and 35˚C, respectively (Fig. 4.1).

The largest maggot measured was 16.2mm long at 25˚C. Larvae reached smaller average sizes at other temperatures (Fig. 4.1) and pupariated at significantly shorter lengths below 20˚C ($F = 79.47$; $p = 0.00$). No larvae survived to pupariation at 15˚C or 45˚C, no pupae eclosed at 17.5˚C, 40˚C or 42.5˚C, and eclosion rates were poor above 35˚C (Fig. 4.1). The highest pupariation survival rate was at 20˚C and 22.5˚C, although both the greatest numbers of puparia (49) and adults (48) were both recorded at 32.5˚C.

Length versus developmental event as a measurement of age

The contour of each developmental event in the isomorphen diagram frequently intersects numerous body length contours of the isomegalen diagram, several of which cross more than one developmental contour (Fig. 4.1).

Thermal summation constant (K) and development zero (D_0)

Development data from extreme temperatures are not used in thermal summation models based on linear regression because these points do not lie on the straight section of the developmental curve, and should therefore be excluded from the regression analysis (Ikemoto and Takai 2000; Higley and Haskell 2001). Using criteria described by Ikemoto & Takai (2000), this analyses was limited to co-linear data points for each developmental event (Fig. 4.2), and calculated D_0 and K for five developmental events. The fit of the models was excellent, with high coefficients of determination (Table 4.1) and narrow confidence intervals (Fig. 4.2). D_0 and K were, respectively, 11.14˚C and 655.63h˚C for 1st ecdysis, 13.00˚C and 984.71h˚C for 2nd ecdysis, 13.92˚C and 2238.98h˚C for onset of wandering, 13.65˚C and 2354.34h˚C for onset of pupariation, and 13.64˚C and 4138.74h˚C for eclosion. The average D_0 for all developmental events was 12.99˚C ($n = 5$ events; std. err. = 0.51˚C).

The same analysis was applied to published studies of development in *C. albiceps* using as many points from each study as met the selection criteria (Ikemoto and Takai 2000). All models showed high R^2 values (Table 4.1) and narrow confidence intervals (Fig. 4.3) except for that of eclosion in the Brazilian fly population. For flies from Moscow

(Marchenko 1988; 2001), the average D_0 was 10.21˚C (n = 2 events; std. err. = 0.00˚C); from Vienna (Grassberger et al. 2003), 10.49˚C (n = 3 events; std. err. = 0.59˚C); and from Rio de Janiero (Queiroz 1996), 15.39˚C (n = 3 events; std. err. = 0.58˚C). None of the 95% confidence intervals overlapped, except between Moscow and Vienna, and the values are in the same rank order as the absolute latitudes of the locations (Fig. 4.4a). Values for K were generally inversely proportional to D_0 (Fig. 4.4b), and therefore proportional to absolute latitude (Fig. 4.4a).

Discussion

Developmental optima

Larvae of the Grahamstown population developed poorly outside a temperature range of 20-40˚C. While growth rates were maximal at the highest temperature (Fig. 4.1), survivorship and condition were better at lower temperatures. Similarly, pupae developed best between 20.0-32.5˚C, which is an even narrower temperature window. The sigmoidal shape of plots of temperature against developmental rate indicates that there is an optimum temperature for development, which would lie at the inflection of the sigmoid. Moving away from this optimum, growth becomes increasingly compromised, so that while larvae grow faster at higher temperatures, they incur increasing levels of physiological stress that are expressed as slightly smaller mature sizes and distinctly lower survivorship. Similarly, at lower temperatures development is increasingly retarded to the point where larvae will pupate at uncharacteristically small sizes rather than spend more time attempting to reach their usual mature size (Fig. 4.1). The decrease in survivorship at lower temperatures is more precipitous than it is at higher temperatures (Fig. 4.2), indicating that the physiological stresses that impede growth are different on either side of the optimum.

Length versus landmarks as a measurement of age

As Dadour et al. (2001) pointed out, the length of a maggot is a poor estimator of its physiological or chronological age. This is true of many animals, but some additional

factors are evident in *C. albiceps*. Maggots of *C. albiceps* regularly diminish in length, not only when they cease wandering and begin to pupariate, but also briefly after each ecdysis. Comparable patterns of fluctuation in mass and length were noted by Wells and LaMotte (1995) and Grassberger and Reiter (2001), respectively. The results from this study (Fig. 4.1) suggest that in conducive environmental conditions, maggots will maximize growth in size to maximize fecundity as adults, since larger females are generally more fecund (Romoser and Stoffolano 1998). When environmental conditions are less favourable, maggots adjust their developmental period in a trade-off between risk of mortality and compromised body size (Fig. 4.1). Only when environmental conditions are extremely cold do maggots completely prioritise development time over size, resulting in dwarfed adults. In *C. albiceps*, this occurred at temperatures below 20˚C (Fig. 4.1).

Additionally, maggots decrease in length as they enter the wandering phase (see Grassberger et al. 2003: Fig. 4.2). This can usually be detected and corrected for by examining the crop contents, which are eliminated at the onset of wandering. The practical implication of these physiological phenomena is that there is no simple relationship between size and chronological age in maggots (Dadour et al. 2001). An isomegalen diagram (Grassberger and Reiter 2001) can capture a great deal of the complexity of the relationship, especially if superimposed on an isomorphen diagram (Fig. 4.1), and provides a means of estimating postmortem intervals from maggot length. Unfortunately, few of these diagrams are available. For these reasons maggot age may not be best estimated from their length given our current knowledge (Dadour et al. 2001; Gaudry et al. 2001).

Thermal summation constant (K) and development zero (D_0)

The developmental zeros for the 2nd ecdysis, onset of wandering, onset of pupariation, and eclosion averaged 13.55˚C (std. dev. = 0.39) and did not differ significantly, with a maximum difference of 0.9˚C between the 2nd ecdysis and the onset of wandering. This consistent D_0 value between developmental events is to be expected because the kinetics

of metabolism is unlikely to vary between developmental stages in insects (Sharpe and DeMichele 1977). The estimated developmental zero for the 1st ecdysis was 2.41˚C lower than those of the other events, but its accuracy is compromised as samples of 1st instar larvae were taken with a lower relative temporal resolution than of the other developmental events due to the rapid growth of 1st instar larvae, particularly at high temperatures. Most published studies sampled at fixed intervals that ranged from 4-12 h (e.g. O'Flynn 1983; Nishida et al. 1986; Grassberger and Reiter 2001, 2002; Grassberger et al. 2003; Lefebvre and Pasquerault 2004). Bearing the current results in mind, future studies should aim for a relative temporal precision of about 10% of the total duration being measured, which translates into a sampling interval of about every 2 h before hatching, about every 3 h before the 1st ecdysis, and about every 6 h before the 2nd ecdysis.

The average D_0 and K for *C. albiceps* varied between studies. Reasons for this variation can be attributed to two possibilities, namely differences in rearing conditions, with particular reference to diet of immature stages, or because the populations in each study differed in geographic latitude. Although rearing media are know to affect duration of development (and therefore K) (Kaneshrajah and Turner 2004; Clarke et al. 2006; Ireland and Turner 2006), it is still possible to compare D_0 values from different studies that have used different rearing conditions. This is because D_0 is a measure of temperature only and is not affected be variables influencing time, such as rearing media. Because K is a measure of time, the different rearing media used in different studies must be taken into account when comparing K values.

Kaneshrajah and Turner (2004) tested development of *Calliphora vicina* on liver, lung, heart, brain and kidney tissue of pigs at 20˚C. They found that development rates were significantly slower on liver with an error of up to 2 days (33%) of development when compared to other tissues for the first 6 days. This is presumably because these were the days when the larvae where feeding. However, once the larvae were post feeding the error in development reduced to as little as 10% as all larvae reached pupariation on all rearing media between 9-10 days. Presumably the error will decrease further on eclosion.

Therefore, it is unlikely that rearing media would significantly affect total development time and because the comparison between studies, in this paper, are restricted to the post feeding stages only (pupariation and eclosion), it is unlikely that the diet used by the different studies will significantly affect the comparisons between *K* values.

If rearing media had a significant effect on *K* values, one would expect the *K* values from this study to be most similar to Grassberger et al. (2003) as both used liver, and the *K* values from Marchenko (1988) to be most similar to Queiroz (1996) as both used meat. However this is not the case. The *K* value, for pupariation and eclosion, for this study are closer to Queiroz (1996) value than to Grassberger et al. (2003) (Table 4.1; Fig. 4.1). Therefore, reasons for the varying *K* values for pupariation and eclosion cannot be attributed primarily to rearing media. An alternative is different geographic populations.

The data also suggest that D_0 and *K* are inversely proportional and that *K* is directly proportional to geographic latitude (Fig. 4.4). The inversely proportional relationship between *K* and D_0 has been shown in a number of different plant and animal species (Trudgill 1994, Trudgill and Perry 1994; Trudgill 1995; Honêk 1996a, b, 1999; Bonhomme 2000; Charnov and Gillooly 2003; Trudgill et al. 2005), including some flies (Honêk and Kocourek 1988; Honêk 1996a), but never demonstrated in species of blowflies. Initially Trudgill and Perry (1994) and Trudgill (1995) explained this relationship by proposing that cold-adapted species of high geographic latitude would possess a lower D_0 and would take a longer time to develop than a warm-adapted species of lower geographic latitude and higher D_0. Honêk (1996a) tested this hypothesis with recalculated *K* and D_0 values from literature on 335 insect species. He found weak but significant correlations indicating that D_0 was inversely proportional to geographic latitude in all developmental events, including hatching ($r^2 = 0.150$), larval growth ($r^2 = 0.142$), pupation ($r^2 = 0.040$) and eclosion ($r^2 = 0.121$). A less obvious relationship existed between *K* and geographic latitude, although a positive correlation was found for hatching ($r^2 = 0.034$). Honêk (1996a) concluded that a high D_0 and low *K* are typical thermal characteristics of warm-adapted (tropical) species.

Although these data support these findings, these D_0s exceeded Honêk's (1996a) data by 1.70-2.82˚C for tropical (0-23˚N or S) populations, by 0.74-2.82˚C for subtropical (24-39˚N or S) populations, and by 1.92-2.35˚C for temperate (≥ 40˚N or S) populations. Reasons for these differences could be that Honêk (1996a) did not separate species on eco-physiological criteria, such as seasonality of development in temperate species (Honêk and Kocourek 1988) and size categories, for the analysis.

The positive correlation between *K* and geographic latitude has often been reported in plants, particularly legumes (Angus et al. 1981; Craufurd et al. 1996; Qi et al. 1999), but rarely in insects (Honêk 1996a). These data (Fig. 4.3) present evidence that *K* is directly proportional to geographic latitude but they are based on only one or two points for each climatic zone. Additional studies are required, either at alternative latitudes or replicates of these latitudes (Fig. 4.3) to confirm the pattern. Should these data be obtained, it may be possible to derive a model for the accurate calculation of *K* and D_0 for *C. albiceps* at any latitude. It is likely that Marchenko's (1988, 2001) *K* value for eclosion is an underestimate, on the basis that his estimate of D_0 for pupariation is more or less consistent with monotonic relationships based on the other published studies (Fig. 4.4).

Acknowledgements

I thank Sue Abraham for assistance with graphics, Kierryn Gendall for laboratory assistance.

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Table 4.1 Development zero and thermal summation constants for five developmental events for *C. albiceps* calculated using the analytical method described by Ikemoto & Takai (2000). Studies are placed in order of absolute latitude within events.

	Developmental zero		Thermal summation constant		R^2	N used for analysis	N rejected from analysis
	˚C	Std. err.	h˚C	Std. err.			
Hatching							
Grassberger et al. (2003)	9.72	0.67	258.30	17.39	0.97	5	0
1st ecdysis							
This study	11.14	0.27	655.63	23.03	0.99	7	5
2nd ecdysis							
This study	13.00	0.74	984.71	84.40	0.98	7	4
Onset of wandering							
Queiroz (1996)	15.01	0.54	1593.72	160.75	1.00	4	0
This study	13.92	0.99	2238.98	210.56	0.98	7	4
Onset of pupariation							
Queiroz (1996)	14.63	0.64	1997.09	212.58	1.00	4	0
This study	13.65	0.68	2354.34	207.55	0.99	7	4
Grassberger et al. (2003)	11.65	0.08	2819.00	15.48	1.00	4	0
Marchenko (1988)	10.21	-	2948.11	-	-	18	0
Eclosion							
Queiroz (1996)	16.52	0.49	2845.89	286.54	1.00	3	0
This study	13.64	1.14	4138.79	429.27	0.97	7	1
Grassberger et al. (2003)	10.10	0.53	7861.04	169.29	0.99	4	0
Marchenko (1988)	10.21	-	4442.40	-	-	18	0

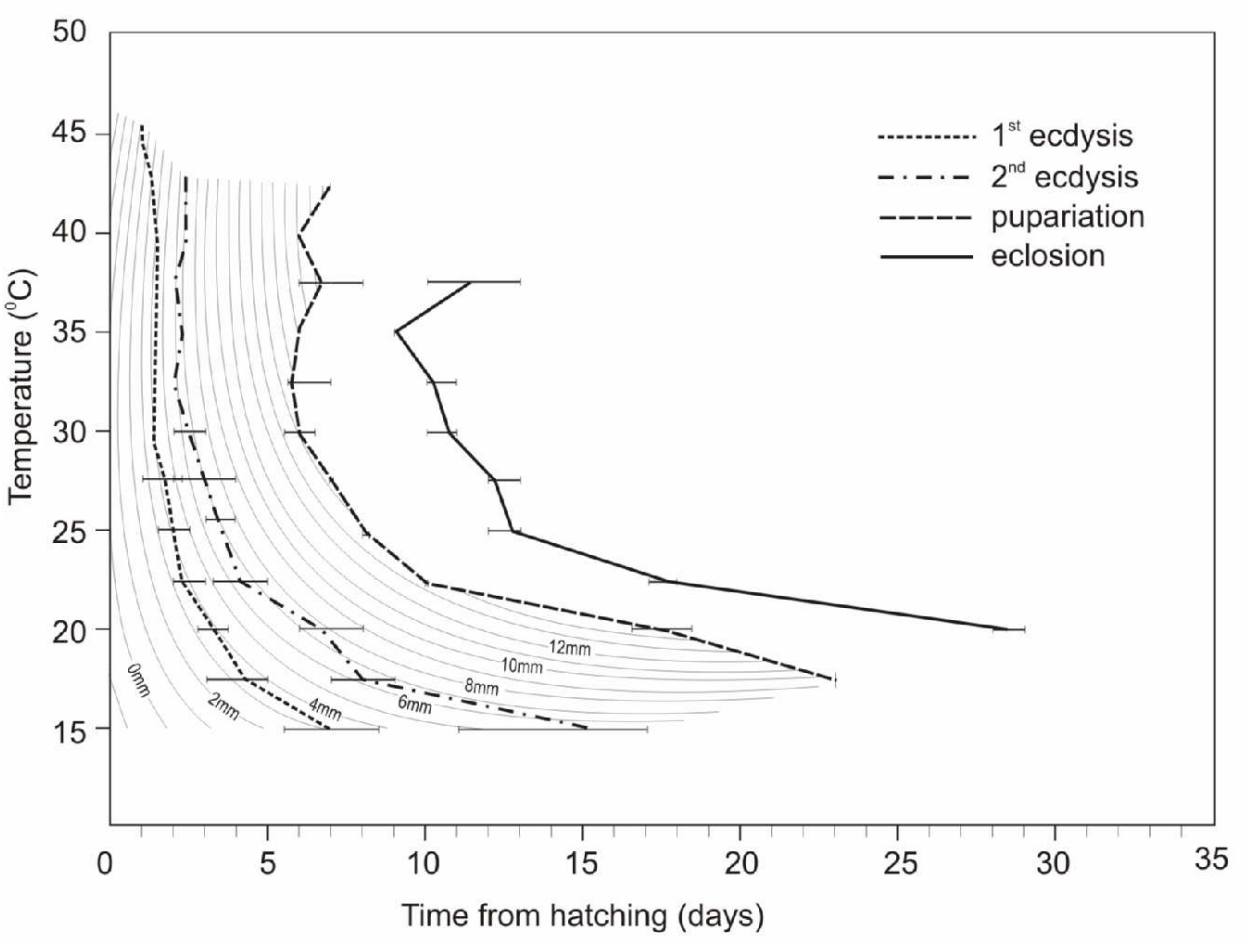

Figure 4.1 Superimposed isomorphen and isomegalen diagrams. The contours of the isomegalen diagram are interpolated using the "distance weighted" method. Error bars on the isomorphen diagram represent 95% confidence intervals. Note the poor correspondence between length and developmental stage at low temperatures.

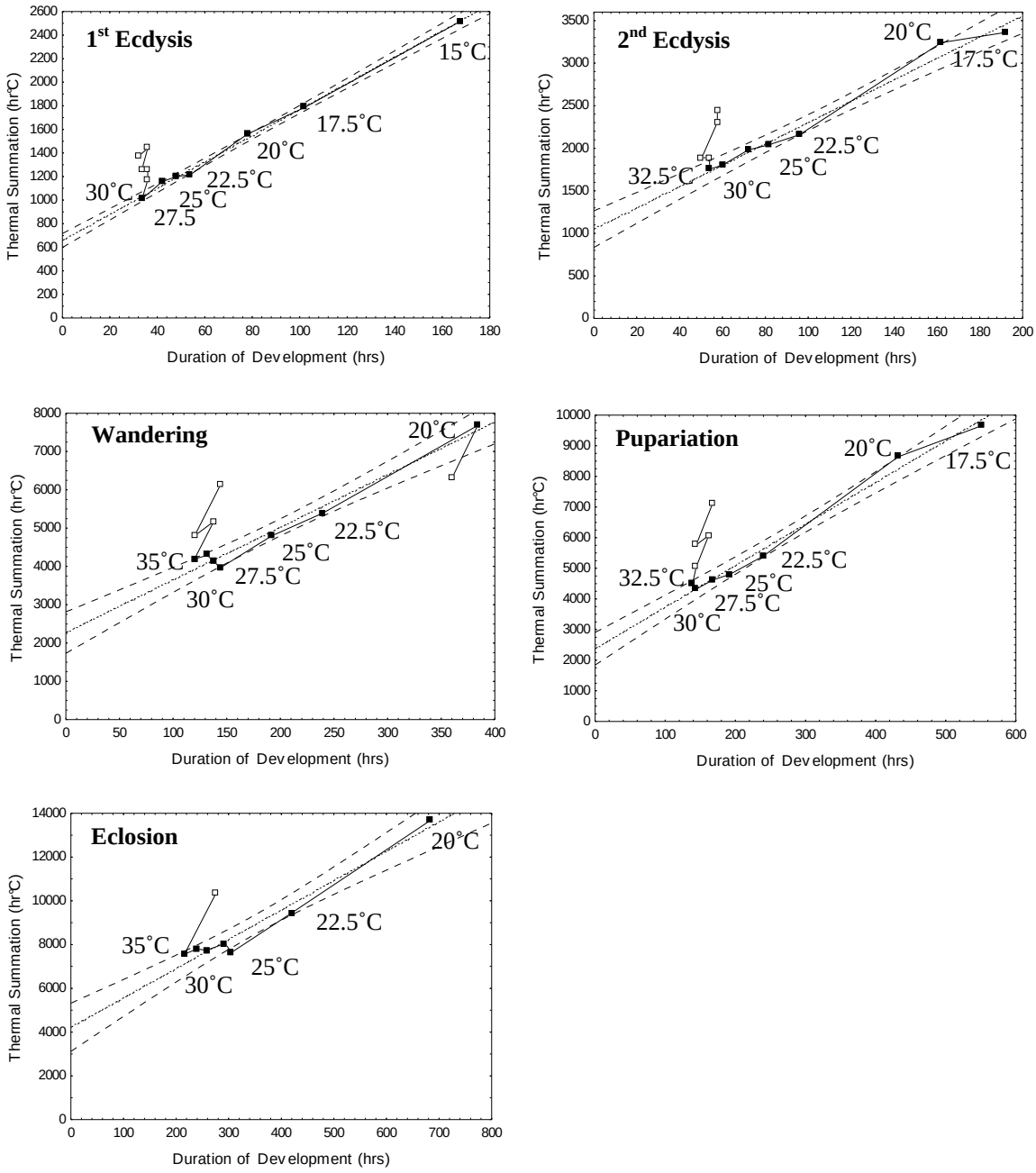

Figure 4.2 S-shaped curves and reduced major axis regression lines used to determine *K*- and D_0 values of *C. albiceps* at 13 different temperatures for the 1st ecdysis, 2nd ecdysis, onset of wandering, onset of pupariation, and eclosion. Black squares indicate points used in the regression calculations, white squares indicate points not used in the calculations because they are not on the linear part of the relationship (Ikemoto and Takai 2000) and dotted lines represent 95% confidence intervals.

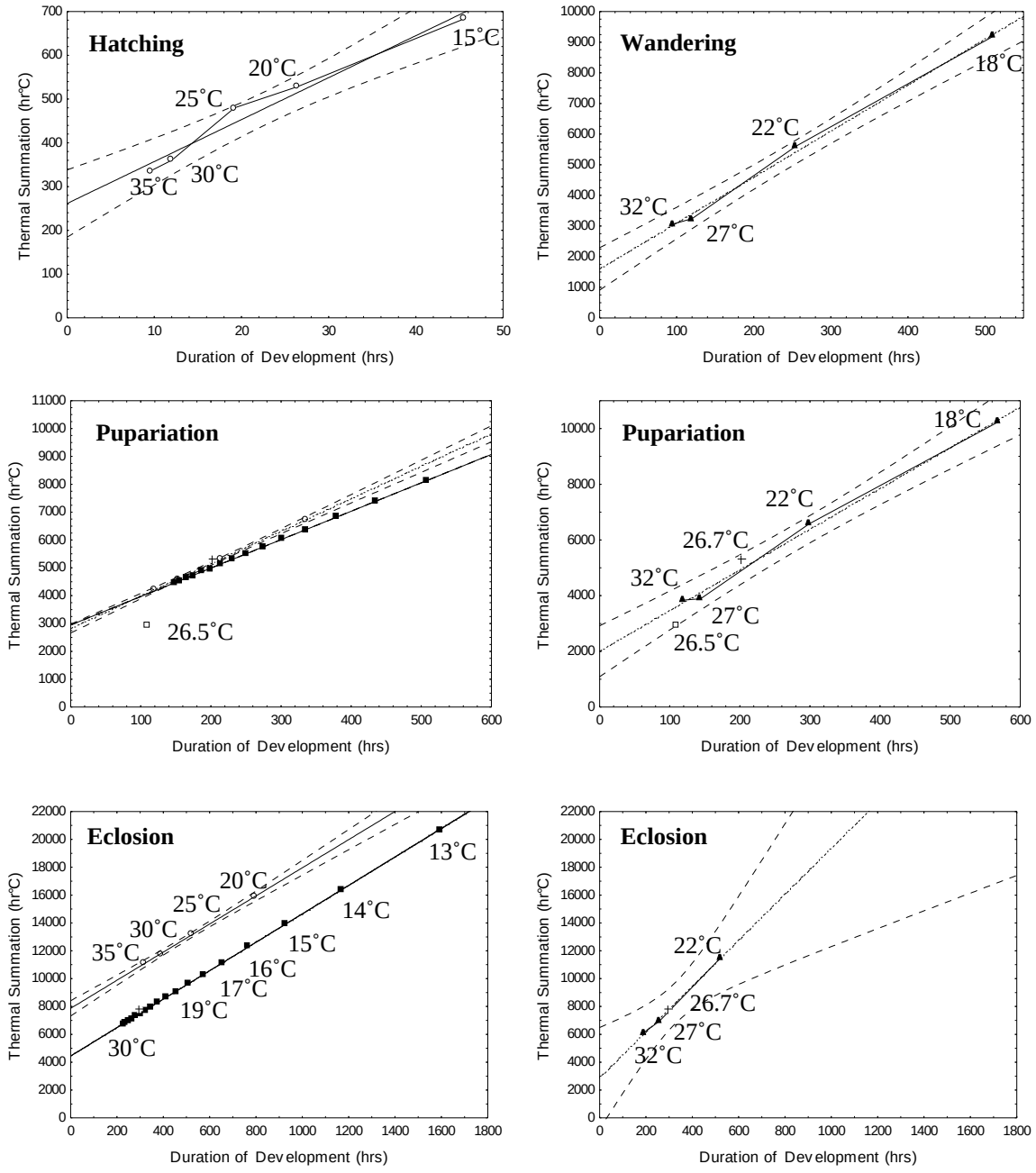

Figure 4.3 S-shaped curves and reduced major axis regression lines used to determine *K* and D_0 values of *C. albiceps* for hatching, onset of wandering, onset of pupariation, and eclosion. Sources: □, Ullyett (1950); +, Prins (1982); ▲, Queiroz (1996); ■, Marchenko (2001); ○, Grassberger et al. (2003). Dotted lines represent 95% confidence intervals.

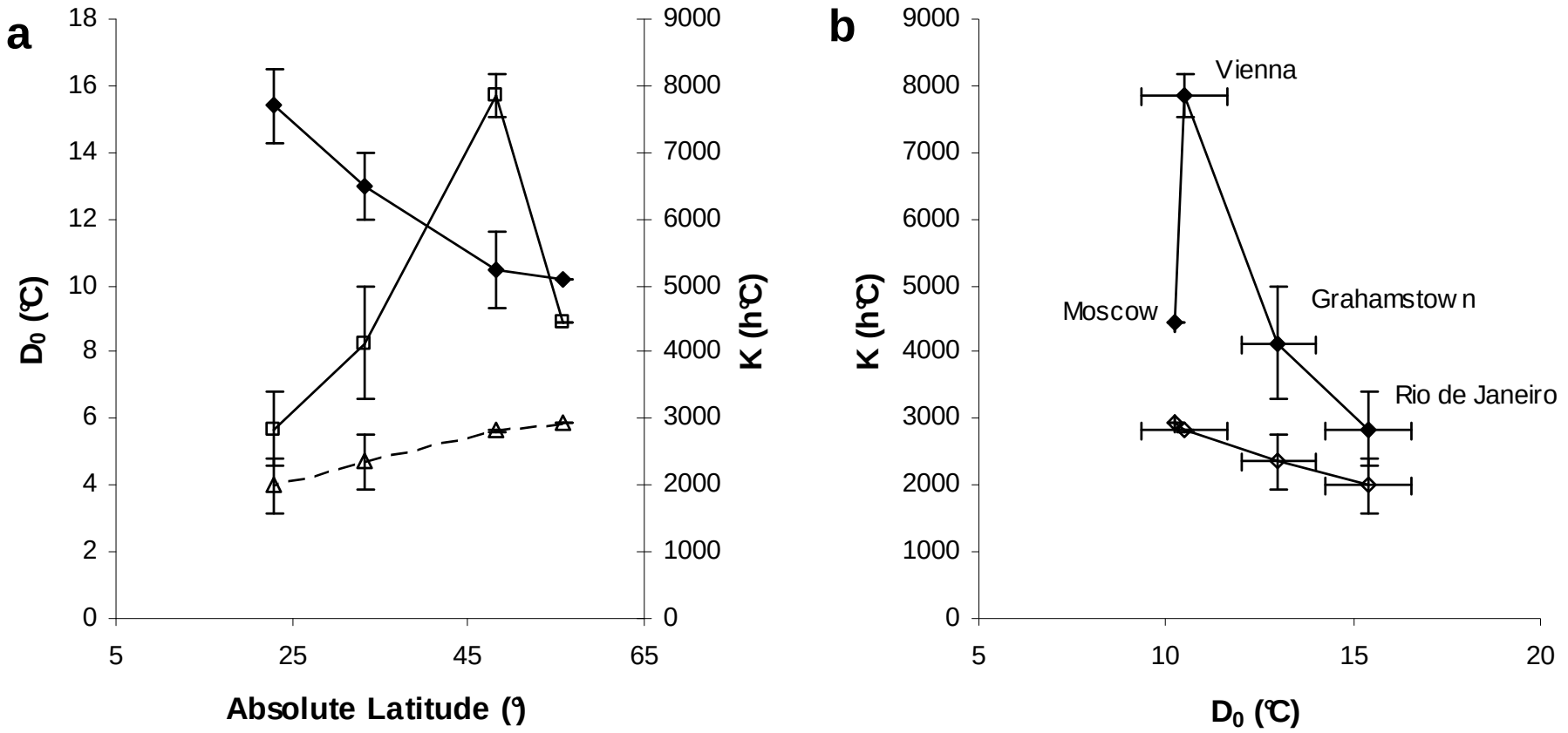

Figure 4.4 a Relationships between absolute latitude and D_0 (filled diamonds), K for pupariation (open triangles) and K for eclosion (open squares). **b** Relationships between D_0 and K. Filled symbols = eclosion, open symbols = pupariation. Error bars represent 95% confidence intervals.

V

Models of development for the blowflies *Chrysomya chloropyga* and *C. putoria* (Diptera: Calliphoridae)

Preface

This chapter uses the knowledge gained in the last three chapters to derive high quality development data and reliable thermal summation constants for two blowfly sister species. This chapter was present at the 33rd bi-annual conference of the Zoological Society of Southern Africa and has been submitted to *Medical and Veterinary Entomology*.

Abstract

Developmental curves for *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae) and *C. putoria* (Wiedemann) (Diptera: Calliphoridae) were established at eight and ten different constant temperatures, respectively, using developmental landmarks and length as measures of age. The thermal summation constants (*K*) and developmental threshold (D_0) were calculated for five developmental landmarks and isomorphen and isomegalen diagrams were also constructed for the purpose of estimating minimum post mortem intervals (PMI). *Chrysomya chloropyga* had an average developmental threshold value (D_0) of 10.91˚C (std. err. = 0.94˚C, n = 5), significantly lower than that of *C. putoria*: 13.42˚C (std. err. = 0.45˚C, n = 5) (paired t-test: t = -4.63, d.f. = 8, p < 0.00). The *K*-values for *C. chloropyga* were larger than those of *C. putoria* for all developmental events except the onset of the wandering phase. These are the first data that can be used

to calculate PMI and predict population growth of *C. chloropyga* and *C. putoria* in Africa and show that data cannot be transferred between species.

Introduction

Chrysomya chloropyga (Weid.) and *C. putoria* (Weid.) are sister species of carrion-breeding blowflies (Wells et al. 2004; Rognes and Paterson 2005) that are widespread in Africa, and *C. putoria* has also become widespread in South America (Laurence 1988; Greenberg and Kunish 2002). *Chrysomya putoria* is commonly found breeding in latrines and cesspits (Conway 1972; Hulley 1983), and both species have medical, veterinary and forensic significance (Ullyett 1950; Zumpt and Patterson 1952; Zumpt 1965; Prins 1982; Hulley 1983; Louw and van der Linde 1993). These disciplines all require information about the life cycle of these insects, but forensic science requires particular focus on the effects of environmental temperatures on their development (Chapter 1 and 2), which is necessary to calculate an accurate minimum post mortem interval (PMI) (Greenberg 1991, Catts 1992). Despite their medical, public health, veterinary and forensic importance, few data have been published on either species. Prins (1982) recorded 1st ecdysis, 2nd ecdysis, pupariation and eclosion for *C. chloropyga* at 23.5˚C and Ullyett (1950) recorded pupariation for *C. chloropyga* at 26.7˚C. Laurence (1988) recorded pupariation at 26˚C for *C. putoria*, while Baumgartner and Greenberg (1984) recorded hatching, 1st ecdysis, 2nd ecdysis, wandering, pupariation and eclosion at 21.7˚C (std. dev. = 1.9˚C) and 26.0˚C (std. dev. = 3.1˚C). No study includes development of both species at more than one temperature.

PMI estimates are calculated using one or more of four models, namely, curvilinear regression (Ullyett 1950; Higley and Haskell 2001), isomegalen diagrams (Reiter 1984), isomorphen diagrams (Grassberger and Reiter 2001) and thermal summation models (Réaumur 1735; Higley and Haskell 2001). Higley and Haskell (2001) dismiss the curvilinear approach as impractical and advocate the use of thermal summation models, which are constrained linear regression models, while Grassberger and Reiter (2001) state that “the use of the isomegalen- and isomorphen-diagrams could provide a quick and

precise estimate of the PMI". Isomorphen diagrams are phase plots of time against temperature, with contours representing developmental transitions or boundaries between the stages of the life cycle (Reiter 1984). Similarly, isomegalen diagrams are phase plots of the same variables, but contoured with the length of the larvae (Grassberger and Reiter 2001). This chapter presents isomorphen and isomegalen diagrams, and thermal summation models from eight and ten different constant temperatures for *C. chloropyga* and *C. putoria*, respectively, and compare the thermal summation constant (K) and developmental zero (D_0) of these sister species, to investigate whether development data can be transferred between species for the purpose of estimating minimum PMI.

Materials and Methods

Data collection

Development data were collected as described in Chapter 2, at eight and ten different constant temperature for *C. chloropyga* and *C. putoria* respectively, starting at 15.0˚C and increasing by 2.5˚C intervals.

Every four hours for the first 48 hours, and every eight hours from then to pupariation, one maggot was removed from each of five random cups at each temperature for both species. These sampling resolutions are in accordance with those recommended in chapters 2, 3 and 4.

Data analysis

Isomegalen diagrams (Grassberger and Reiter 2001), or three-dimensional contour plots of larval length, temperature and development time, were constructed for each species from these data using a spline function in the computer software programme 'Statistica 7'. Similarly, isomorphen diagrams (Reiter 1984) were constructed from the median times to reach 1st ecdysis, 2nd ecdysis, onset of wandering, onset of pupariation and eclosion. These median values were also used to calculate the developmental threshold (D_0) and thermal summation constant (K) of a standard thermal summation model

(Chapter 3) using the reduced major axis regression method described by Ikemoto and Takai (2000).

Results

Isomorphen and isomegalen diagrams

Because the isomegalen diagram lies within the phase space representing the larval stages in the isomorphen diagram, the two plots were combined (Fig. 5.1). Development rates for both species increased steadily with increasing temperature. Larvae of *C. chloropyga* grew consistently well at all temperatures tested (Fig. 5.1), but those of *C. putoria* developed poorly at temperatures below 20˚C and failed to reach pupariation at 15˚C (Fig. 5.1). Larvae of *C. chloropyga* reached a maximum size of 17.0mm at 20˚C, 22.5˚C and 25˚C, and 16.8mm at 27.5˚C and 32.5˚C (Table 5.1), while those of *C. putoria* reached a maximum length of 18.7mm at 37.5˚C (Table 5.1). These maximum sizes are not represented in the isomegalen diagram, which reports median data and corresponding 95% confidence intervals.

Pupariation and eclosion was recorded at all temperatures tested for *C. chloropyga*, while *C. putoria* larvae reached pupariation at all temperatures except 15˚C, and eclosion was recorded at all temperatures except 15˚C, 17.5˚C and 37.5˚C. The pupation survival rate for *C. chloropyga* was high for all temperatures with a mean of 84%, while the rate for *C. putoria* decreased dramatically from a mean of 91% between 20˚C and 27.5˚C to a mean of 55% between 30˚C and 35˚C, with an average of 76% for all temperatures, but no significant difference was found between the two species (paired t-test: $t = 2.08$, d.f. = 16, $p > 0.05$).

Thermal summation constants

A minimum of six temperatures were used in the regressions analyses to calculate the D_0 and K values for both species, which satisfied the minimum sample size required for

regression analysis (Chapter 2). The coefficients of determination (R^2) values for all regression models were above 0.95 (Table 5.2).

Chrysomya chloropyga had an average developmental threshold value (D_0) of 10.92˚C (std. err. = 0.94˚C, n = 5), lower than *C. putoria's* 13.42˚C (std. err. = 0.45˚C, n = 5). The D_0 values for all life stages of *C. chloropyga* were significantly lower than those of *C. putoria* (paired t-test: t = -4.63, d.f. = 8, p < 0.00).

All thermal summation constants (*K*) for *C. chloropyga* were higher than those of *C. putoria* except for the onset of the wandering phase (Table 5.1). The largest 95% confidence intervals for D_0 for *C. chloropyga* occurred at wandering, while the largest 95% confidence intervals for *K* for *C. chloropyga* occurred at eclosion. Both the largest 95% confidence intervals for D_0 and *K* for *C. putoria* occurred at eclosion (Fig. 5.2).

Discussion

These are the first data that can be used to calculate minimum PMI and predict population growth of *C. chloropyga* and *C. putoria* in South Africa. The use of isomorphen and isomegalen diagrams to estimate the age of larvae was explained by Grassberger and Reiter (2001), and the application of linear thermal summation models was explained by Higley and Haskell (2001). Because *C. chloropyga* and *C. putoria* are sister species (Wells et al. 2004), a comparison of their developmental dynamics has special interest.

Isomorphen and isomegalen diagrams

Severe curving of the contours in the isomegalen diagram for *C. putoria* at temperatures below 20˚C suggests that larvae struggle to maintain a consistently healthy (normal) growth rate in cool environments and are forced into premature wandering behaviour. This poorer development at cool temperatures is expected because *C. putoria* has a more tropical distribution throughout the central and eastern African countries (Zumpt 1965, Laurence 1988; Rognes and Paterson 2005) and is therefore inherently adapted to warmer

climates (Chapter 4). Contrary to this, an absence of exaggerated curvature in the contours in *C. chloropyga* data suggest that *C. chloropyga* larvae are better able to develop in cooler climates, and the species accordingly has a distribution that includes the more temperate regions of South Africa where *C. putoria* is absent (Zumpt 1965, Rognes and Paterson 2005).

Thermal summation models

Amendt et al. (2007) support the exclusivity of species specific development data to estimate minimum PMI, but simultaneously advocate the use of congeneric development data when the former data set is not available. The results of this study contend the latter argument and suggest that development data are not transferable between *C. chloropyga* and *C. putoria* (discussed below), two closely related species, and therefore authors should assume differences in development data between other closely related species.

K and D_0 values differed significantly between the two species in three of the five developmental events tested (Fig. 5.2), and the average D_0 value for *C. putoria* was 2.51˚C higher than that of *C. chloropyga*. These data also suggest that *C. putoria* is adapted to warmer environments than *C. chloropyga* (Chapter 4). Similarly, the cultures used in this study were caught in the same location, but *C. putoria* was most active at the site in late summer (February/March) (34˚10’28”S:22˚05’13”E) and absent in early spring (September), while *C. chloropyga* was caught abundantly in September and only in smaller numbers in February and March. These trapping times also support the contention that these species are adapted to different temperature conditions.

The developmental thresholds from 1st ecdysis to wandering for *C. chloropyga*, and from 1st ecdysis to eclosion for *C. putoria*, remained consistent throughout all developmental events (Table 5.2; Fig. 5.2). This was expected because the kinetics of metabolism is unlikely to vary between developmental stages in insects (Sharpe and DeMichele 1977). However, D_0 for *C. chloropyga* did decrease by 1.98˚C between the onset of wandering and eclosion. This might suggest that the D_0 for *C. chloropyga* is closer to 10˚C than the

average of 10.92˚C as the developmental threshold is expected to drift to its true value as the relative error in measuring duration of development decreases for later developmental events (Chapter 3).

The *K*-values for *C. chloropyga* were larger than those of *C. putoria* for all developmental events except the onset of the wandering phase. This is to be expected because, if *C. chloropyga* can tolerate cooler climates, they would inherently need a longer time to grow to reach each developmental landmark (Chapter 4; Trudgill and Perry 1994; Trudgill 1995) because the kinetics of metabolism is affected by temperature and thus would slow down in cooler climates, requiring more time to reach a developmental landmark (Fig. 5.1).

Acknowledgements

I thank Sue Abraham (Rhodes University) for assistance with graphics.

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Table 5.1 Mean and maximum larval length, and pupation survival rate for *C. chloropyga* and *C. putoria* at eight and ten different constant temperature, respectively.

Temperature (˚C)	Species	Larval length (mm)		Pupation survival rate (%)
		Mean	Maximum	
15	*C. chloropyga*	12.1	16.0	79.4
	C. putoria	6.8	7.4	0
17.5	*C. chloropyga*	13.3	16.4	93.1
	C. putoria	10.7	13.2	0
20	*C. chloropyga*	12.9	17.0	62.5
	C. putoria	12.1	16.1	100.0
22.5	*C. chloropyga*	13.6	17.0	93.1
	C. putoria	12.3	15	80.0
25	*C. chloropyga*	13.5	17.0	100.0
	C. putoria	12.2	15.8	100.0
27.5	*C. chloropyga*	13.5	16.8	92.9
	C. putoria	12.5	16.4	85.7
30	*C. chloropyga*	13.5	16.2	71.1
	C. putoria	12.9	15.5	57.1
32.5	*C. chloropyga*	13.4	16.8	80.5
	C. putoria	13.4	15.2	50.0
35	*C. chloropyga*	N/A	N/A	N/A
	C. putoria	12.3	15.6	57.1
37.5	*C. chloropyga*	N/A	N/A	N/A
	C. putoria	12.8	18.7	0

Table 5.2 Development threshold (D_0) and thermal summation constants (K) for five developmental events for *C. chloropyga* and *C. putoria*, calculated using the method described by Ikemoto and Takai (2000).

	Developmental threshold (D_0)		Thermal summation constant (K)		R^2	N used for analysis	N rejected from analysis
	˚C	Std. Err.	h˚C	Std. Err.			
1st ecdysis							
C. chloropyga	11.16	0.53	273.70	18.02	0.95	7	1
C. putoria	13.11	0.35	227.50	15.11	0.99	10	0
2nd ecdysis							
C. chloropyga	11.30	0.22	599.71	17.15	0.99	8	0
C. putoria	13.29	0.12	508.71	15.18	0.99	7	3
Onset of Wandering							
C. chloropyga	11.86	0.60	1075.51	89.05	0.98	8	0
C. putoria	12.52	0.71	1251.64	102.94	0.98	6	3
Onset of Pupariation							
C. chloropyga	10.38	0.37	1999.44	79.94	0.99	8	0
C. putoria	13.76	0.39	1314.30	74.17	0.99	6	3
Eclosion							
C. chloropyga	9.88	0.35	3972.71	140.57	0.99	8	0
C. putoria	13.27	1.42	3093.13	406.51	0.94	6	1

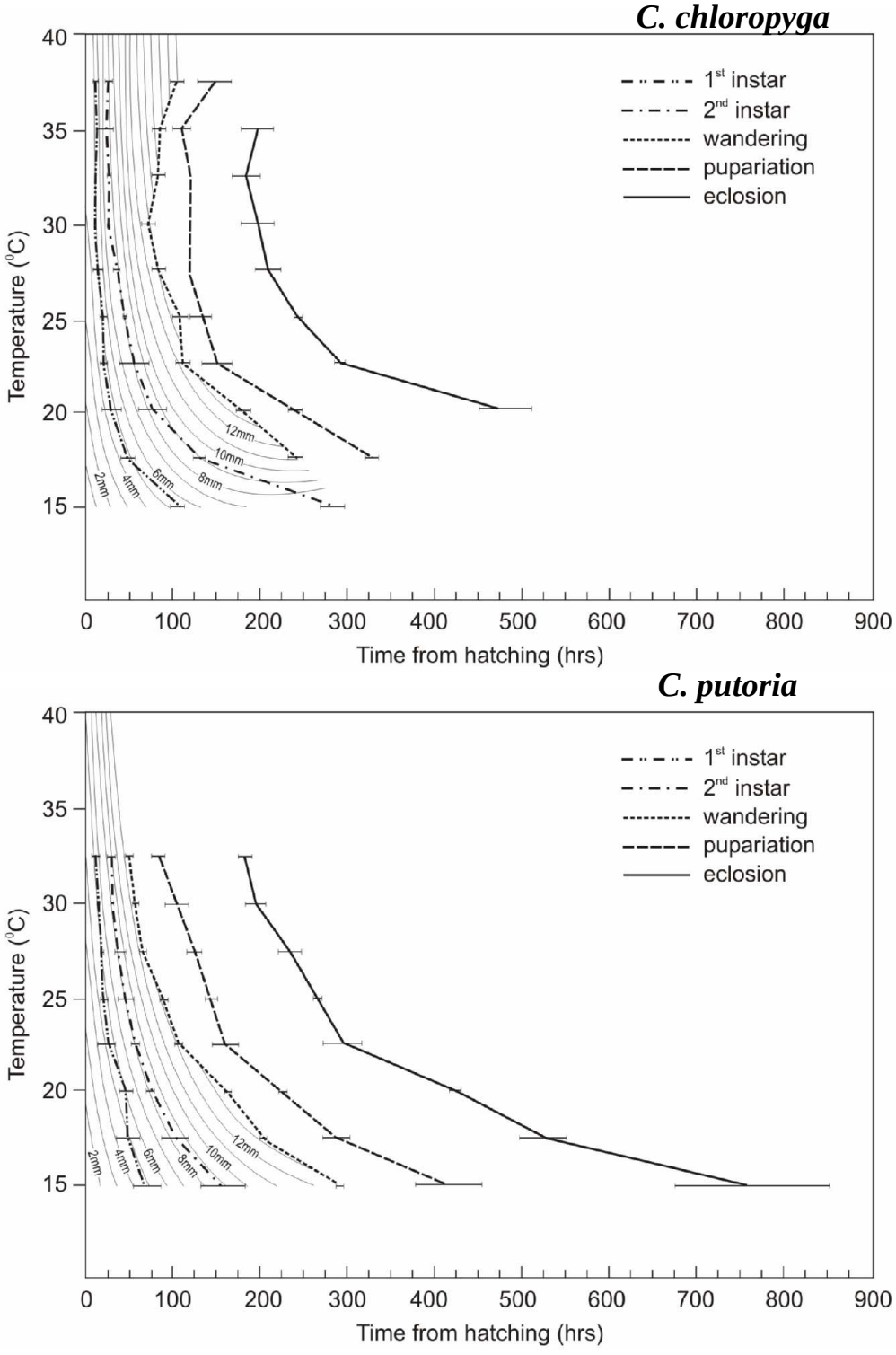
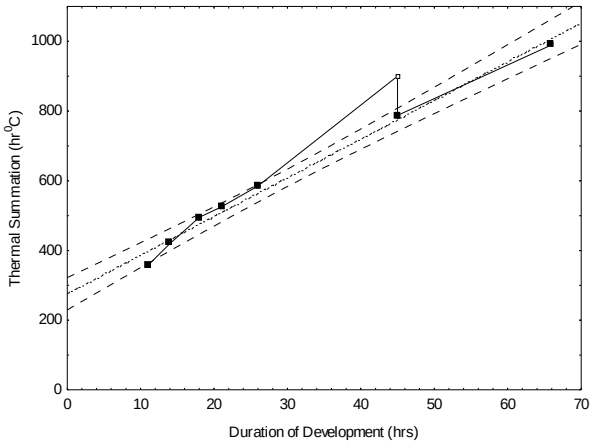
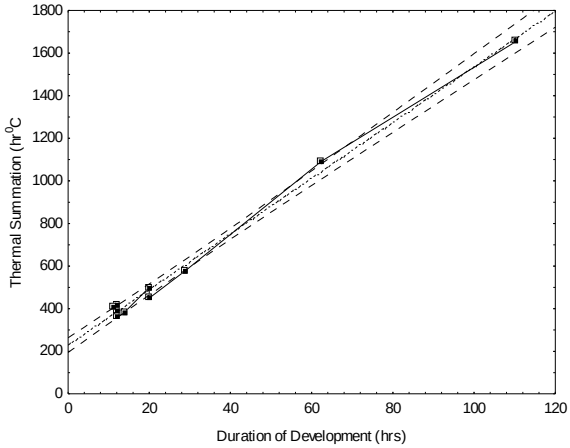


Figure 5.1 Combined isomorphen diagram (contours of life stages) and isomegalen diagram (body length contours for larvae) for *C. chloropyga* and *C. putoria*. Error bars represent 95% confidence intervals for developmental transitions.

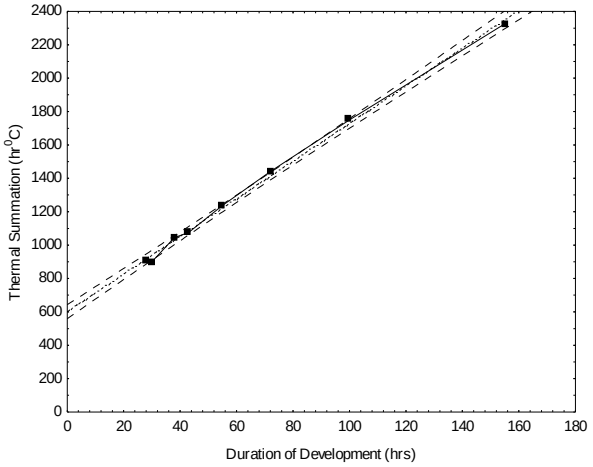
1st ecdysis



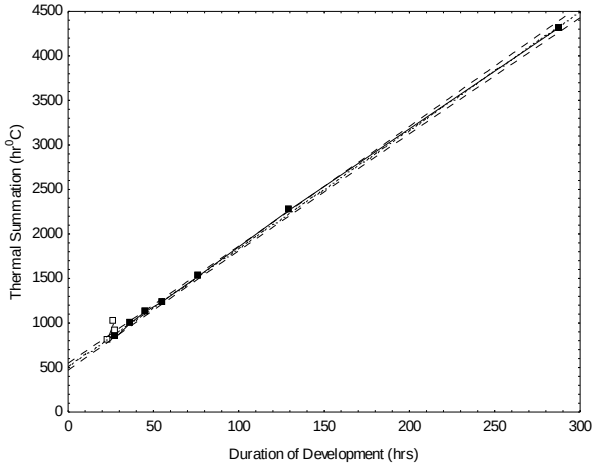
1st ecdysis



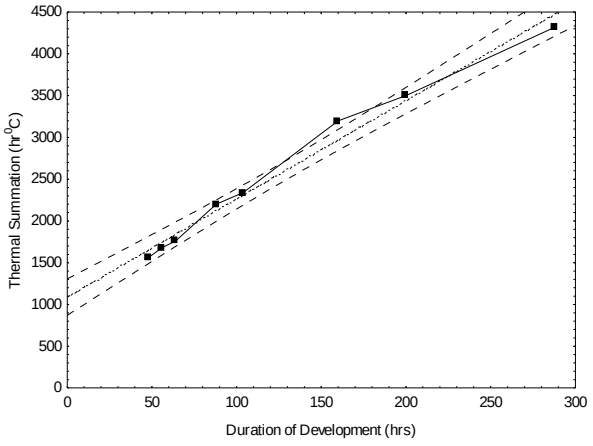
2nd ecdysis



2nd ecdysis



Wandering



Wandering

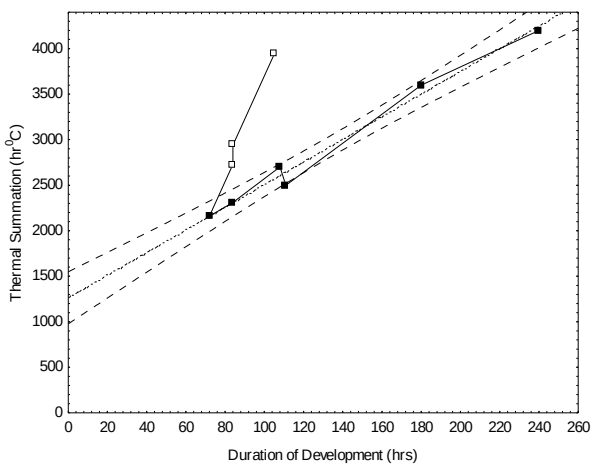


Figure 5.2 continued

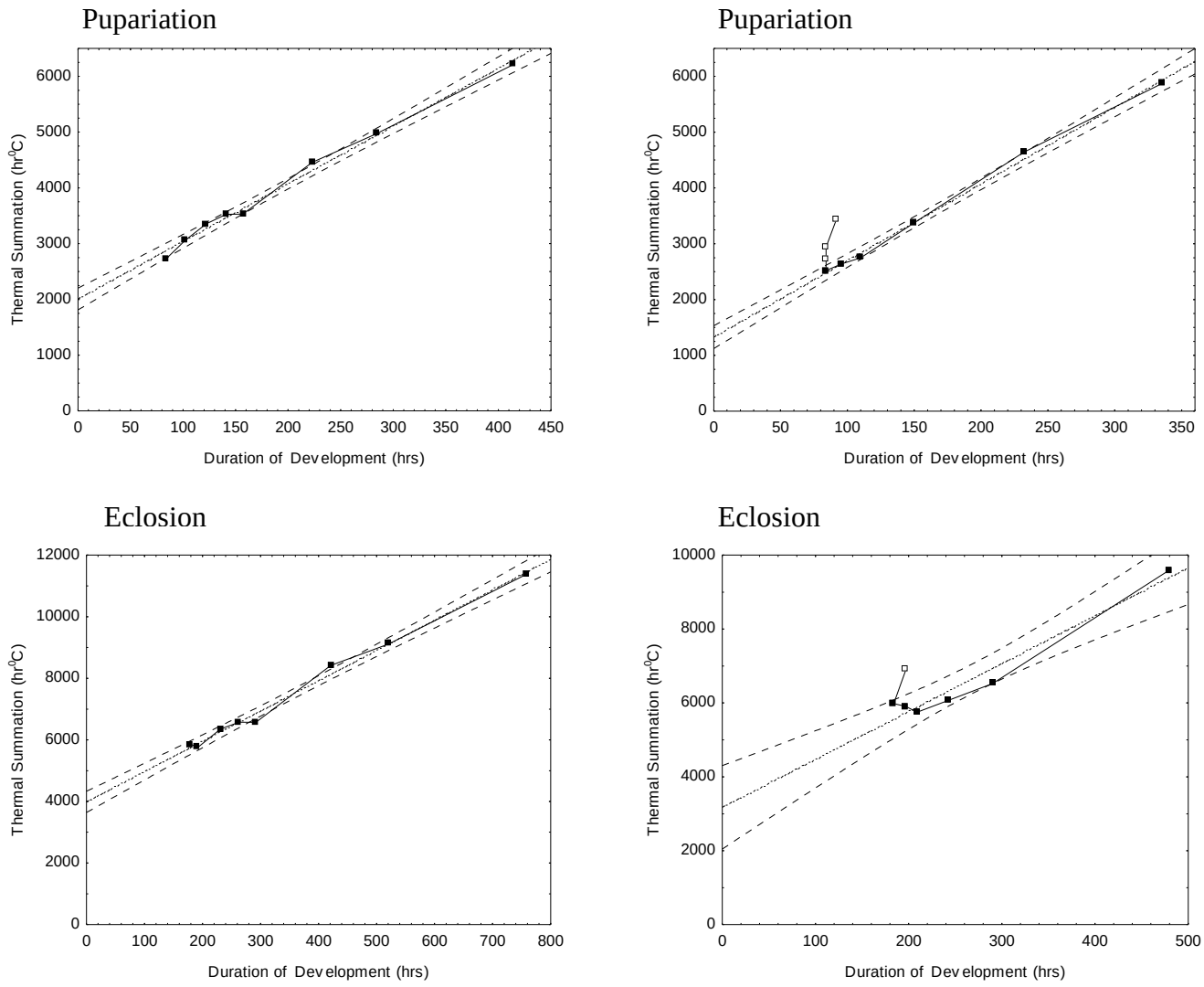


Figure 5.2 Developmental curves and reduced major axis regression lines (dotted lines) used to determine *K*- (the regression intercept) and D_0-values (the regression slope) for 1st ecdysis, 2nd ecdysis, onset of wandering, onset of pupariation, and eclosion of *C. chloropyga* (left column) and *C. putoria* (right column). Black squares indicate points used in the regression calculations; white squares indicate points excluded from the calculations because they are not on the linear part of the relationship (Ikemoto and Takai 2000; Higley and Haskell 2001); dash lines represent 95% confidence intervals.

VI

Thermal ecophysiology of seven carrion-feeding blowflies (Diptera: Calliphoridae) in South Africa

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Preface

This chapter quantifies numerous larval, pupal and adult temperature thresholds for seven forensically important blowfly species and compares those to developmental thresholds calculated in previous chapters. It aims to identify common trends between the temperature thresholds of larvae, pupae and adults of different species. This chapter has been submitted to *Entomologia Experimentalis et Appicata*.

Abstract

A variety of temperature thresholds for larvae, pupae and adults of seven African species of carrion-feeding blowflies (Diptera: Calliphoridae) were measured and compared to understand their basic thermal biology and the influence of temperature on their behaviour. *Calliphora croceipalpis* (Jaennicke) had a consistently significantly lower temperature threshold than all other species tested for all larval, pupal and adult temperature thresholds. *Chrysomya marginalis* (Robineau-Desvoidy) had significantly higher larval and adult upper lethal temperature thresholds than all other species and weighed significantly more than all other species. *Chrysomya albiceps* (Wiedemann) and *Lucilia sericata* (Meigen) had similar pupal and adult temperature thresholds, while *C. putoria* (Wiedemann), *C. chloropyga* (Wiedemann) and *C. megacephala* (Fabricius) had

inconsistent rank temperature thresholds between the larval, pupal and adult stages. With a few minor exceptions, female adult flies responded at higher temperature thresholds and weighed consistently more than conspecific male flies for all species tested. These data suggest that there is a phylogenetic component to the thermal biology of blowflies. Comparisons were made between these temperature thresholds and the distributions of blowfly species present on two rhinoceros carcasses. These comparisons suggest that blowfly larvae with high upper lethal temperature thresholds dominate in interspecific competition in favorable thermal environments by raising maggot mass temperature above the thresholds of other carrion-feeding blowflies, through maggot-generated heat.

Introduction

Flies, in general, are seen as pests that carry and spread diseases, although only a few fit this profile. Several of these are blowflies of the family Calliphoridae, well known by livestock and game farmers for their myiasis-causing capabilities (Zumpt 1965). Two species that have legendary reputations in this regard are *Lucilia sericata* (M.), known to cause sheep strike (Ullyett 1950; Wall et al. 1992), and *Chrysomya bezziana* (Villeneuve) or screw-worm, which is an obligate myiasis breeder (Zumpt 1965). Other species, e.g. *C. putoria* (Wied.) and *C. megacephala* (F.), are closely associated with latrines and are know to be vectors of several enteric diseases (Zumpt and Patterson 1952; Sulaiman et al. 1988). Not all calliphorid species are pests and some of them can be useful to man. For example, African members of this family that breed in carrion, e.g. *L. sericata*, *C. albiceps* (Wied.), *C. chloropyga* (Wied.), *C. marginalis* (R-D.), *C. megacephala*, *C. putoria* (Wied.) or *Calliphora croceipalpis* (J.), are used by forensic entomologist to estimate time since death (Williams & Villet 2006), known as post mortem interval (PMI) (Greenberg 1991, Catts 1992). Because blowflies are ectotherms, temperature influences much of their physiology and behaviour and may therefore be a major limiting factor in their ecology. Thus, the Calliphoridae of South Africa have veterinary, medical and forensic importance and it is of practical and theoretical significance to study their biology.

Temperature is one of the most significant environmental influences on the biology of ectotherms like blowflies (Sharpe and de Michele 1977). It affects when and where they are active, and the nature of that activity. For these reasons, this study focuses on the thermophysiology of the larval, pupal and adult life stages of the seven African carrion-breeding blowfly species mentioned above. Common trends may be expected between thermal thresholds of larvae, pupae and adults of different species and they may be related to phylogeny.

Materials and methods

Chrysomya marginalis and *C. albiceps* adults were obtained from a dead kudu (*Tragelaphus strepsiceros* Pallas) and a white rhinoceros (*Ceratotherium simum* Burchell) in Kwandwe Game Reserve (33°3'S:26°26'E). *Calliphora croceipalpis* adults were caught periodically at various indoor locations in Grahamstown (33°17'S:26°31'E), and *C. chloropyga*, *C. megacephala*, *C. putoria* and *L. sericata* adults were caught on multiple occasions, in Red top® fly traps set up at the Grahamstown municipal dump. Flies were maintained on a diet of granulated sugar, milk powder and water *ad libitum*. After approximately 1 week, the flies were supplied with fresh chicken liver and within two days the females had laid eggs.

A Physitemp MT–29/1 thermocouple (0.33mm diameter) and a Physitemp BAT 12 electronic digital thermometer were used to measure all temperatures for all experiments. All data were analysed in the computer programme 'Statistica 7'.

Rate of warming in maggots

Maggots experience a wide range of temperatures in a carcass due to fluctuating ambient temperatures and maggot generated heat (Greenberg 1991, Ames and Turner 2003). Knowing the rate of warming of maggots will reflect how quickly maggots thermo-conform to these constantly changing environmental temperatures. This will aid in determining accurate development temperatures of maggots in the environment, e.g. carcass or incubator.

Ten maggots of *C. megacephala* and five each of *C. albiceps*, *C. marginalis* and *C. croceipalpis* were placed in separate Petri dishes, one maggot per Petri dish. Each Petri dish was placed on ice for 5 min, after which it was transferred to a 30°C control environment room where the cuticle temperature of the maggot was recorded every 5 sec until it reached room temperature. A regression line was fitted to a scatterplot of thermal gradient versus rate of warming, to find the rate of warming for each specimen of each species. Significant differences of rate of warming were tested between species using ANOVA and Tukey's post hoc test.

Upper lethal temperature thresholds of 3rd instar maggots

Six groups of ten 3rd-instar larvae of *L. sericata* were placed in separate Petri dishes and incubated at each of eight constant temperatures, ranging from 38°C to 59°C at intervals of 3°C, in Labcon 3104U incubators. One Petri dish was removed from each incubator every 10 minutes for a total duration of 1 hour. Mortality was recorded at each time interval by assessing maggots' responses to prodding: if no response was seen immediately and after 30 minutes of recovery time at 20°C, death was recorded. This procedure was replicated twice to total three replicates and 1440 maggots, and the whole experiment was repeated for *Ca. croceipalpis*, C. *albiceps*, *C. chloropyga*, *C. megacephala*, *C. putoria* and *C. marginalis*.

Data were analysed by Analysis of Covariance, relating temperature treatment to percentage mortality, treating time as a covariate.

Preferred pupariation temperatures

A Labcon WBM601 water bath was heated to within 5°C of 75°C and another was cooled to within 1°C of 8°C using a Lauda ETK 30 cold finger. Eight 50 cm-long copper troughs (3cm diameter) were placed between the two water baths, each end with a copper leg in one of the water baths to conduct heat, for 2 hours, which allowed a temperature gradient (10°C - 60°C) to form along the troughs. The hot water bath was covered to

minimize evaporation. The bottoms of the copper troughs were covered 1-2mm deep with river sand (deep enough for maggots to pupate in), which was allowed to heat to equilibrium with the temperature gradient.

In a separate experiment for each species (*Ca. croceipalpis*, *L. sericata*, C. *albiceps*, *C. chloropyga*, *C. megacephala*, *C. putoria* and *C. marginalis*), 10 maggots were first incubated at 35°C for 10 minutes and then placed in each trough where the sand temperature was 35°C (middle of the trough). The troughs were then covered with tinfoil to darken them to encourage pupariation. After five days all maggots had pupariated and the temperature of the sand under each puparium was recorded. The data were analysed with a Kruskal-Wallis ANOVA and a multiple comparison test.

Thermophysiological thresholds of adult blowflies

Ten male and ten female *Ca. croceipalpis*, *L. sericata*, C. *albiceps*, *C. chloropyga*, *C. megacephala*, *C. putoria* and *C. marginalis* flies were anaesthetized on ice, after which each immobile fly was stabbed off-centre through the side of the mesothorax with the thermocouple. The flies were then allowed to warm up 30cm from, and directly in front of, a Maxamatic CT 250 WATT infrared lamp. This method is similar to the method for measuring body temperature of ants, cicadas, blowflies and cicadas by Christian and Morton (1992), Sanborn and Phillips (1996), Williams (2002) and Sanborn et al. (2003), respectively. Temperature at onset of the following behaviours was recorded for each fly: twitching of mouthparts (initial movement, indicating the onset of nervous activity), standing (presumed onset of coordinated muscle activity), and death (cessation of neuromuscular activity, assumed on no response after prodding). After death, the flies were weighed to the nearest 0.1mg on an Ohaus Adventurer™ micro-balance. The data were analyzed in an ANCOVA with body mass as the covariate, and Tukey's HSD post-hoc test.

Developmental zero

Developmental zero (D_0) is the theoretical temperature below which development ceases and can be considered as a lower temperature threshold (Chapter 2). The D_0 of all seven species where obtained from various studies (Fig. 6.1) and compared to the larval and pupal temperature thresholds of this study. In addition, the D_0 of *Ca. vicina* (Robineau-Desvoidy) and *Ca. vomitoria* (Linnaeus) were calculated from Williams & Richardson (1984) and Davies and Ratcliffe (1994), respectively. A spearman's correlation matrix was performed on all temperature thresholds and D_0 of all species to identify any trends in the thresholds of each life stage.

Field observations

Two dead white rhinoceros bulls were autopsied in two different game reserves (Site 1 in summer and Site 2 in autumn) and left in the field (one bull per site). The vegetation and climatic zones of the two sites were typical of the Kowie River Thicket vegetation of the Eastern Cape (Mucina and Rutherford 2006). Approximately one week later five ambient shade air temperatures and five maggot mass temperatures were recorded from each of the forequarters, middle and hindquarters of the carcass. Temperatures were recorded at a depth of 1cm in the maggot layer and the depth of the maggot layer was measured to the nearest 5mm. Three large masses, approximately 1 – 1.5 litres, of 3rd-instar maggots (between 15,000 and 20,000 maggots), were taken from each measuring site and raised to adulthood for identification. *Chrysomya albiceps* larvae were collected from separate masses on the periphery of the carcass (Fig. 6.2).

Results

Rate of warming in maggots

Ambient temperature remained fairly constant for the duration of the experiment (mean = 31.49°C; s.d. = 1.07°C; n = 20). *Chrysomya megacephala* and *C. marginalis*, and *C. albiceps* and *Ca. croceipalpis* had very similar mean rates of passive warming (Fig. 6.3). A significant difference was established ($F = 3.79$; $df = 3, 16$; $p < 0.032$) between species

using ANOVA, but Tukey's and Scheffe's post hoc tests showed no significant difference between any of the species.

Upper lethal temperature thresholds of 3rd instar maggots

As expected, longer exposure to heat had a significant effect on mortality for all species ($F = 82.94$, d.f. = 1, $p < 0.00$). *Chrysomya marginalis* (LT_{50} = 50.1°C) and *Ca. croceipalpis* (LT_{50} = 42.9°C) had the highest and lowest upper lethal temperature thresholds respectively (Fig. 6.1) and were significantly different from each other and all other species tested. *Lucilia sericata* had the second lowest lethal temperature thresholds and differed significantly from all other species. *Chrysomya putoria* differed significantly from all other species except *C. albiceps*, while *C. megacephala*, *C. chloropyga* and *C. albiceps* did not different significantly from one another (Fig. 6.1).

Preferred pupariation temperatures

There was a significant difference between the preferred temperature of some of the species tested ($H_6 = 315.46$, $n = 527$, $p < 0.00$). *Chrysomya putoria* and *C. chloropyga* had the greatest and smallest range of preferred pupariation temperatures respectively, while *C. megacephala* and *Ca. croceipalpis* had the highest and lowest mean temperature preference (Fig. 6.4). Pupariation temperatures of *Ca. croceipalpis* and *C. chloropyga* differed significantly from one another and from all other species tested ($p < 0.00$), while *C. megacephala* differed from all species ($p < 0.00$) except *C. putoria* ($p = 0.06$). No significant differences occurred between *C. albiceps* and *C. marginalis* ($p = 1.00$), *C. albiceps* and *L. sericata* ($p = 1.00$), and *L. sericata* and *C. marginalis* ($p = 0.19$). The greatest non-outlier range was 9.6°C (*Ca. croceipalpis*) and no species pupariated higher than 29.7°C (*C. megacephala*).

Thermophysiological thresholds of adult blowflies

With a few minor exceptions, females responded at consistently higher temperatures for "twitching", "standing", and "death" thresholds in all species (Fig. 6.5). Female blowflies

weighed consistently more than male blowflies for all species except *Ca. croceipalpis*, but were only significantly larger for *C. marginalis* ($F_{6, 126} = 8.31$, $p < 0.00$). Hence, the covariate (mean = 0.0604g) had no significant effect on "twitching", "standing", or "death" thresholds (Fig. 6.5).

Chrysomya marginalis had the second lowest twitching and standing temperature thresholds but the highest lethal threshold. Similarly, *C. megacephala* had the third lowest temperature threshold for standing but the second highest temperature threshold for death. *Chrysomya putoria* and *C. chloropyga* had similar and significantly higher temperature thresholds than all other species, for both twitching and standing (Fig. 6.5) but the 3rd and 4th highest temperature thresholds respectively for death, with *C. chloropyga* not differing significantly from those low thresholds of *L. sericata* and *C. albiceps*. The temperature thresholds for *L. sericata* and *C. albiceps* did not differ significantly from one another for all three responses, while *Calliphora croceipalpis* had significantly lower temperature threshold for twitching, standing and death when compared to all other species (Fig. 6.5).

Developmental zero

Ca. croceipalpis was the only species' to have the same rank for D_0 and all temperature thresholds (Table 6.1), otherwise the pattern between species' D_0s differed considerably from the pattern of species' larval, pupal and adult temperature thresholds (Fig. 6.1). Similarly, only one significant correlation was found between ranks for 'twitching' and 'standing' thresholds (Table 6.2). Although the larval and pupal thresholds had a strong correlation to the adult lethal thresholds, they were not significant (Table 6.2).

Field observations

At the site of rhinoceros 1, maggot mass temperatures were as high as 46.9°C and averaged 43.0°C (std. dev. = 2.11°C) when ambient temperature averaged 30.4°C (std. dev. = 0.13°C). *Chrysomya albiceps* masses on the periphery of the carcass had a considerably lower average temperature of 38°C (std. dev. = 1.59°C). The large majority

of the maggots inside the carcass were identified as 3rd-instar *C. marginalis*, while only about 50 where identified as *C. megacephala*. At the site of rhinoceros 2, maggot mass temperatures reached as high as 40.9°C and averaged at 34.2°C (std. dev. = 4.1°C) when ambient temperature averaged 18.3°C (std. dev. = 0.2°C). *Chrysomya albiceps* masses were again noted on the periphery of the carcass and on the intestines and stomach which lay approximately 1m away, and their temperature averaged 22.3°C (std. dev. = 2.41°C). Thousands of *C. albiceps, C. marginalis, C. megacephala, C. chloropyga* and *C. inclinata* were reared from rhinoceros 2, along with a couple of hundred *C. putoria* and *Lucilia* species. Temperature records of *C. albiceps* masses on the periphery of both carcasses had considerably lower temperatures than maggot masses on the interiors.

Maggots were observed feeding in a saturated environment in both carcasses, probably comprising of blood and larval excrement. The average depth of the maggot layers, which completely covered the interior of the carcasses and excluded *C. albiceps*, was 2.0cm (std. dev. = 0.3; n = 15). Most commonly the maggot layer was only one maggot deep, comprising of a mat of maggots protruding perpendicularly from the carcass with their posterior ends exposed (Fig. 6.6). In other instances, a second layer of maggots were present crawling on top of this feeding layer (Fig. 6.6).

Discussion

Rate of warming in maggots

The rate of warming of maggots was shown to be largely invariant within and between the species tested (Fig. 6.3). Thermal equilibration with the environment was exceptionally quick within all four species, suggesting that maggots rapidly thermo-conform (in roughly 3 minutes from 3°C - 30°C) to environmental temperatures. It is likely that this result would remain true for all species of blowfly. Their small size limits their thermal inertia below a threshold where they can retain any heat they generate (Sharpe and de Michele 1977). This thermo-conformity means that they cannot use thermal inertia to thermoregulate and proves that the environmental temperatures they experience accurately reflect the temperatures at which they are developing.

Thermophysiological trends

Calliphora croceipalpis had a significantly lower larval temperature threshold, mean pupariation temperature, and adult temperature thresholds than all six other species tested (Table 6.1). Similarly, *Ca. vicina* (R-D.) (6.62°C (std. err. = 5.12°C)) and *Ca. vomitoria* (L.) (5.44°C (std. err. = 0.64°C)) also had significantly lower D_0 values than all temperature thresholds for all *Chrysomya* and *Lucilia* species tested. This is not surprising as the genus *Calliphora* is characteristically from the north temperate zones, and is not typically (sub)tropical. They are therefore adapted to cooler environments, which is reflected in their behaviour and the distribution of *Ca. croceipalpis* in South Africa. This species reputedly breeds in small carcasses (Meskin 1980, 1986), e.g. bird and rat carcasses (Prins 1982), although it has been reported from human bodies (M.W. Mansell, pers. comm.). Small carcasses can support substantially fewer maggots than larger carcasses, implying a reduced capacity for maggot-generated heat production (Goodbrod and Goff 1990). This might mean that *Ca. croceipalpis* maggots experience lower temperatures more regularly than some other blowfly species, which is apparently more suited to its physiological temperature thresholds (Fig. 6.1-6.3). The low temperature thresholds for *Ca. croceipalpis* detailed in Figs. 6.1-6.3 and Williams (2002), are reflected in the distribution of *Ca. croceipalpis* in South Africa, as it is one of the few species to reside at higher altitudes (Zumpt 1965), and is most active in the winter months (Prins 1982; Williams 2002).

Lucilia sericata was introduced to southern Africa from the temperate climates of the Palaearctic and also belongs to a predominantly Holarctic genus (Harvey et al. 2003). They too have adapted to cooler environments but this is not as obvious in the results of this study as it was for *Ca. croceipalpis* (Table 6.1). There may be a strong phylogenic component to the thermal biology of these flies, which could explain why the temperature thresholds of *Calliphora* were vastly different to those of *Lucilia* and *Chrysomya*. *Lucilia* and *Chrysomya* are in the same subfamily (Chrysomyinae) and are therefore more closely related to one another than to *Calliphora*, which is placed in another subfamily (Calliphorinae) (Zumpt 1965; Scholtz and Holm 1985; Harvey et al. 2003). The genus *Chrysomya* is a phylogenetically compact and recently evolved group (Stevens & Wall

2001). The results show that *Chrysomya* expressed the highest thresholds for all life stages tested, which suggests that this genus is better suited for warmer climates than *Lucilia* and *Calliphora*.

The results from the preferred pupariation temperatures and upper lethal temperature thresholds for larvae and adults show no clear thermophysiological trend across all species (Table 6.1 and 6.2), presumably because their thermal environments differ considerable between life stages. These results are similar to those of Sanborn et al. (2003), who found no trend between minimum flight temperatures and shade-seeking temperatures in 22 species of Eastern Cape Cicadas across five different biomes. Despite this, numerous thermophysiological trends were found between the three life stages of certain species: with *L. sericata* and *C. megacephala* consistently had the lowest and highest recorded mean temperature thresholds respectively between *L. sericata, C. putoria* and *C. megacephala*; *C. albiceps* consistently had a lower temperature threshold than *C. megacephala*; and *C. chloropyga* consistently had lower temperature thresholds than *C. marginalis*; between *L. sericata*, C. *albiceps* and *C. marginalis*, for larval and pupariation threshold only, *L. sericata* and *C. marginalis* consistently had the lowest and highest recorded mean temperatures respectively.

Larval upper lethal temperature thresholds and interspecific competition

Surprisingly, the trends in the different species D_0s were dissimilar to those of the upper lethal thresholds. These results show that some species, particularly *C. megacephala* and *C. chloropyga* have a wide temperature range between upper (LT_{50}) and lower (D_0) thermal thresholds for larvae. This is advantageous for them as it allows both species to develop at higher and lower temperatures relative to other blowfly species. But, perhaps the importance in understanding thermal limitations in the microhabitats of larvae is not with D_0 (which affects their distribution in the environment), but with the upper lethal thresholds. This is because blowfly larvae frequently feed in large masses (Figs. 6.2 and 6.6) that produce heat as a result of metabolic activity (Deonier 1940; Payne 1965; Sharpe and de Michele 1977; Ames and Turner 2003). Therefore, maggots, particularly

those in 2nd and 3rd instar in larger carcasses, are more likely to experience temperatures closer to their upper lethal thresholds than to their D_0.

Of all the species tested, *Ca. croceipalpis*, L. *sericata*, *C. putoria* and *C. albiceps* had the four lowest larval upper lethal temperature thresholds respectively. Similarly, these four species have all adopted unique breeding strategies that utilize alternative food resources. *Calliphora croceipalpis* is commonly associated with small carcasses (Meskin 1980, 1986; Prins 1982), *L. sericata* is a facultative ectoparasite on mammals, particularly sheep (MacLoud 1943; Bauch et al. 1984; Stevens and Wall 1997; Anderson and Huitson 2004) but are frequently recorded on small carcasses (Meskin 1980; Prins 1982), *C. putoria* is most commonly associated with latrines but naturally breeds in faeces and small carcasses (Hulley 1983; Laurence 1988), and *C. albiceps* is a facultative predator on other maggots (Del Bianco Faria et al. 1999; Grassberger et al. 2003) and is commonly found on the periphery of carcasses (Fig. 6.2). As a result, the immature stages of these species may not experience significant maggot-generated heat on a regular basis and thus has a reduced larval upper lethal temperature threshold when compared to other species feeding on carrion.

Chrysomya megacephala, *C. chloropyga* and *C. marginalis* have adopted the more 'conventional' breeding strategy of carrion-breeding flies and are all attracted to medium-sized to large carcasses (Prins 1982; Braack 1984; Braack and Retief 1986) and experience intense interspecific and intraspecific competition (Ullyett 1950). Their larvae commonly experience high temperatures from maggot-generated heat and it is no surprise that these three species have the three highest larval upper lethal temperature thresholds. However, *C. marginalis* has a significantly higher larval upper threshold than the other two species. This would be advantageous when maggot mass temperatures reach above the thresholds of *C. megacephala* and *C. chloropyga*, as illustrated in the following example.

A classical example in which the upper thermophysiological threshold is the determining factor for one species prevailing over another is a defense mechanism called "heat-

balling", employed by Asian honeybees (*Apis cerana* (Hymenoptera: Apidae) against the false wasp, *Vespa velutina* (Hymenoptera: Vespidae). *Vespa velutina* is a predator of *A. cerana* and is known to kill 20-30% of bee colonies on each hunt (Sakagami 1960). As a defense mechanism, numerous individuals of *A. cerana* overpower individual wasps by covering them in a ball of honeybees (Matsuura and Sakagami 1973). Through metabolic heat generated by their wing muscles, the honeybees then actively raise the temperature within the ball (Esch 1960) above the wasp's upper lethal threshold, resulting in the wasp's death (Matsuura and Sakagami 1973). The thermophysiological threshold of the honeybees is 50°C while the thermophysiology of the wasps is only 46°C (Ken et al. 2005). I suggest that maggots of one species might similarly use maggot-generated heat to out-compete species on a limited food resource.

Ullyett (1950) suggested that *C. albiceps, C. chloropyga* and *Lucilia* sp. maggots have a distinct advantage over *C. marginalis* when competing for food source, as total developmental time of the latter species exceeds that of the above-mentioned species at any particular temperature. However, *C. marginalis* larvae can withstand higher temperatures than all other species tested. If maggot mass temperatures were to exceed the thermophysiological thresholds of all other species, *C. marginalis* would have a distinct advantage over those species and achieve dominance at the food resource. It is unlikely that this method of overcoming interspecific competition is a species-specific collective behavioural strategy. Instead its happening could be credited to the basic biology of each species.

The two rhinoceros carcasses provide evidence to this model. At rhinoceros 1, maggot mass temperatures reached as high as 47°C when ambient shade temperature was 30°C (± 0.3°C). This carcass temperature is unfavourable for *L. sericata* (LT_{50} = 47.8°C) but tolerable for *C. marginalis* (LT_{50} = 50.1°C). The results show that by far the majority of maggots collected from the carcass were *C. marginalis.* This clear dominance of *C. marginalis* was despite the abundant diversity of adult carrion-feeding flies of at least five species around the carcass. At rhinoceros 2, maggot mass temperatures reached 40°C, sufficient for all immature blowfly species to thrive. The results show that vast

numbers of maggots from at least six blowfly species were present, including *C. marginalis* and *L. sericata.* These results are preliminary evidence that maggot-generated heat may indeed shape carcass communities. *Chrysomya marginalis* most commonly lays eggs on large carcasses (Braak & Retief 1986), which might be because a large carcass can support a large number of maggots, leading to a high-temperature environment that allows it to out-compete other species. Small carcasses may not assist the species to generate such beneficially high temperatures.

Blowfly maggots may manipulate microhabitat temperature through maggot-generated heat for two reasons. First, it promotes more rapid growth rate during a vulnerable life stage (larval stage) (Catts 1992), which minimizes risk by passing through it as quickly as possible, and second, it can help to dominate competing species in interspecific competition in favorable environmental conditions. Both promote the survival of the species.

Thermophysiology of pupae

The thermal environment of pupae is very different to that of larvae or adults. All seven species of blowfly tested in this study leave the food source at post-feeding and pupariate in the soil either surrounding the food source or directly under the food source (in the case of *C. albiceps* (Grassberger et al. 2003) at a depth between 2-10cm. Soil temperatures at these depths remain fairly constant through time (Charman and Murphy 2007. Because of this D_0 may not be as important for understanding the thermal environment of pupae as preferred pupariation temperatures, but it is interesting to note that the pattern in D_0 for pupae was similar to that of larvae with the exception that pupal D_0s had larger inherent variation. The patterns in preferred pupariation temperatures were dissimilar to those in D_0, which suggests that no clear relationship exists between the developmental temperature thresholds and preferred development temperatures of a single life stage.

The mean preferred pupariation temperatures ranged between 16.6°C (*Ca. croceipalpis*) and 26.1°C (*C. megacephala*), with five of the seven species being within 1.5°C of 25°C. This close association of preferred pupariation temperatures to a moderate temperature may reflect the relatively constant temperatures of the pupal environmental in soil.

Thermophysiology of adult blowflies

Thermophysiological trends were found between "twitching" and "walking" thresholds for all species tested (Fig. 6.5). These data suggest that *C. putoria* and *C. chloropyga* are intolerant of cool conditions (below 14°C) and are likely to favor warmer areas, that *L. sericata, C. albiceps* and *C. megacephala* can tolerate a wider range of climatic temperatures than *C. putoria* and *C. chloropyga* (between 11°C and 50°C), that *Ca. croceipalpis* are able to tolerate cold areas (as low as 7.5°C), as they have supposedly adapted to cool temperate climates (as discussed above), and the combination of low "twitching", low "walking" and significantly higher lethal thresholds than for *C. marginalis* might suggest a cosmopolitan fundamental niche.

Conclusion

The thermal biology of blowfly larvae is apparently very important in understanding the community structure in the micro-environment of a carcass. If environmental conditions are favorable, species with a high thermal tolerance may use maggot-generated heat to overcome interspecific competition.

Thermal biology may not only be important in understanding distribution in a micro-environment but may also be useful in understanding geographic distribution on a meso- or global scale. This is evident in the low and significant low temperature thresholds of *Lucilia* and *Calliphora* species respectively, which are originally distributed throughout the cool temperate zones of the Northern Hemisphere.

Acknowledgements

I thank Philip S. Weyl for Figure 6; to Kerri-Lynne Kriel, Lucy Glover, Bradley Whittington, Mark Lewis, Tarryn Martin and John Midgley for assistance in both the laboratory and the field.

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Table 6.1 Rank order of temperature threshold for three life stages and D_0 for seven blowfly species.

	Rank order						
		Larval	Pupal	Adult thresholds			Mean
Species	D_0	LT_{50}	preference	Twitching	Walking	Death	rank
Ca. croceipalpis	7	7	7	7	7	7	7.0
L. sericata	4	6	3	3	3	4	3.8
C. chloropyga	4	2	6	1	1,2	4	3.1
C. albiceps	1	2,5	4	3	2,3	4	3.0
C. megacephala	4	2	1	3	3	1,4	2.6
C. marginalis	1	1	3,4	6	3	1	2.6
C. putoria	1	5	2	1	1	1,4	2.1

Table 6.2 Spearman's rank order correlation matrix. Bold correlations are significant at $p < 0.05$.

	Developmental zero	Larval Threshold	Pupal Threshold	Twitching	Standing
Larval Threshold	0.3143				
Pupal Threshold	0.0286	0.3929			
Twitching	-0.2943	0.1819	0.2910		
Standing	-0.0286	0.2143	0.0714	**0.8729**	
Death	0.2571	0.7500	0.7500	0.2910	0.0357

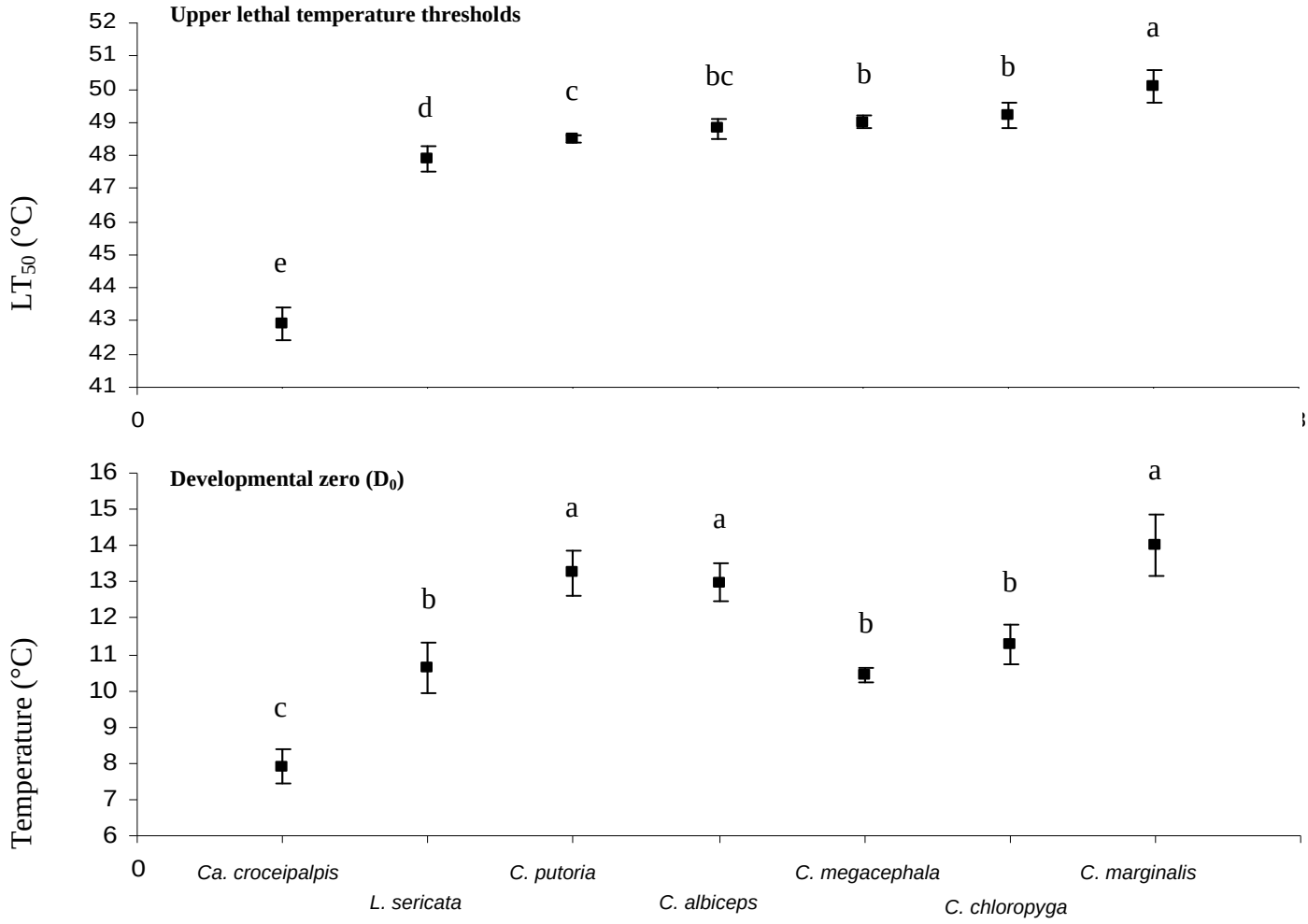

Figure 6.1 Temperature at which 50% of individuals died (LT_{50}) and corresponding developmental zeros (temperature below which development ceases) of seven blowfly species. Vertical error bars denote 95% confidence intervals. Developmental zeros for the following species were taken from: *Ca. croceipalpis* (Forsyth et al. unpublished), *L. sericata* (Richards and Villet unpublished), *C. putoria* (Chapter 5), *C. albiceps* (Chapter 4), *C. megacephala* (Chapter 2), *C. chloropyga* (Chapter 5), and *C. marginalis* (Weyl et al. unpublished).

Figure 6.2 Distinct segregation between *C. albiceps* and *C. marginali*s on a warthog (*Phacochoerus africanus*, Pallas) carcass in the Andries Vosloo Kudu Reserve in the Eastern Cape. Photograph by Cameron Richards.

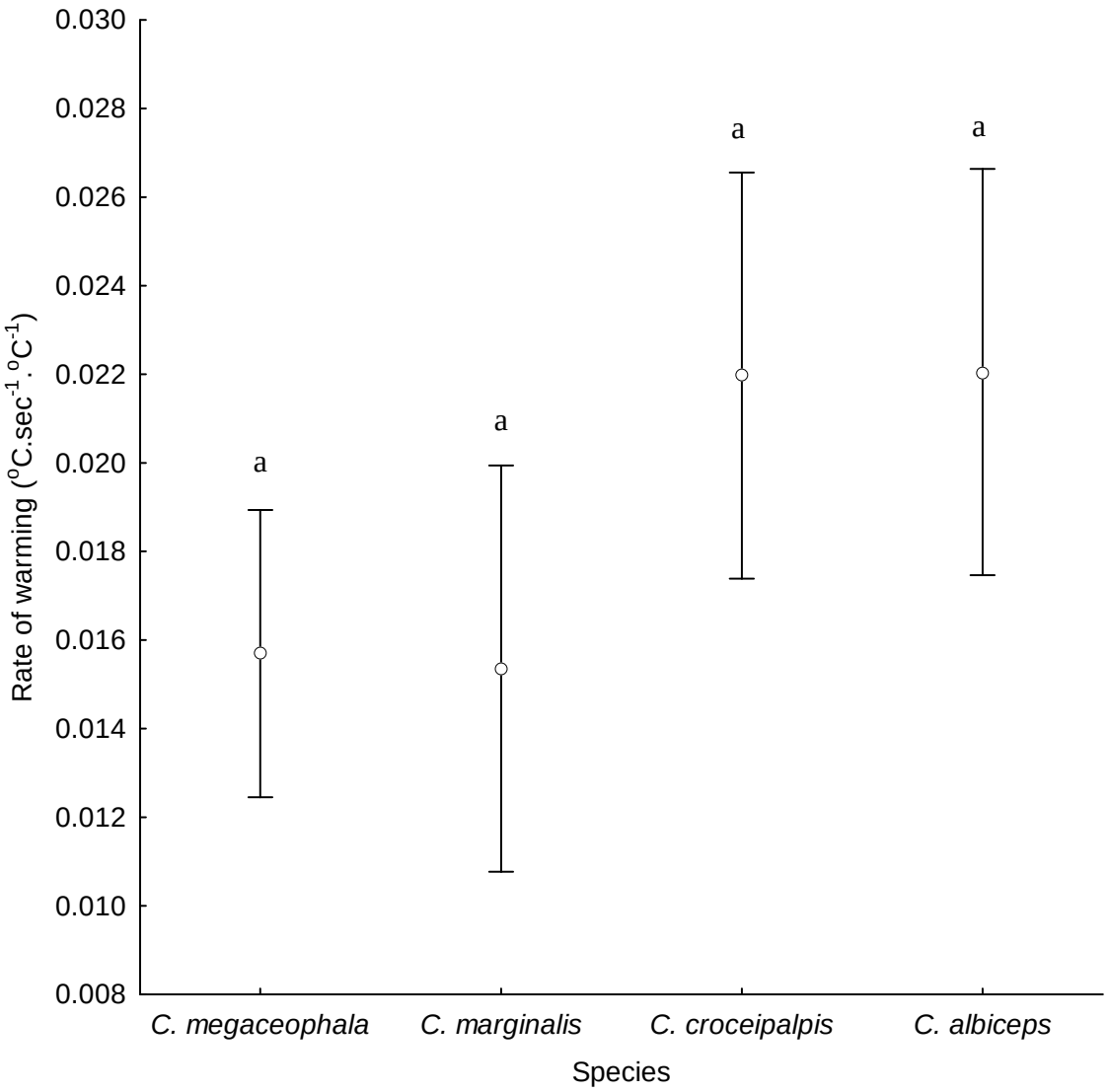

Figure 6.3 Mean rates of warming between four species of blowfly. Vertical bars denote the 95% confidence intervals.

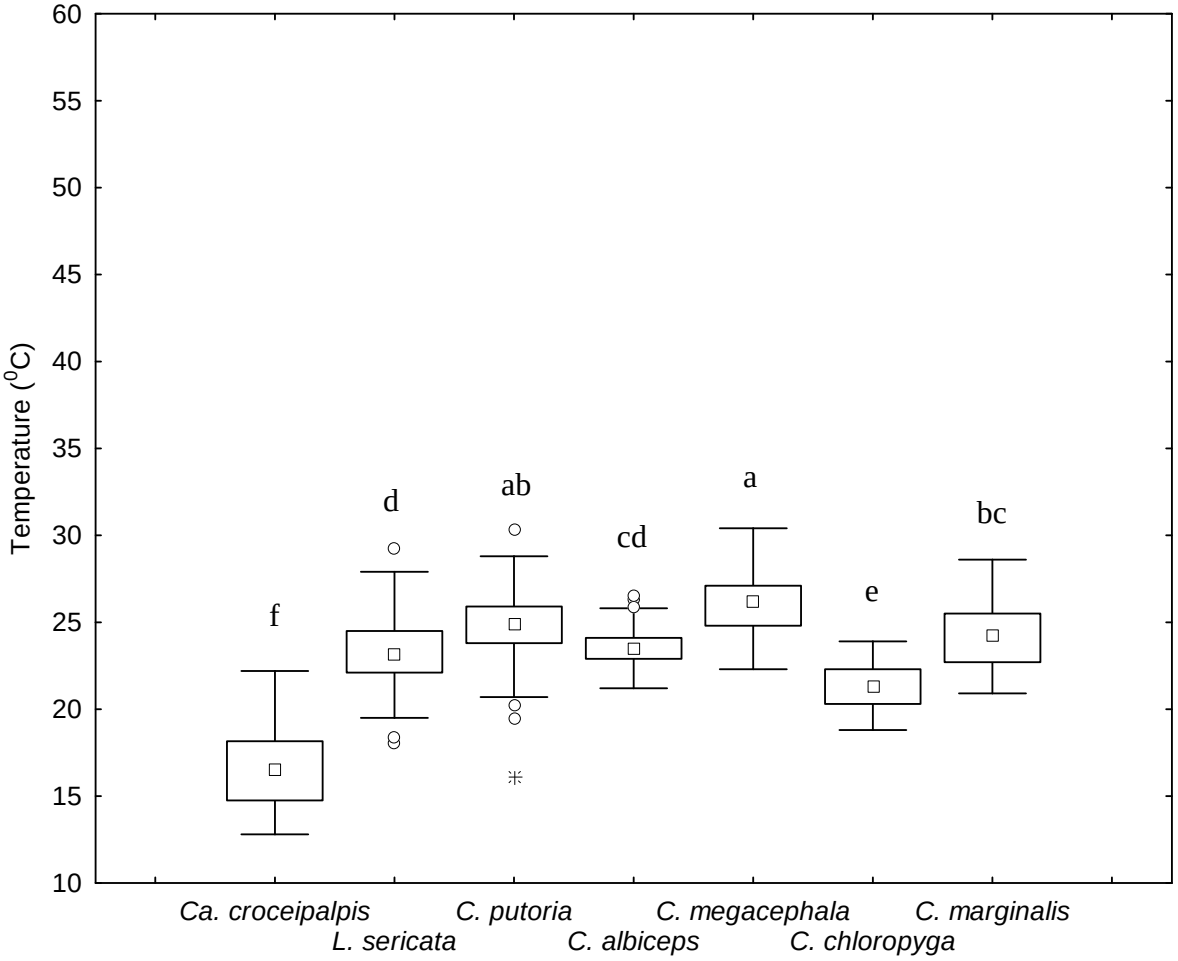

Figure 6.4 Box and whisker plot of recorded pupariation temperature of seven blowfly species represented by the median and 25-75 percentiles, and non-outlier range. Outlying and extreme values are represented by open circles and stars, respectively. The y-axis represents the temperature range that was available to maggots in the thermal gradients.

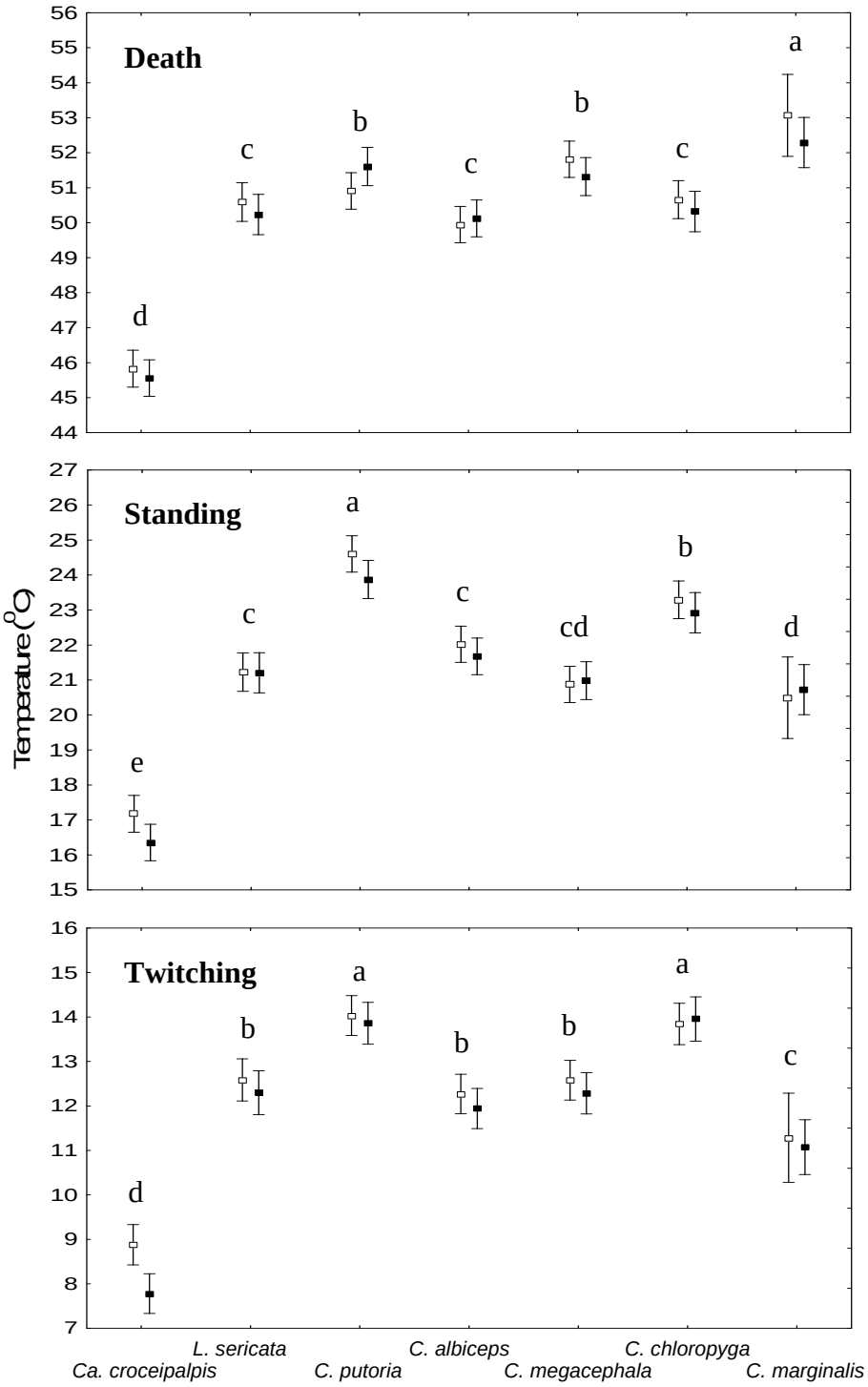

Figure 6.5 Temperature of adult of seven blowfly species at three different behavioural thresholds; twitching, standing, death. Open squares represent females' data and closed squares represent males' data. Vertical bars denote 0.95 confidence intervals.

Feeding maggot layer with posterior end exposed

Second maggot layer crawling on top of feeding layer

Feeding maggot layer with posterior end exposed

Figure 6.6 Photographs detailing structure of a typical 3rd instar maggot mass. Photographs taken by Phillip Weyl.

VII

The significance of temperature for the geographic distribution of seven blowfly species (Diptera: Calliphoridae) in South Africa

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Preface

This chapter aims to determine the significance of temperature on the geographic distribution of seven forensically important blowflies in South Africa. The results include maps of the predicted geographic distribution of these flies in South Africa, based on a suite of 11 climatic variables. This chapter has been submitted to *African Entomology*.

Abstract

The hypothesis that temperature is a primary determinant of the geographic distributions of seven ectothermic blowfly species in South Africa, a country with very large climatic and environmental gradients, was tested using maximum entropy models incorporating eleven climatic variables. It is shown that moisture, and particularly humidity, was in fact usually paramount. *Chrysomya albiceps* (Wiedemann) and *C. marginalis* (Robineau-Desvoidy) had the most widespread geographic and climatic distribution, while the forest-associated *C. inclinata* (Walker) was the least widespread. *Calliphora croceipalpis* (Jaennicke) and *C. chloropyga* (Wiedemann) were the only species predicted to occur at high altitudes. *Chrysomya putoria* (Wiedemann) and *C. megacephala* (Fabricius) had very similar predicted distributions that were restricted mainly to Limpopo, KwaZulu-

Natal and the coastline of the Eastern Cape. Localized distributions were predicted better than widespread ones, presumably because species with extremely widespread distributions in the study area occupy nearly the whole range of most predictor variables, leaving little for the maximum entropy modeling method to discriminate with.

Introduction

Numerous members of the family Calliphoridae (Diptera) are carrion-breeding flies that have veterinary, forensic and medical importance (Zumpt 1965). Some species are myiasis breeders (Baker et al. 1968, Hall et al. 2001), while others are known vectors of several enteric diseases (Sulaiman et al. 1988). These species are considered harmful or detrimental to humans and human society. Other carrion-breeding species may be used by forensic entomologist to detect the postmortem translocation of corpses (Smith 1986; Catts 1992). It is therefore essential to know the geographic distribution of these flies to focus control measures and to improve our basic understanding of their forensic significance.

Because blowflies are ectotherms, temperature heavily influences their development, behaviour and physiology, but the influence of temperature on their geographic distribution is unknown. To test the hypothesis that temperature would have a role in shaping where species occur, I predicted the known distribution of seven species using a suite of climatic variables and a maximum entropy technique implemented in the Maxent 3.0.2BETA (July 2007) software (Phillips et al. 2006). Maxent is the only predictive biogeography program that provides an analysis of the degree of influence of each environmental layer on the predicted distribution. It is also one of a few programs which does not need absence data to build a predictive model and it has been advocated by several recent publications, (Elith et al. 2006; Hernandez et al. 2006; Pearson et al 2007).

South Africa was chosen as the study area for several reasons. First, the country has large environmental gradients, from tropical forest to desert, from coastal forest to montane grassland, and from summer to winter rainfall. Second, digital maps with a 1’ resolution

(about 1.6 x 1.6 km) are available for a wide array of climatic variables (Schulze et al. 1997). Third, despite its climatic variability, the country is sufficiently small that any areas not represented by museum specimens can be surveyed relatively easily. This chapter presents maps of the predicted distributions for seven species of carrion-associated Calliphoridae in South Africa using the computer modeling program Maxent and comment on the climatic variables most influencing the distribution of adult blowflies.

Materials and Methods

Locality data

Locality records for seven different species of Calliphorids were initially collected from three different sources: personal contacts (listed in the Acknowledgements) (presence and absence data); literature searches (Baker et al 1968; Paterson 1968; Braack and Retief 1986; Braack 1991; Louw and van der Linde 1993; Williams and Villet 2006) (presence data only); and museum records compiled from the collections of the South African Museum (Cape Town); Rhodes University (Grahamstown); Albany Museum (Grahamstown); Durban Natural Sciences Museum (Durban); Natal Museum (Pietermaritzburg); Kruger National Park Museum (Skukuza); and Onderstepoort Veterinary Institute (Pretoria).

After collecting and combining these data, five field trips were designed to collect data in areas with few or no locality records. Red-top® fly traps (Miller Methods, Ltd.) were used to catch blowflies on all field trips. They were modified by making a horizontal cut in the base of the trap. Half-litre jars containing 125g of fresh chicken liver were attached to the trap at the horizontal cut with a 10x10cm gauze mesh placed between the trap and the opening of the bottle. This allowed the flies to detect the odour of the liver and enter the trap but prevented them from becoming fouled in the liver, making them easier to handle and identify. One modified fly trap was set every 50km along the survey route and left for no fewer than 4 days. Blowflies were also collected from any dead animals found

between traps. Field trips were conducted in all seasons excepting winter to optimize trapping and to account for any possible seasonal trends (Braack and De Vos 1987).

The area between Ixopo (KwaZulu-Natal) and Sani Top border post (Lesotho) was chosen for a survey of altitudinal distribution for two reasons; 1) it is one of the few places in South Africa with access to high altitudes, and 2) all species of interest occur in this region. Four modified traps, placed approximately 100m apart, were set at each of eight sites, separated by 300m altitude, starting in Ixopo (900m a.s.l.) and ending at Sani Top border post (3000m a.s.l.), during January, 2006. Traps were collected after five days and the contents was identified and counted.

Climatic variables

Eleven climatic predictor variables were selected (Table 7.1). Digital maps of the variables for South Africa, developed by Schulze et al. (1997), were created by interpolating from point data obtained from a network of weather stations distributed throughout South Africa and averaged over 10 years, to produce continuous digital maps (or 'layers') at a resolution of 60 pixels per degree (Schulze et al. 1997). Each climatic variable was originally represented by a map for each month, a total of 72 layers. To reduce the dimensionality of the climatic data set to rationalize computing effort, principal component analyses (PCA) were performed of the twelve monthly maps of each variable, and the factor scores for the first two principle components were kept, resulting in two summary layers for each variable (Table 7.1). The first variable (PCA axis 1) represented the magnitude of the climatic variable, while the second (PCA axis 2) represented seasonal variation in the same variable. The resulting layers were the same as those employed by Robertson et al. (2001, 2003, 2004).

Statistical analysis

Maps of distributions in South Africa were constructed using "Maxent" version 3.0.2-BETA (July 2007) (downloadable from http://www.cs.princeton.edu/~schapire/maxent/), eleven climate variables (Table 7.1) and the locality data for *Chrysomya albiceps*

(Wied.), *C. marginalis* (R-D.), *C. megacephala*, *C. putoria* (Wied.), *C. chloropyga* (Wied.), *C. inclinata* (Wa.), and *Calliphora croceipalpis* (Jaen.).

Maxent uses only presence data and a maximum entropy method for predicting geographic distribution. It estimates the probability distribution that is closest to uniform, or most spread out (maximum entropy), subject to the constraint that the expected value of each environmental variable matches the average values for the set of locality records taken from the target distribution (Phillips et al. 2006).

Phillips et al (2006) list three implicit ecological assumptions in the set of environmental variables used for modeling which the user should meet for the most representative predictions. First, "temporal correspondence should exist between occurrence localities and environmental variables". Locality records used in the analyses were no older than 50 years, which is within the temporal precision of the climate variables used in this study (Hijmans and Graham 2006). Second, "the variables should affect the species' distribution at the relevant scales". The variables used in this study include fundamental climatic descriptors that are appropriate to ectotherms at global and meso-scale (Mackey and Lindenmayer 2001, Robertson et al. 2003), which is relative to the region under study. Since the species are generalist feeders that do not occur throughout the study area, it is likely that climate will shape their distributions more than food or breeding resources at this scale. Third, "the choice of variables to use for modeling also affects the degree to which the model generalizes to regions outside the study area". Because the entire modeling area had been surveyed, this study was concerned with interpolation within the study area rather than extrapolation outside it, so this requirement is not violated.

Default parameters of Maxent were employed for the 'regularization multiplier' (1), 'maximum iterations" (500), 'convergence threshold' (0.00001) and 'maximum number of background points' (10000), with accumulative output type to construct the predictive distribution maps. Additionally, random seed analysis and a random test proportion of 20%, instead of the default 0%, were used. This meant that 20% of all data points were reserved from building the predictive models and instead were used to measure predictive

success. These data are termed 'test' data, while the data used to build the model are termed "training" data (Pearson et al. 2007). Jackknife analyses and response curves (area under curve (AUC)) for each species were also constructed using Maxent. Detailed descriptions of these two analyses are described in Pearson et al (2007). Jackknife is used to identify the most influential predictive variable(s) and to assess the predictive success of the model. This is achieved by removing one climatic variable and making a prediction from the remaining data set, until each variable has been tested. The change in fit of the prediction is a measure of the influence of the omitted variable. Default Maxent jackknife procedures are performed on training data, test data and AUC values. Results in this paper focus on jackknife validations of AUC values only. The AUC is commonly used as a measure of models' overall performance with values commonly ranging from 0.50 (random) to 1.00 (perfect discrimination) (Hernandez et al. 2006). Values below 0.50 are considered to be worse than random and the predictive result is ignored. Other measures of predictive success are available (Fielding and Bell 1997), but I have used AUC simply because it is built in to the Maxent software package.

Results and Discussion

Locality records

The final data set included 66 species records from 45 localities from the literature, 60 species records from 24 localities from personal contacts, 427 species records from 214 localities for the five field trips, and 615 species records from 336 localities for the seven museum and university collections, a total of 1168 species records from 512 different localities in South Africa (Fig. 7.1, Table 7.2). This was sufficient to cover all climatic zones in the country although sampling effort was not equal in each zone.

Climatic variables

Before computer-based predictive biogeography programmes were available, numerous authors (Smit and du Plessis 1926; Ullyett 1950; Zumpt 1956, 1965; Pont 1980; Prins 1982) made anecdotal statements about blowfly distribution without mention of any specific environmental variable. Because blowflies are ectotherms, temperature strongly

influences their physiology and development (Higley and Haskell 2001; Ames and Turner 2003). It was therefore assumed that temperature would be the environmental variable most influencing geographic distribution of blowflies. However, the jackknife validations indicate that the variables of least overall influence on blowfly distribution were maximum temperature, minimum temperature, and seasonal variation in humidity, with respective mean ranks of 8.1, 7.6 and 8.9 out of 11 (Table 7.3).

The jackknife validations also indicated that for all species, the climate variables relating to moisture e.g. humidity and evaporation, had more influence on distribution than other variables (Table 7.3). More specifically, the amount of relative humidity had a mean rank of 2.3 out of 11, and for four of the seven species humidity (Humd1) had the largest AUC value, while the remaining three species had humidity as their 2^{nd}, 4^{th} and 5^{th} most influential variable, respectively (Table 7.3). The remaining climatic variables had means ranks of about 5-6 out of 11, and larger standard deviations in rank than amount of humidity, indicating less consistency in their rank. These patterns became even more polarized if the analysis omitted *C. albiceps* and *C. marginalis*; these species had slightly atypical, relatively poorly fitted models for reasons discussed at the end of this paper.

Basic feeding behaviour of blowfly larvae in a carrion environment often results in maggots occupying a saturated microhabitat, and for this reason studies involving the immature stages of development mostly ignore moisture content, but authors often report ambient humidity for studies involving adult flies (Al-Misned 2001; Grassberger et al. 2003; Anderson 2004; Clark et al. 2006). Adult blowflies more readily die from even a brief absence of water than from extended cold temperatures (6-9˚C) (personal observations). Clearly, water loss is more influential than temperature. Humidity is also probably more of a limiting factor to egg development than temperature, as blowfly eggs desiccate easily and blowflies generally lay eggs on areas of a carcass that are very sheltered, especially crevices, body orifices and junctions between the ground and body (personal observations). These observations therefore support the primacy of humidity over temperature and offer two physiological mechanisms in different life stages to explain it.

Individual species' predictions

There is very little literature available on the geographic distribution of these flies in South Africa and what is available is either anecdotal, fragmentary or outdated (Smit and du Plessis 1926; Ullyett 1950; Zumpt 1956, 1965; Pont 1980; Prins 1982). The most recent publication on this topic is by Williams and Villet (2006), who provide a detailed account of locality records for two invasive calliphorids, *C. megacephala* (F.) and *Calliphora vicina* (Meigen). Although this is a comprehensive paper, it accounts for only two species and provides limited insight into the potential geographic distribution of these two species, which are apparently still spreading through the country. The predictions in this chapter provide both detailed maps and insight into the biology of seven blowfly species.

***C. albiceps* and *C. marginalis*.** These two species had the widest predicted geographic and climatic distributions of all seven blowfly species tested and it is likely that they can be found in almost all parts of South Africa expect in areas of high altitude (Fig. 7.2). The results from the altitude survey support these findings and imply that both species may occur only as high as 2400m but are most common below 1800m (Fig. 7.3). Jackknife validations for both species show that no single climatic variable to be identified as most influential on the geographic distribution of both blowfly species as jackknife validation values for all climate variables were between 0.43 and 0.62 for both species (Table 7.3). Together with this, 8 and 10 of the 11 variables yielded an AUC value within 0.04 of one another for *C. marginalis* and *C. albiceps*, respectively.

C. putoria. This species is commonly referred to as the "tropical latrine blowfly" for its originally tropical distribution throughout the central, eastern and southern African countries (Table 7.4) and Neotropical countries (Guimarães et al. 1978, Baumgartner and Greenberg 1984, Laurence 1988, Rognes and Paterson 2005), and for its renowned feeding and breeding behaviours around faeces and latrines (Laurence 1981, 1988; Braack 1991, Wells et al 2004). It was introduced into Southern Brazil during the 1970s (Guimarães et al. 1978) and has since spread north through the tropical regions of South and Central America (Wells et al 2004, Rognes and Paterson 2005). The data from the

jackknife validations suggest that the climate variables of most influence on the distribution of *C. putoria* are degree of humidity, variation in maximum temperature, and amount of rainfall (Table 7.3). These three variables describe a warm, moist, wet environment that is characteristic of tropical environments and therefore validates its common name of "tropical latrine blowfly".

The predicted distribution of *C. putoria* in South Africa is mainly along the eastern coastline of South Africa, Mpumalanga and the central parts of Limpopo province (Fig. 7.2). The Drakensberg mountain range appears to act as a natural barrier to the inland spread of *C. putoria*, probably because it plays a major role in creating a rain shadow in the interior of South Africa. The fact that few species were recorded in the altitude survey (Fig. 7.3) suggests that this species prefers areas of low altitudes (below 1200m). This predicted distribution is supported by a high AUC value for both training (0.92) and test (0.91) data, indicating a good fit.

C. megacephala. This is another latrine fly that has a wide distribution in the Oriental and Australasian regions (Bohart and Gressitt 1951; Wijesundara 1957; Zumpt 1965; Khole 1979; Levot et al. 1979; O'Flynn 1983; Nishida 1984; Nishida et al. 1986; Wells and Kurahashi 1994). Its distribution in Africa is not well documented and only two studies on the African variety has been published, from Egypt (Gabre et al. 2005) and South Africa (Williams and Villet 2006), while Zumpt (1965) mentions records on islands off the east coast of Africa, including Madagascar, Réunion and Mauritius. There are only four other African locality records, from four different countries, in the data for this study (Table 7.5), which illustrates the lack of understanding of the distribution of *C. megacephala* in Africa. Furthermore, the 285 collecting events for *C. megacephala* revealed only 38 specimens at 35 different localities in the whole of South Africa (Table 7.2). This might suggest that *C. megacephala* does not occur in abundance in South Africa and that its distribution might be more extensive than predicted simply due to the lack of locality records, which are unevenly distributed throughout the country (i.e. weighted in the north eastern and eastern provinces). Similarly, it is possible that *C. megacephala* has not spread to the limits of its tolerance and that their geographic

distribution may continue to expand (Williams and Villet 2006). The evident low abundance of this species calls into question the absence of *C. megacephala* in the altitude survey. Absences can be explained by far more reasons than presences (Fielding & Bell 1997; Robertson et al. 2003), and is therefore difficult to draw any conclusions about the limits of their distribution in this regard (Fig. 7.3).

Chrysomya megacephala has a very similar geographic distribution to *C. putoria*, with a predominant occurrence along the eastern coastline, Mpumalanga and Limpopo but extends into the Northern Free State, and along the entire coastline of the Western Cape (Fig. 7.2). Zumpt (1965) noted that the distribution of *C. megacephala* in Australia extended along much of the east coast, as far south as Bateman's Bay, but was not recorded at any distance from the coast. A similar trend of geographic distribution is evident in the results of this study with a large proportion of its geographic distribution occurring along the coastlines of South Africa. Humidity (magnitude) was the most influential environmental variable on the geographic distribution of *C. megacephala* (Table 7.3). Humidity is essentially the amount of water vapor present in the atmosphere and is measured as a proportion relative to the maximum quantity of water vapor that the atmosphere can support (Fellows 1985). This is termed 'relative humidity' and is expressed as a percentage. Relative humidity is affected in one of two ways: (1) a decrease in ambient temperature causes an increase in relative humidity, even though the amount of water vapor in the atmosphere remains constant, and (2) the presence of a large water surface increases relative humidity through evaporation (Strahler 1975). Therefore, relative humidity is often highest along coastlines due evaporation from the ocean. This explains why *C. megacephala* is found along the coastlines of Australia and now South Africa and not at any distance from the coast.

C. inclinata. This fly inhabits dense, forest-like vegetation, as trapping this species is always most successful in these habitats. This is strongly reflected in its predicted distribution and its absence in the altitude survey. The geographic distribution of *C. inclinata* was most influenced by degree of humidity (Table 7.3) and is therefore highly unlikely to occur in Gauteng, Northwest, Free State and Northern Cape provinces (Fig.

7.2). Furthermore, it is mostly absent from the Western Cape where it is entirely restricted to the eastern coastline, north of Mossel Bay (34˚10'28"S:22˚05'13"E). The distribution expands into the Eastern Cape limited to areas within approximately 80km of the east coast. The distribution further expands into KwaZulu-Natal, resisted to areas east of Pietermaritzburg in the south and Vryheid in the north, and may continue along the east coast well into Mozambique, as far north as Ethiopia (Zumpt 1965).

Ca. croceipalpis. Zumpt (1965) states that *Ca. croceipalpis* "is a common fly in South Africa, but in tropical parts is probably restricted to higher altitudes". Maxent predicted that *Ca. croceipalpis* does not have as wide a distribution as *C. albiceps, C. marginalis, or C. chloropyga* (Fig. 7.2) and is certainly more common at areas of higher altitude. This is strengthened by the fact that the only *Ca. croceipalpis* specimen caught in the altitude survey was at the highest altitude (3000m). It is unlikely that *Ca. croceipalpis* occurs on the coastline of KwaZulu-Natal and it appears to be limited to areas west of Pietermaritzburg (29˚38'S:30˚26'E) (> 640m altitude). Unfortunately the altitude survey could not confirm this as I did not trap at altitudes lower than 900m. The most northern point that *Ca. croceipalpis* is likely to occur on the eastern coastline is Port St. Johns (31˚37'S:29˚32'E) in the Eastern Cape. Furthermore, and it is highly unlikely that *Ca. croceipalpis* will occur north or east of Umfuli (KwaZulu-Natal) (28˚50'S:30˚28'E) except in the northern Drakensberg mountains. *Calliphora croceipalpis* is prevalent throughout Gauteng, Eastern Cape, and Western Cape including the rainforests of Nature's Valley (33˚59'S:23˚33'E) and Tsitsikamma National Park (34˚01'S:23˚52'E). It is largely absent from the Northern Cape (limited to the Cederberg mountains only), Northwest Province (limited to the border of Gauteng), Limpopo (limited to the northern Drakensberg), Mpumalanga (limited to the northern Drakensberg), and the Free State (limited to the Lesotho border) (Fig. 7.2).

Of the 169 *Ca. croceipalpis* records used in this analysis, 118 (70%) of those were caught from July to October, which supports Prins's (1982) finding that this species is "more active during the winter months and early spring".

Calliphora croceipalpis and *C. chloropyga* are the only species in which 'humidity' was not listed as one of the top three most influential climatic variables. Similarly, both species are the only species to be most affected by evaporation and least affected by seasonal variation in minimum temperature (Table 7.3). *Calliphora croceipalpis* has been referred to as a cold-adapted species (Zumpt 1965; Prins 1982) and the results from this study show that it resides at higher altitudes in South Africa (Fig. 7.2 and 3).

C. chloropyga. It is evident that *C. chloropyga* has a much wider distribution than *Ca. croceipalpis* in South Africa (Fig. 7.2). This species does not favour hot and very dry climates e.g. the Kalahari. It is found throughout Gauteng, Mpumalanga, Kwa-Zulu Natal, Eastern Cape and Western Cape, and throughout most of the Northwest Province and the Free State. It is limited to the eastern and southern areas of the Northern Cape and the southern area of Limpopo.

The predicted distribution map for *C. chloropyga* suggests a distribution at higher altitudes. The results from the altitude survey support these findings as *C. chloropyga* was the only species to reside at all altitudes and was the most common species trapped at altitudes higher than 2400m (Fig. 7.3). This might imply that *C. chloropyga* is able to tolerate cooler climates than other *Chrysomya* species.

Predictive success of Maxent

Computer modeling programs such as Bioclim (Nix 1986), Domain (Carpenter et al. 1993), FloraMap (Jones and Gladkov 1999), GARP (Stockwell and Peters 1999) and Maxent, have been used extensively to map predictive distributions of many animal and plant species, but no author has used these programs to model blowfly distribution in South Africa. I did not compare Maxent with other software, but did notice that Maxent performs better for some species than for others. *Chrysomya albiceps* and *C. marginalis* were shown to have extremely widespread distributions in the study area, and therefore occupy nearly the whole range of most predictor variables. This seems to have challenged the predictive power of the maximum entropy modelling method as both species had the two lowest AUC values for training and test data (Table 7.3). But, species with a

localized geographic distribution had the highest AUC value for both training and test data, implying nearly perfect models. It seems that Maxent is most capable in predicting geographic distribution for species with limited climatic distributions and that prediction for species with widespread climatic distributions are less successful. These conclusions support the findings of other studies (Stockwell and Peterson 2002; Segurado and Araujo 2004; Hernandez et al. 2006; Pearson et al. 2007).

Stockwell and Peterson (2002) and Hernandez et al. (2006) explain that different habitat preferences of sub-populations within a species of widespread geographic and climatic distribution exist as a distinct climatic range and that when the species is modeled as a whole, the resulting distribution is an overestimate of the species' realized niche. This results in the prediction appearing more random than not. Therefore, adaptation by local populations to surrounding environments may decrease the accuracy of the predictive model. Evidence of such local adaptation has been found in the developmental ecology of *C. albiceps* (Chapter 4), lending support to this explanation.

Acknowledgements

I thank Ivan Horak, Claire Jackson, Bret Lunt, Nicola Lunt, John Midgley, Nkululeko Nyangiwe, Kyla Orr, Jane Pistorius, Carol Simon, Kyle Smith, Tamara Smith, and Gareth Whittington-Jones for blowfly locality records; Ashley Kirk-Spriggs (Albany Museum, Grahamstown), Kirstin Williams (Durban Natural Sciences Museum, Durban), Hendrik Sithole (Kruger National Park Museum, Skukuza), Simon van Noort (South African Museum, Cape Town), Gert Venter (Onderstepoort Veterinary Institute, Pretoria) for locality records from museum collections; Mikhail Mostovski (Natal Museum, Pietermaritzburg) for data from the museum's database.

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Table 7.1 Climate variables used for building predicted geographic distribution maps.

Variables	Abbreviations	Predictor variable
Minimum Temperature 1	Mint1	Component axis 1 (magnitude of temperature) of a PCA on 12-monthly minimum temperature surfaces
Minimum Temperature 2	Mint2	Component axis 2 (amplitude of temperature fluctuations) of a PCA on 12-monthly minimum temperature surfaces
Maximum Temperature 1	Maxt1	Component axis 1 (magnitude of temperature) of a PCA on 12-monthly maximum temperature surfaces
Maximum Temperature 2	Maxt2	Component axis 2 (amplitude of temperature fluctuations) of a PCA on 12-monthly maximum temperature surfaces
Rainfall 1	Rain1	Component axis 1 (magnitude of rainfall) of a PCA on 12-monthly rainfall surfaces
Rainfall 2	Rain2	Component axis 2 (season of rainfall) of a PCA on 12-monthly rainfall surfaces
Relative Humidity 1	Humd1	Component axis 1 (magnitude of humidity) of a PCA on 12-monthly humidity surfaces
Relative Humidity 2	Humd2	Component axis 2 (seasonal variation in humidity) of a PCA on 12-monthly humidity surfaces
Frost	Frost	Number of days with frost
Evaporation 1	Evap1	Component axis 1 (magnitude of evaporation) of a PCA on 12-monthly evaporation surfaces
Evaporation 2	Evap2	Component axis 2 (seasonal variation in evaporation) of a PCA on 12-monthly evaporation surfaces

Table 7.2 Number of specimens and locality records collected and used to construct distribution maps for seven species of Calliphoridae.

Species	# of Specimens	# of locality records
C. albiceps	292	261
C. chloropyga	275	256
C. putoria	111	101
C. inclinata	40	39
C. marginalis	254	227
C. megacephala	38	35
Ca. croceipalpis	165	141

Table 7.3 AUC values for whole models and Jackknife validations for individual climate variables used to predict the distributions of seven species of Calliphoridae. AUC values indicate the quality of the predicted distribution map, while the Jackknifed models identify the environmental variable(s) most influential on the predicted distribution of each species. Values nearest to 1.00 represent a perfect validation; those near 0.5 represent a random fit. The predictor variables are arranged by mean rank across all species. Numbers in bold correspond to variables of most influence in a particular species while numbers in italics correspond to variables of least influence.

	Rank		AUC						
	Mean	S.D.	*C. albiceps*	*C. chloropyga*	*C. inclinata*	*C. marginalis*	*C. megacephala*	*C. putoria*	*Ca. croceipalpis*
Whole model									
Training data			0.77	0.85	0.98	0.79	0.90	0.92	0.92
Test data			0.55	0.81	0.97	0.71	0.75	0.91	0.89
Variables									
Humd1	2.3	1.6	0.51	0.67	**0.96**	**0.62**	**0.83**	**0.86**	0.77
Evap1	4.7	3.5	0.49	0.72	0.91	*0.48*	0.82	0.78	**0.83**
Rain2	5.2	3.3	0.50	0.71	0.92	0.58	0.59	0.81	0.75
Maxt2	5.4	2.2	*0.43*	0.62	**0.96**	0.60	0.72	0.85	0.74
Frost	5.6	3.2	0.49	0.59	0.95	0.61	0.80	0.77	0.71
Evap2	5.8	2.8	0.49	**0.73**	0.86	**0.62**	0.52	0.66	0.76
Rain1	5.9	3.1	**0.52**	0.60	0.93	0.54	0.70	0.81	0.68
Mint2	6.6	3.5	0.47	0.68	0.88	0.50	0.74	0.75	0.80
Mint1	7.6	3.0	0.49	*0.50*	0.89	0.60	0.68	0.76	*0.55*
Maxt1	8.1	2.5	0.48	0.66	*0.62*	0.59	0.46	*0.61*	0.79
Humd2	8.9	1.9	0.49	0.60	0.80	0.59	*0.39*	0.64	0.65

Table 7.4 Locality records of *C. putoria* in Africa, excluding South Africa.

Country	Collection/ Reference
Botswana	Albany Museum, Rhodes University, Paterson 1968, Rognes and Paterson 2005
Cameroon	Natal Museum, Paterson 1968, Rognes and Paterson 2005
Democratic Republic of the Congo	Paterson 1968, Rognes and Paterson 2005
Eritrea	Paterson 1968
Ethiopia	Zumpt 1965
Gambia	Natal Museum, Rognes and Paterson 2005
Ghana	Natal Museum, Paterson 1968, Rognes and Paterson 2005
Kenya	Natal Museum, Rhodes University, Rognes and Paterson 2005
Liberia	Paterson 1968, Laurence 1988
Madagascar	Natal Museum, Rognes and Paterson 2005
Malawi	Natal Museum
Mauritius	Natal Museum, Rognes and Paterson 2005
Mozambique	South African Museum, Paterson 1968
Namibia	South African Museum, Kurahashi and Kirk-Spriggs 2006
Nigeria	Paterson 1968
Senegal	Paterson 1968, Rognes and Paterson 2005
Sierra Leone	Paterson 1968, Rognes and Paterson 2005
Sudan	Paterson 1968
Swaziland	Rognes and Paterson 2005
Tanzania	Laurence 1988, Paterson 1968, Rognes and Paterson 2005
Uganda	Natal Museum, Rognes and Paterson 2005
Zambia	Albany Museum, Rhodes University, Rognes and Paterson 2005
Zimbabwe	Albany Museum, Natal Museum, Rhodes University

Table 7.5 Locality records of *C. megacephala* in Africa, excluding South Africa.

Country	Locality	Date	Collector	Collection
Cote d'Ivoire	Abidjan	21 April 1989	J.G.H. Londt	Natal Museum
Kenya	Sigor	31 Jan 1973	I. Bampton	Natal Museum
Zimbabwe	Harare	12 Dec 2000	N. Lunt	Rhodes University
Zambia	Kitwe	18 July 2001	N. Mkize	Rhodes University

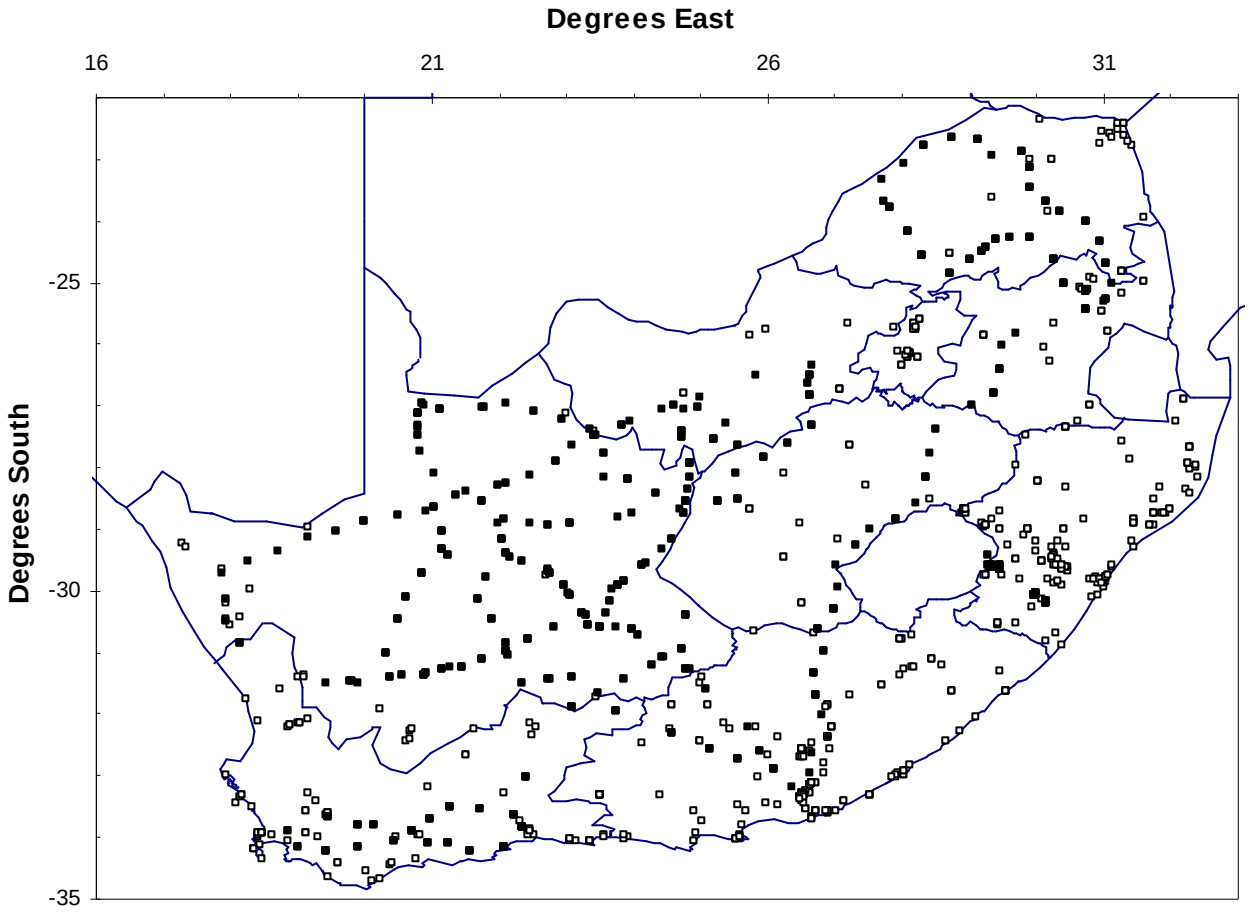

Figure 7.1 Distribution of all locality records used in this study for all species tested. Closed squares represents data collected on field trips (as described in the text) and open squares represents data obtained from museums, literature or personal contacts.

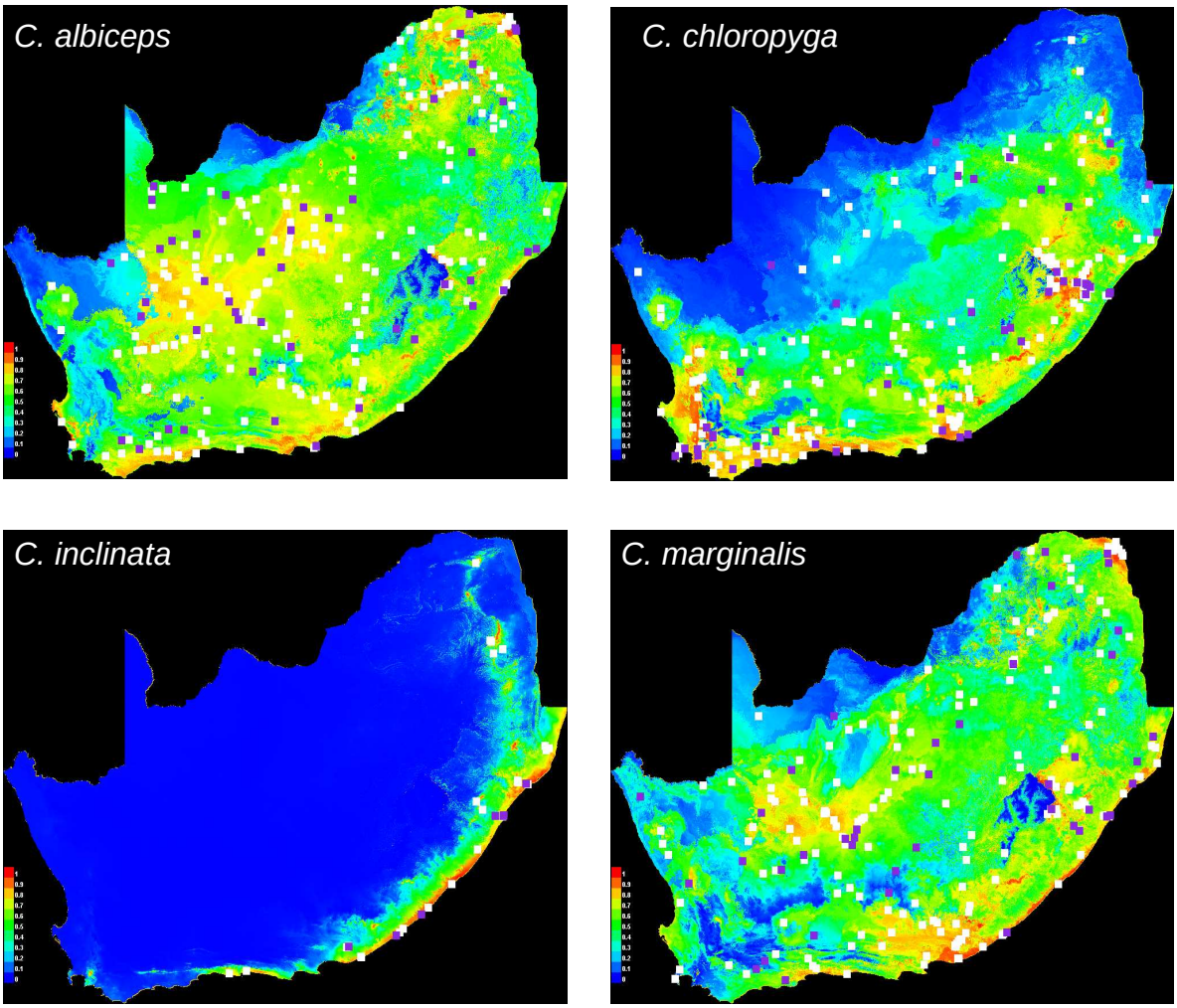

Figure 7.2 continued

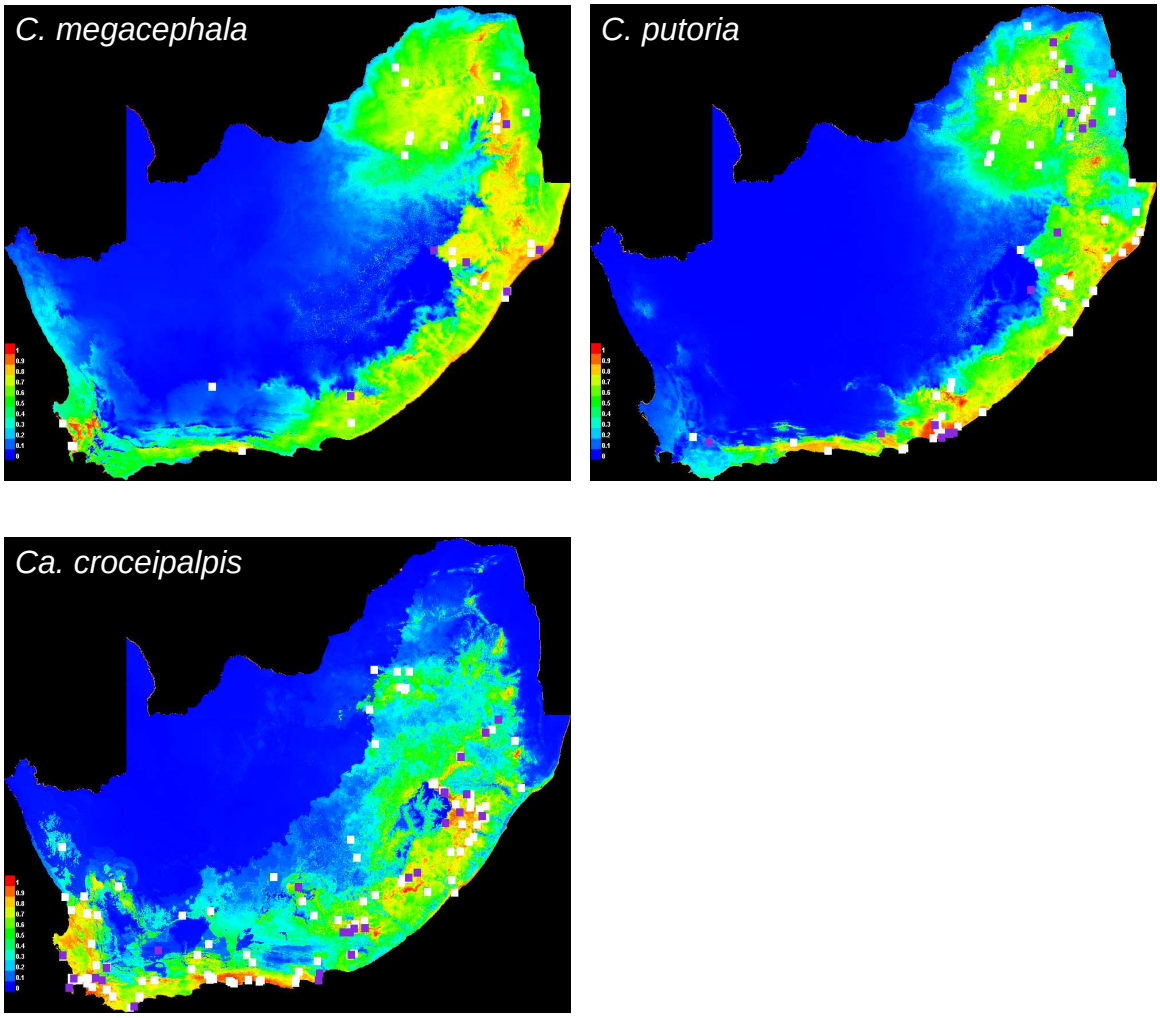

Figure 7.2 Maximum entropy modelling results of predictive distribution maps for seven blowfly species. Open (white) squares represent training data and closed (lavender) squares represent test data. The colour scheme on each map represents the probability of blowfly distribution ranging from dark blue (most unlikely to occur) to red (most likely to occur).

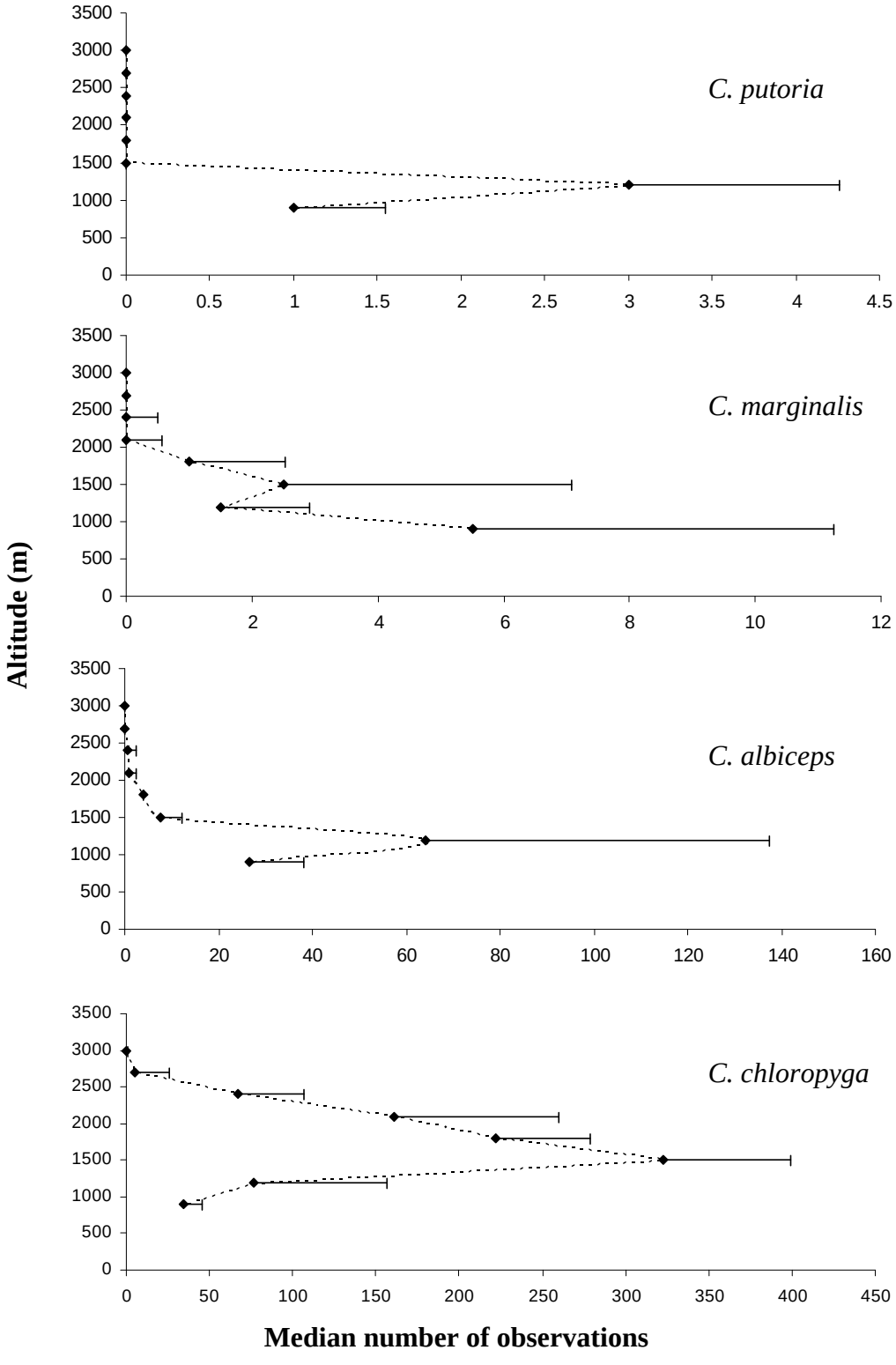

Figure 7.3 Altitudinal distribution profiles of four species of blowfly.

VIII

General Discussion

Preface

The aims of this thesis (listed in chapter 1) are discussed in corresponding order in each subsequent chapter. The aim of this chapter is to condense these conclusions and discuss some applications in future research.

Because minimum PMI estimates are based on the calculation of growth rate or development of immature insects, much of the forensic literature focuses on factors influencing development (Grassberger and Reiter 2001, 2002; Greenberg and Kunish 2002; Kaneshrajah and Turner 2004; Clarke et al. 2006; Nabity et al. 2006, 2007). These include effects of drugs and toxins (reviewed by Campobasso et al. 2004), maggot-generated heat (Turner and Howard 1992; Slone and Gruner 2007), nocturnal oviposition (Greenberg 1990a), diet (Clarke et al. 2006; Ireland and Turner 2006), and ambient site temperature and weather station data correlation (Archer 2004), etc. Furthermore, recent work has focused on the analysis of development data such as the regression model described by Ikemoto and Takai (2000) to try to limit the error derived in development models. Limited attention has been given to methods for development data collection and for this reason the bulk of this thesis has focused in that direction.

To improve the accuracy and precision of an estimate, it is imperative to quantify biological variation (which can be conceptualized as error in the organism), and the error in basic data collection and summary, to resolve a single, reliable approach that yields the

least error. With such an approach, it will also make comparing data sets more reliable. The following conclusions drawn from this thesis focus on error in basic development data collection and summary and will aid in describing such an approach for forensic entomology.

Because the most common development model used to calculate minimum PMI is thermal summation (Higley and Haskell 2001), this approach will be described in that forensic context.

Refining thermal summation constants

It is important to rear immature stages at as wide a temperature range as possible, within the developmental temperature extremes (Chapter 2 and 4), to increase accuracy of the thermal summation constant (*K)* and developmental zero (D_0) (Chapter 2). This is particularly important when deriving *K* and D_0 from fewer than six temperatures, which is not recommended (Chapter 2). However, the relationship between development rate and temperature is generally sigmoidal, and temperatures above the upper and lower developmental threshold are left out of the linear regression, resulting in a loss of development temperatures in the regression. To avoid losing such data, authors should identify the upper and lower developmental threshold of the species they are working with. The calculated D_0 for *Chrysomya* species in South Africa is between 10°C and 14°C (Chapter 2, 4, 5, and 6). Because D_0 is a lower developmental threshold, I suggest a slightly higher temperature, such as 15°C, be used as a minimum temperature threshold. This threshold would presumably be lower for *Lucilia* and *Calliphora* species (Chapter 6) as well as *Chrysomya* populations residing at higher latitudes (> 45°) (Chapter 4).

The upper developmental threshold for *Chrysomya* species in South Africa is between 27.5°C and 32.5°C (Chapter 2, 4 and 5), limiting experimental temperature range to between 15°C and 32.5°C for thermal summation models. But, because carcass temperatures have been recorded above 40°C (Chapter 7; Deonier 1940; Payne 1965; Greenberg 1990b, 1991), which is not on the linear part of the summation graph, rearing

maggots at higher temperatures is encouraged (Chapter 5). Rearing larvae at these high temperatures is particularly important when constructing isomorphen and isomegalen diagrams as development become significantly altered at these temperatures, which prevents accurate interpolation of results in thermal summation models. Similarly, this threshold would be lower for *Lucilia* and *Calliphora* species (Chapter 6) and presumably for *Chrysomya* populations residing at higher latitudes (> 45°C) (Chapter 4).

The precision of the estimate of age accounting for temperature is affected by the number of constant temperatures used in the regression analysis and minimal regression models use no fewer than six points along the linear section of the relationship (Ikemoto and Takai 2000; Sokal and Rohlf 2005). Therefore, once the upper and lower temperature extremes have been identified (as described above), it is important to rear developing stages at at least four additional temperatures at equal intervals between those extremes, bringing the minimum total number of temperatures used to six (Ikemoto and Takai 2000). It is not important that absolute temperatures be standardized between studies to allow for reliable comparisons, because different population of the same species possess different developmental temperature optimums and extremes (Chapter 4), but it is important that each study uses a minimum of six temperatures. This minimum will provide an acceptable error to allow for reliable comparisons (Chapter 3).

It is important to use identical experimental methods for development data collected from all temperatures to standardize precision between temperatures (Chapter 3). Rearing media, sample size, temporal sampling resolution, and summary statistics, some of which influence the precision and accuracy of the true duration of development (Chapter 3) and all of them compromise comparisons between different studies (Chapter 2). Perhaps the most important point is that all of them can be experimentally or statistically controlled (discussed below).

Rearing media are of particular importance when calculating minimum PMI from feeding stages of development, as different rearing media can have a significant effect on the duration of development at these stages (Kaneshrajah and Turner 2004; Clarke et al.

2006; Ireland and Turner 2006). Although this topic was not dealt with directly in this thesis, we can make some recommendations for minimum PMI estimates and future studies. Liver is the most frequently used medium in development studies (Nabity et al. 2007), which offers a potential standard between studies and gives reason for future studies to use liver. But immature stages develop significantly slower on liver in the feeding stages than on heart, lungs, kidney or brain (Kaneshrajah and Turner 2004), presumably because the liver is a detoxifying organ and therefore may house toxins or enzymes that alter larval development. Generally, PMI estimates are made from maggots taken from more than one source on the body and it is likely that each source will provide a different dietary medium. It is also unlikely that larvae will feed on a single tissue on a cadaver to reach post-feeding stages due to the rapid consumption rate of gregarious larval masses (personal observation). The dietary media used to obtain development data in the laboratory should preferably match the dietary media of the larvae (i.e. off the cadaver) used to make the estimate during the feeding stages of development (Byrd 2001). Then, perhaps the most comparable media would be a mixture of organs. Future work could determine the diet of particular larval masses on cadavers, including the migratory behaviour of larvae, and assess the difference between development rate of organs and a mixture of organs. It is important to remember that estimates made from post-feeding larvae can use development data obtained from any diet media (Chapter 4).

Once a diet medium had been decided, the sample size, temporal sampling resolution and summary statistics should be chosen. Whether the author uses the temperature/rate graph or the improved method described by Ikemoto and Takai (2000) (discussed in Chapter 2), "time" is a common variable in both models. For this reason, time and the number of sampling points are the only variables that will influence the precision and accuracy of the regression (Chapter 3; Ikemoto and Takai 2000). Sample size will not influence the regression model as much as temporal sampling resolution because sample size is not a direct estimator of time (Chapter 3), but rather a variable within each sampling event. Therefore, to increase the precision of the K and D_0 values, and subsequent minimum PMI estimates, it is more important to sample frequently using fewer samples than to sample less frequently using more samples. A relative error of about 10% in the

resolution of temporal sampling of the total development time for each event is an optimal trade-off between effort and accuracy (Richards et al. 2007) because it represents an improvement in precision of an order of magnitude; to improve a further order of magnitude would generally be logistically intractable.

Data acquired for each developmental event at each temperature will then be summarized into a single point and plotted. As long as the temporal sampling resolution is high enough (≤ 10% relative error), the different summary statistics (minimum, mean, etc) will not differ enough to compromise comparisons between data sets. Having said that, median data are the most representative measure of the duration of development when analyzing both symmetrical and skewed data, as they are the most statistically robust to the effects of outlying values, and would provide the most reliable routine summary data for estimating K and D_0.

Amendt et al. (2007) recommended that when developmental constants for a particular species were not available, they could be approximated by the constants of congeneric species. It is clear from Chapter 5 that even for sister species this is not necessarily wise, while the results in Chapter 4 show that even conspecifics from different areas may show phenotypic plasticity that will confound forensic analyses. Phenotypic plasticity in blowfly thermobiology is an important adaptive trait in all developmental stages that promotes survival in the changing environment. However, the limits of phenotypic plasticity appear to be governed by genetics, which help to define a species' fundamental niche. Inevitably, plasticity will have implications on the accuracy of minimum PMI estimates. Because blowflies are highly mobile (Wells and Lamotte 2001; Harvey et al. 2003), groups of individuals of one population could experience different climates to one another, and potentially express different thermal thresholds (Ayrinhac et al. 2004). This would certainly have an effect on a minimum PMI estimate, for example, a single population may have different D_0s for summer and winter. Similarly, the results of Chapter 4 suggest that an increase of 5° in latitude results in a decrease of 1°C in D_0. Future work could focus on the degree of error induced by phenotypic plasticity on minimum PMI estimates.

Thermobiology and distribution

Although *K* and D_0 are acquired primarily for minimum PMI estimates, the D_0 can be used to as a physiological index to better understand the basic thermobiology (Chapter 6) and possibly the geographic distribution of these flies (Chapter 7; Klok and Chown 2003). In this thesis D_0s of South Africa blowflies were shown to be species-specific and the thermobiology of these flies seems to be constrained by phylogeny (Chapter 6). Indeed species from cold-adapted lineages, like *Calliphora croceipalpis* (Calliphorinae), had significantly lower larval, pupal and adult temperature thresholds than species from warm-adapted lineages, like *Chrysomya* (Chrysomyinae). But D_0*s* also seem to vary significantly between populations of the same species separated by significant latitudinal variation (Chapter 4). It is unlikely that these populations were significantly genetically different (Harvey et al. in press), and that this variation in thermal thresholds is rather a result of phenotypic plasticity. Ayrinhac et al. (2004) suggest that in *Drosophila melanogaster* (Meigen), a cosmopolitan fly, phenotypic plasticity influences its thermobiology more than does genetic variation. This degree of plasticity may hold true for blowfly species, because the difference between D_0s of two *C. albiceps* populations (4.9°C) (Chapter 4) was similar to the difference between the D_0s of *C. albiceps* (Chrysomyinae) and *Ca. croceipalpis* (Calliphorinae) (5.75°C), two blowfly species that are genetically dissimilar. Additionally, phenotypic plasticity may be constrained by genetics as cold-adapted species (*Ca. croceipalpis*) residing in a subtropical climate had a significantly lower D_0 (7.91°C) (Chapter 6) than a warm-adapted species (*C. albiceps*) residing in a temperature climate (10.46°C) (Chapter 4).

Because temperature is widely cited as the primary climatic variable limiting geographic distribution of numerous insect species (Leather et al. 1993; James and Partridge 1998; Klok and Chown 2003), it is possible that the thermobiology of blowflies is reflected in their geographic distribution in South Africa. Both *C. albiceps* and *C. marginalis* were shown to have similar, widespread distributions in South Africa (Chapter 7) but possessed very different larval, pupal and adult thermal thresholds (Chapter 6). Similarly, *C. putoria* and *C. megacephala* shared nearly identical geographic distributions but possessed significantly different larval and adult thermal thresholds. These comparisons

suggest that it is difficult to predict the geographic distribution of a blowfly species based exclusively on their thermobiology at different life stages. Furthermore, no correlation was found between the thermal thresholds of the different life stages (Chapter 6). This is probably because geographic distribution is influenced by more than one climatic variable (Chapter 7), effects of predation, parasitism, interspecific competition, terrain, etc (Phillips et al. 2006), and because climatic variables relating to moisture content, specifically relative humidity, were shown to be more influential on the geographic distribution of blowflies than temperature (Chapter 7).

Although blowfly thermal biology is not reflected on the macro-scale, i.e. geographic distribution, it may help to understand the behavior and distribution within the micro-environment. Larval temperature thresholds were shown to be a limiting factor in the carrion feeding environment. Therefore, by knowing both the larval temperature thresholds and the temperature of a maggot mass, it may be possible to predict the species present in the larval mass (Chapter 6).

Conclusion

Forensic entomologists can improve the usefulness of their minimum PMIs by careful data collection from the species which they are actually basing their estimates, preferably using specimens from the same population. The thermal tolerances of different species may affect the community ecology and succession patterns of corpses, which needs to be taken into account in using succession data for late minimum PMI. Although temperature has a crucial influence on blowflies at the physiological and ecophysiological level, this does not extend to the biogeographical level as is sometimes asserted. Thermobiology is a useful tool in helping forensic entomologists to understand the meaning of the behaviour and distribution of blowfly larvae and pupae, and it demands a level of sophistication that is still becoming nuanced in this nascent discipline.

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