Effect of temperature on development of the blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae)

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Abstract

The blowfly *Lucilia cuprina* is a primary colonizer of decaying vertebrate carrion, and its development provides a temperature-dependent clock that may be used to estimate the post-mortem interval of corpses and carcasses in medicolegal forensic investigations. This study uses the development of *L. cuprina* raised on a substrate of chicken liver at six constant temperatures from 18 to 33 °C to calibrate a thermal accumulation model of development for forensic applications. Development was optimal near 24 °C; above this temperature, survival of post-feeding life stages was increasingly compromised, while below it, development was increasingly retarded. The lower developmental threshold (~12 °C) and thermal summation constants of *L. cuprina* are distinct from those reported for *Lucilia sericata*, verifying that it is essential to identify African *Lucilia* specimens accurately when using them to estimate post-mortem intervals.

Keywords *Lucilia cuprina*, temperature, development, post-mortem interval

Introduction

The most ecologically and forensically important arthropods associated with decomposing vertebrates are flies (Diptera) and beetles (Coleoptera), which can usually be used to estimate the time of death or post-mortem interval (PMI) of an associated corpse or carcass, especially when the usual postmortem indicators such as livor mortis and rigor mortis are no longer meaningful [1–5]. The minimum post-mortem interval (PMI_{min}) is estimated from the age of necrophagous insects that could not have been present before the body died [1–6]. The earliest time at which the carcass or corpse was exposed to insects and, thus, the latest time of environmental exposure can be estimated by investigating larval development at several constant temperatures [1–4]. The effects of temperature on blowfly (Diptera: Calliphoridae) development rate have been studied extensively [4], most notably in the genera *Calliphora* Robineau- Desvoidy, 1830, *Chrysomya* Robineau-Desvoidy, 1830, and *Lucilia* Robineau-Desvoidy, 1830. An optimal temperature range for most species has been identified to be between 20 and 30°C, with development and survival being compromised at temperatures outside this range [7, 8]. *Chrysomya putoria* (Wiedemann, 1830) and *Chrysomya chloropyga* (Wiedemann, 1818) barely develop below a temperature threshold of 14 °C [9], while *Calliphora croceipalpis* Jaennicke, 1867 exhibits very low temperature thresholds [9]. The most temperature - tolerant species have been identified a *Chrysomya albiceps* (Wiedemann, 1819) and *Chrysomya megacephala* (Fabricius, 1794) capable of surviving and developing at temperatures from 11 to 50°C, a trait also found in *Lucilia sericata* (Meigen, 1826) [9]. *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) is a blowfly that causes myiasis in humans and livestock, usually sheep [10, 11], and is commonly found around garbage and decaying flesh [2]. As such, *L. cuprina* has medical, veterinary, agricultural, public health, and forensic significance [12–16], but there are few published data on the postembryonic development of this species [10, 16–18], with most applied studies focusing on the effect of *L. cuprina* on sheep production. *L. cuprina* is closely related to *L. sericata*, another forensically important species with which it may be confused due to very similar morphology [19, 20]. The aim of this study was to provide data on the development for *L. cuprina* at various constant temperatures for more accurate estimation of PMI_{min} in medicolegal forensic investigations. Developmental parameters were compared with those published for *L.*

sericata to establish the need for the two species to be discriminated when estimating PMI_{min}.

Methods

Culture establishment

Adults of *L. cuprina* were collected in Johannesburg (26° 09′ 31″ S 28° 01′ 43″ E) using traps baited with frozen free-range chicken breasts for 2–5 days. Adult flies were accumulated in rearing cages at 24°C under a lighting cycle of 12:12 (L/D), with water, milk powder, and sugar as food sources until 30– 50 females were present. Chicken liver was placed in the adult holding cage to permit oviposition, and these eggs were used to rear the next generation of the laboratory culture.

Protocol

Eggs of known age were obtained by removing the oviposition medium from the laboratory culture for 2–3 days and then placing fresh chicken liver in the cages during the day. The medium was checked every hour for oviposition activity and removed at night. Eggs from various mothers were transferred in clusters of 20 to separate experimental feeding containers. Each feeding container was composed of 50g of catering-grade chicken liver in a 125-ml Styrofoam cup placed in a container with washed sand approximately 3.0–3.5 cm deep in which larvae could pupate and covered with mesh to confine larvae and exclude parasitoids. The stocking density (0.4 larvae/g) minimized the accumulation of maggot-generated heat [21] that might have stimulated growth [1–4] and avoided stunted growth associated with isolation of larvae [22]. Fifteen cups were prepared in this manner for each temperature. These were held in an incubator (model 0102A: AFH Devers & Co. (Pty) Ltd., Johannesburg) at 18°C (±1°C). One larva was sampled from ten random cups every 3h for the first 48h and, thereafter, every 6h until they pupated. Each larva sampled was immediately placed in boiling water for 30s and then transferred into 70% ethanol for 1–2h, which killed them but did not allow them to contract or swell [23]. The length of each larva was then measured using a pair of high accuracy digital calipers (Series 1101, Insize Co., China), and its developmental stage was determined by examination of its posterior spiracles under a dissecting microscope [1–3]. During pupation, sand was sifted to recover pupae. Once larvae had successfully pupated,

they were transferred individually into microcentrifuge tubes to record the time required for pupal development and rates of adult eclosion. The time taken to reach each developmental landmark and the survivorship of each replicate at each sampling event was calculated. Survivorship was expressed as the percentage of individuals in each previous life stage reaching the next developmental landmark based on known numbers of larvae removed throughout development and those remaining at the beginning of the post feeding stage. The above procedure was repeated in the same incubator at constant temperatures of 21, 24, 27, 30, and 33°C in random order, eventually involving eggs drawn from five different generations of the laboratory culture. Thus, any potential incubator effect was strictly controlled, any genetic effects were highly randomized, and the treatments were well replicated and randomized.

Data analyses

The means and standard errors of the median time to reach each developmental landmark were calculated from the developmental stage data [24]. The reduced major axis regression analysis of Ikemoto and Takai [25] was applied to these data to calculate lower developmental thresholds (D_0) and thermal summation constants (K) (and their confidence intervals) for each developmental landmark [24]. This approach also accommodates uncertainty in the values of the predictor variable, i.e., imprecision of the incubator's thermostat [25]. Grassberger and Reiter [8] calculated and tabulated the mean minimum duration of each developmental stage for L. sericata from data collected twice daily from each of ten replicates of each of ten temperatures from 15 to 34°C. Reduced major axis regression analysis [25] was used to calculate the lower developmental thresholds (D_0) and thermal summation constants (K) and their confidence intervals for each developmental landmark. We did parallel analyses of our data using the minimum time to reach each developmental landmark to match Grassberger and Reiter's sampling procedure [8] to compare the thermal summation models of the two species strictly. Chi-square analyses of association were performed in Microsoft Excel (Microsoft Corporation 2010) to assess the differences in survivorship for pupation and eclosion across the temperatures tested. These tests could not be performed for larvae reaching the post-feeding stage, as the number of larvae reaching this stage for each temperature was uneven due to the differing developmental periods and differences in egg hatch across temperatures.

Results

Development rates and thermal summation constants

Larvae took 8.4 days to reach the wandering stage at 18°C and 2.5 days at 33°C (Fig. 1). The duration of pupation did not differ greatly across temperatures with the exception of 18°C (Fig. 2) at which it took approximately twice as long as at the higher temperatures. The greatest individual larval length was observed at 27 °C, and the average larval length for all wandering larvae across all temperatures was 12–14mm (Fig. 1). There was a significant effect of time after hatching on mean larval length ($F_{1,141}$=1718.37; p<0.001), but also a significant interaction between temperature and time after hatching on mean larval length ($F_{5,141}$=106.14; p<0.001) that reflected a faster increase in length as temperature increased. Across all temperatures, length peaked towards the end of the larval stages before decreasing slightly when larvae entered the wandering phase. Because survivorship of pupae was low, especially at higher temperatures, there were insufficient data to calculate a thermal summation model for adult eclosion. The thermal summation models estimated for median times for first and second ecdysis, onset of wandering, and onset of pupation all yielded good fits and narrow confidence intervals, although onset of wandering had a broader confidence interval (Fig. 3). The developmental thresholds calculated from medians for all four developmental events did not differ greatly (Table 1). However, when compared to the developmental thresholds calculated from minima (Table 1), there was an increase of approximately 1°C for each developmental event, with the exception of second ecdysis, where there was a decrease in the D_0 value.

Survivorship

Larval survivorship peaked at 24°C, with temperatures further from this increasingly compromising development in the post-larval stages (Fig. 4). At this temperature, the highest percentage of larvae pupated (85.1%) and eclosed successfully (77.5%). There was a significant difference in the pooled number of post-feeding larvae that successfully pupated across the temperature range (χ^2=13.4, df=5, p=0.020) and a significant difference in the pooled number of adults that eclosed successfully across the temperatures tested (χ^2= 31.87, df=5, p<0.001). Wandering larvae were more likely to reach pupation, and pupae were also most likely to eclose at 24°C.

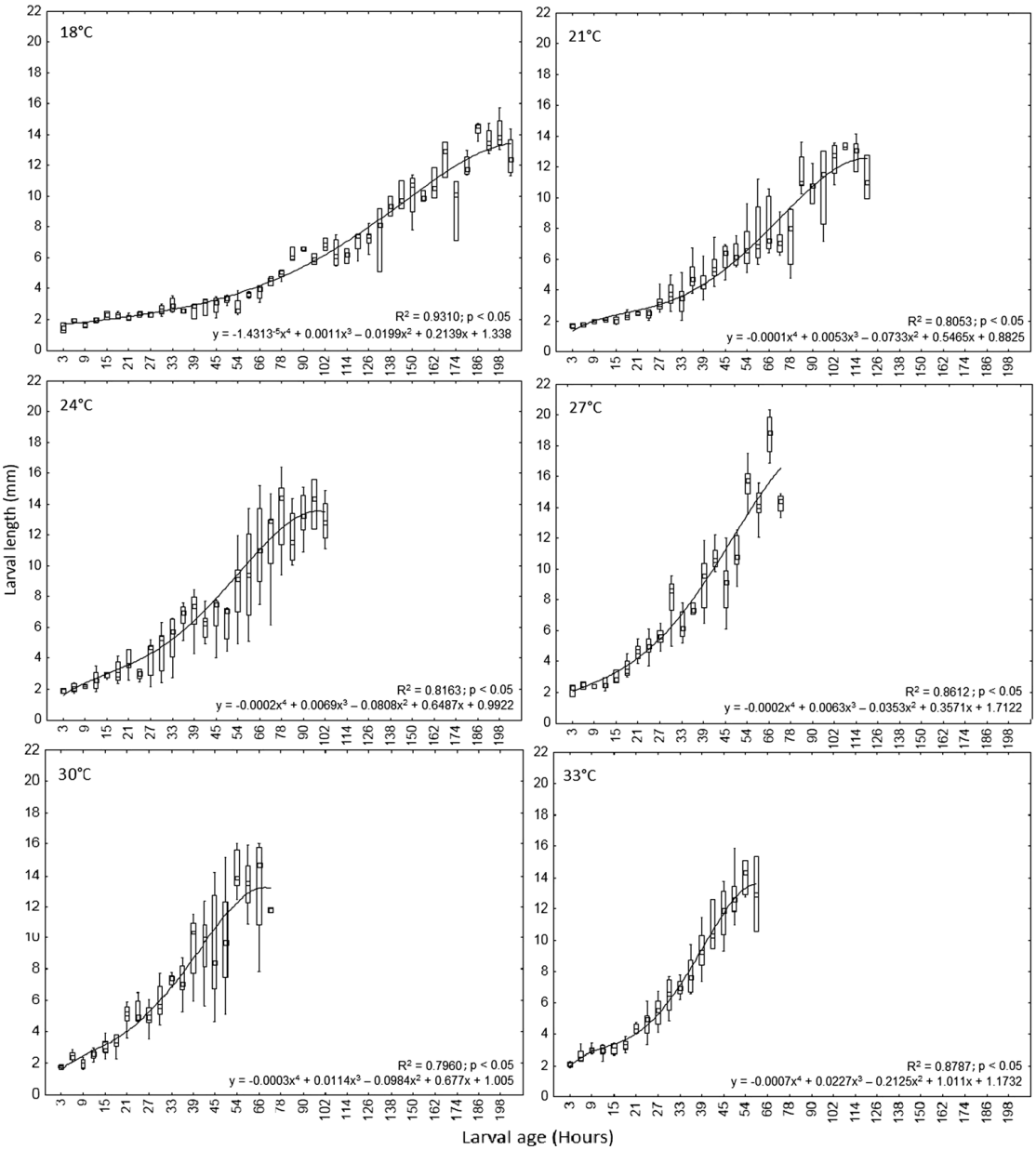

Fig. 1: Boxplots of median, interquartile range, minimum, and maximum body length of *Lucilia cuprina* development at six constant temperatures. Each line represents the quadratic polynomial function for that temperature with the equation and fit presented in each panel

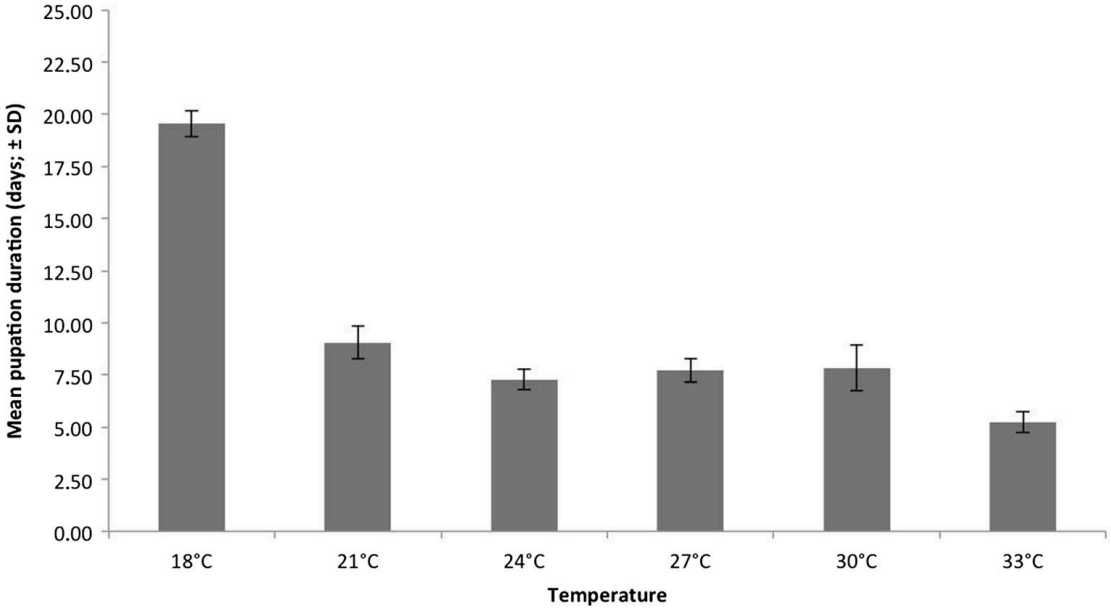

Fig. 2: Mean (± SE) duration of pupariation for *L. cuprina* at six constant temperatures

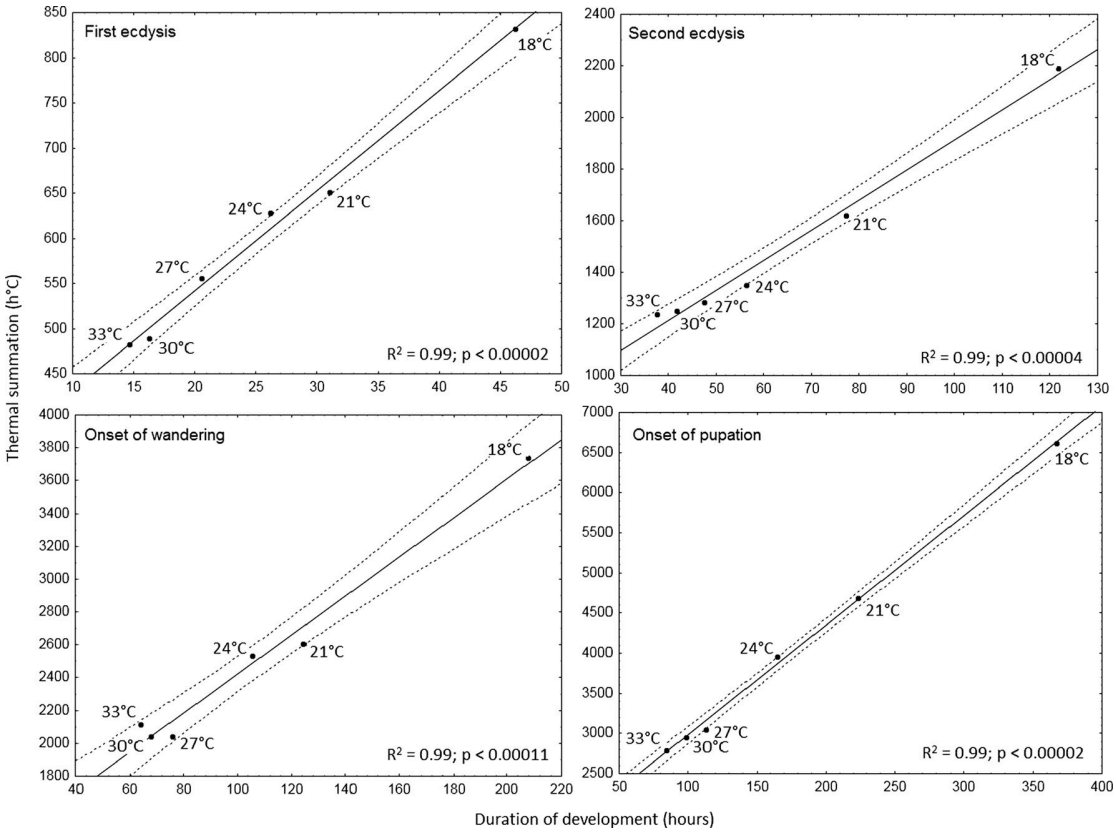

Fig. 3: Regressions used to determine D_0 and K of *L. cuprina* at six constant temperatures for median times to reach first ecdysis, second ecdysis, wandering, and pupation. Dashed lines represent 95 % confidence intervals

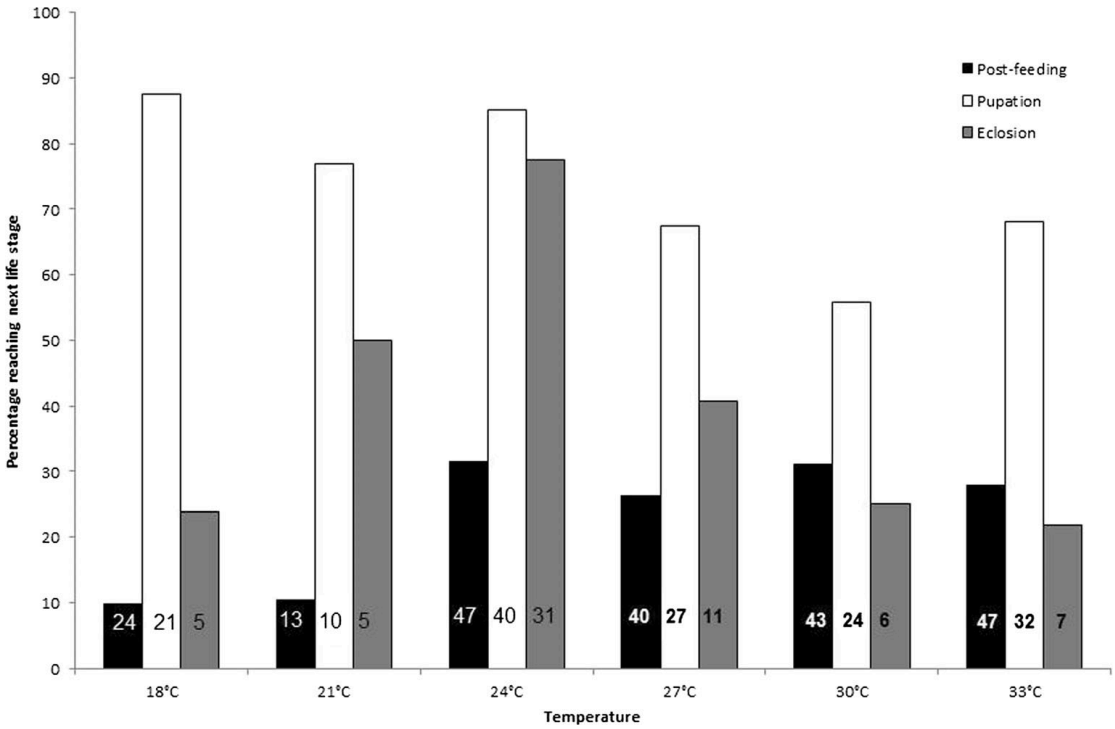

Fig. 4: Survivorship of postlarval stages of *L. cuprina* at six constant temperatures. Values are expressed as the percentage of the previous life stage that successfully reached the developmental landmark. The number in each bar represents the sample size for that particular developmental landmark (total numbers used were: 18°C: 244, 21°C: 125, 24°C: 149, 27°C: 152, 30°C: 138, and 33°C: 169). The number for larvae reaching the post-feeding stage differs due to differences in developmental time and consequent differences in number of larvae removed, as well as differences in egg hatch, across each temperature

Discussion

Growth rate and survivorship

Larvae from the *L. cuprina* population used in this study survived best at 24°C and mean larval length peaked at 27°C (Fig. 1). At lower temperatures, larval development was slower and survivorship was compromised, especially at 18°C, with many larvae dying as they reached the wandering phase. As temperatures rose from 24°C, growth was increasingly compromised, and the physiological stress of developing at more extreme temperatures was evident in decreased survivorship. Dallwitz [16] reported that pupal survivorship for *L. cuprina* did not drop below 75 % for temperatures below 30°C. However, in this study, pupal survival at temperatures below 30°C varied between 25 and 80%. This variation in pupal survival may be attributed to the number of generations that each studied culture had been kept under

Table 1: Parameters of thermal accumulation models for the median and minimum time of occurrence of four developmental events in *L. cuprina* (this study) and the minimum time of occurrence of the same developmental events in *L. sericata* [8] Values are reported as the means of the median and minimum times taken for each sample at each life stage, with the standard error of the mean in parentheses

	Developmental milestone			
	1st ecdysis	2nd ecdysis	Wandering	Pupariation
Developmental zero (°C)				
L. cuprina				
Median time	11.8 (0.8)	11.9 (0.9)	11.6 (1.9)	12.8 (2.7)
Minimum time	12.9 (1.6)	11.3 (1.5)	12.7 (1.6)	14.0 (1.3)
L. sericata				
Minimum time	16.7 (0.9)	13.8 (0.6)	11.4 (1.3)	10.2 (3.2)
Thermal summation constant (h°C)				
L. cuprina				
Median time	307.3 (23.0)	740.6 (62.1)	2176.2 (595.3)	1275.1 (219.1)
Minimum time	217.2 (38.0)	637.7 (87.5)	1010.3 (169.7)	1422.5 (2298)
L. sericata				
Minimum time	126.8 (18.6)	218.6 (15.8)	498.5 (56.7)	1485.3 (369.9)

laboratory conditions. The culture used by Dallwitz [16] had been in laboratory culture for 17–69 generations, whereas our colony had been cultured for only three to eight generations before experimentation began. The greater number of generations (which were reared under similar conditions of air-dried soil) may have increased the ability of pupae to withstand increasingly unfavorable conditions by allowing each generation to adapt to water stress, thus increasing survival over many generations when kept in the same conditions. Here, it must be noted that the conditions under which post-feeding larvae pupated were not conducive to their survival, which may also have contributed to their poor survival, especially at higher temperatures. Under natural conditions, the soil in which larvae pupate is never completely dry, and especially if there has been seepage from the corpse, the soil surrounding the crime scene is generally relatively moist. Many studies describe using a dry substrate for pupation [7, 8, 26], and keeping this in mind, the increased pupal mortality seen in this study may be attributed to the dry soil, which may in fact have desiccated the pupae.

Lower developmental threshold and thermal summation

The lower developmental thresholds based on medians for all four developmental events (first ecdysis, second ecdysis, onset of wandering, and onset of pupation)

averaged 12.02°C (standard deviation=0.51°C), with the greatest difference between any two events being 0.9°C between the second ecdysis and onset of pupation. The lower developmental thresholds based on minima for all four developmental events averaged 12.74°C (standard deviation=1.1°C), with the greatest difference between any two events being 1.7°C between the onset of pupation and second ecdysis. The lower developmental thresholds based on data obtained from Grassberger and Reiter's [8] study averaged 13.03°C (standard deviation= 2.81°C), with the greatest difference of 6.5°C being between first ecdysis and the onset of pupation. Consistency between all developmental events is expected because the energetics of metabolism are unlikely to vary with development [27]. During this study, it was also noted that the time spent wandering decreased with increasing temperature before the larvae pupated. At the two lowest and two highest temperatures, larvae were noted to be largely inactive. This may suggest that compromising temperatures experienced during early larval stages may have pronounced effects on development and hinder the onset of pupation. Greenberg [28] reported dispersal radii as much as 8.1 m from the food source for *L. sericata* and *Calliphora vicina*. Furthermore, Pitts and Wall [29] emphasized the importance of soil depth on survival of larvae in diapause and pupae. In this study, the containers in which the larvae were incubated had a diameter of 14cm, with soil at a depth of 3.5cm. These conditions, combined with the low humidity and high risk of desiccation, may have affected the time that post-feeding larvae spent searching for a suitable site to pupate and, if no suitable site was found, may also have contributed to the high mortality rate noted, especially when higher temperatures imposed additional physiological stress. Day and Wallman [17] reported that at 24°C, *L. cuprina* took an average time of 48 h to reach first ecdysis, 72h to reach second ecdysis, 96h to begin wandering, and 144 h to begin pupation. These values vary from those obtained in this study, which showed that at 24°C, larvae took on average 26.2h to reach first ecdysis, 56.2h to reach second ecdysis, 105.6h to begin wandering, and 165.0h to begin pupation. For all four developmental events, the thermal accumulation values from Day and Wallman fell outside the 95% confidence intervals of the models calculated using medians in this study. The minimum values reported by Day and Wallman [17] fell within the confidence intervals for the models derived in this study for minimum time to reach wandering and pupation, but fell outside the confidence intervals of the models for first and second ecdysis. Even though the minimum time taken to reach post-feeding stages show similar

developmental thresholds, the differences noted in the earlier instars may be attributed to variation in development rates in blowfly populations at different latitudinal and altitudinal ranges [7]. The difference in latitude and altitude between Wollongong, Australia (34°S; approx. 5–150m above sea level) and this study, conducted in Pretoria, South Africa (26°S; 1300–1400m above sea level) may be a contributing factor to developmental differences in this species. Additional studies are required to explore latitudinal and altitudinal effects on *L. cuprina* development rate at different temperatures. In addition, the thermal summation constants recorded for this study were compared to those found for *L. sericata*, a species closely related to *L. cuprina* [18], using data obtained by Grassberger and Reiter [8]. For the first ecdysis and onset of pupation, the developmental constants fell within the 95% confidence intervals obtained in this study, while the developmental constants for second ecdysis and onset of wandering fell outside of the 95 % confidence interval calculated for this study. Ash and Greenberg [18], reporting the median time required for egg to pupation, also found that *L. cuprina* and *L. sericata* (reported under the synonyms *Phaenicia pallescens* and *P. sericata*, respectively) showed differences in developmental patterns. However, in that study, which used cultures from populations from the eastern USA, *L. sericata* developed at a slower rate than *L. cuprina*. Median development times for *L. sericata* were 53.9 days at 19°C, 13.5 days at 27°C, and 11.3 days at 3°C; for *L. cuprina*, the median development times were 30.3 days at 19°C, 12.8 days at 27°C, and 10.4 days at 35°C. These differences in development show that even though the two species are closely related when both are present on carrion, it is not satisfactory to use the developmental constants for one species as proxies for those of the other, a conclusion also noted for other pairs of closely related species [30, 31]. As discussed above, variation in the direction of differences in development rate between the two species also illustrates the need to more fully explore phenotypic variation [7, 32].

Conclusions

This study provides forensic entomology and maggot debridement therapy with a calibration of the effect of temperature on the development rate of *L. cuprina* and survivorship of its post feeding life stages. Higher temperatures accelerate growth but compromise development later in the life cycle, and lower temperatures extend larval

development, but also compromise development and survival. The results differ to varying degrees from those published for other populations of the species, and from those of its sister species, *L. sericata*, which can have important implications for forensic estimates of post-mortem intervals.

Acknowledgments

We thank Daniel Hart, PJ Jacobs, Nina Parry, and Ashleigh Donaldson for their assistance in sampling; Ludwig Eksteen for laboratory assistance; Deon Bakkes for laboratory assistance and statistical advice; and particularly Roelf Coertse and the security team at the LC de Villiers Sport Grounds and University of Pretoria Experimental Farm for accompanying ZK during after-hours access to these locations. Research was funded by a University of Pretoria Research Development Program grant awarded to CWW.

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