Larval development of the carrion-breeding flesh fly, *Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart (Diptera: Sarcophagidae), at constant temperatures

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Larvae of *Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart were raised on chicken liver under six different constant temperatures. Maximum survival indicated an optimal developmental temperature of near 20 °C, while trends in mortality, larval length and larval mass implied that the thermal window for successful development lay between 15 °C and 30 °C. Using a recently described method to estimate a simple thermal summation model, it was found that the timing of the end of the feeding phase could be estimated by a developmental zero (D_0) of 5.2 °C (S.E. = 1.21) and a thermal summation constant (K) of 106.4 d°C (S.E. = 8.31) and of the end of the wandering phase by D_0 = 4.1 °C (S.E. = 0.39) and K = 126.7 d°C (S.E. = 3.28). Published development times at constant temperatures were compiled for 19 other species of flesh flies, and the developmental constants were calculated for six species for which sufficient data were accumulated.

Key words: *Sarcophaga* (*Liosarcophaga*) *tibialis*, larval developmental rate, thermal summation model, forensic entomology, public health.

INTRODUCTION

Studies of the development of carrion-breeding flies at different temperatures are of particular use in estimating the duration of larval development, which can be used by forensic scientists to estimate the post mortem interval (PMI) between death and discovery of a maggot-infested body (Smith 1986; Lord 1990; Catts & Goff 1992), and by animal and public health officers in designing control programmes for disease vectors. Such studies have tended to focus on blowflies (Calliphoridae) and houseflies (Muscidae) (*e.g.* Kamal 1958; Nishida *et al.* 1986; Davies & Ratcliffe 1994) because of their importance to public health, medicine, veterinary science and forensic entomology, but flesh flies (Sarcophagidae) are also relevant to all of these concerns. Knipling (1936) reported growth rates at unrecorded temperatures for 11 species of flesh fly, and various authors (Table 1) have reported growth rates at known constant temperatures for a total of 18 species. A thermal summation model relating larval growth rate to temperature has been published for only four species (Marchenko 1981, 1988, 2001; Chen *et al.* 1987; Amoudi *et al.* 1994; Grassberger & Reiter 2002).

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The carrion-breeding flesh fly *Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart is found throughout the Afrotropical Region, Madagascar and parts of Europe (Zumpt 1965, 1972; Dear 1980; Pape 1996). It has been reported to breed in carrion and faeces (Aspoas 1991) and to cause traumatic dermal myiasis (Zumpt 1965), potentially giving it significance in forensic investigations and as a vector of disease (Zumpt & Patterson 1952). Little has been published about its development (Aspoas 1991; Musvasva *et al.* 2001). This study reports the developmental rate of *S. tibialis* larvae over a range of constant temperatures in order to create a standard thermal summation model that will be useful to forensic and medical science, and reviews the published data on rates of development of sarcophagid larvae.

MATERIAL AND METHODS

Larvae of *S. tibialis* were obtained from a second- and third-generation laboratory culture established from wild flies trapped on carrion in Grahamstown, South Africa. They were therefore minimally affected by artificial selection from laboratory conditions. The culture was maintained in a constant environment room at 25 °C under a lighting

Table 1. Published durations of larval development (larviposition to pupariation) of sarcophagid flies. Nomenclature follows Pape (1996).

Species	Larval duration (days)	Temperature (°C)	Location	Source
Sarcophaga (*Alisarcophaga*) *gressitti* Hall & Bohart	5	29.5	Guam	Bohart & Gressitt 1951
S. (*Bercaea*) *africa* (Wiedemann)	4	?	South Africa	Zumpt 1965
= *S. cruentata* Meigen	9–10	23–28	Nigeria	Madubunyi 1986
= *S. haemorrhoidalis* (Fallén)	7.0–9.0	25	South Africa	Aspoas 1991
	6.5	25	Florida, U.S.A.	Byrd & Butler 1998
S. (*Boettcherisca*) *peregrina* (Robineau-Desvoidy)	6.3+	22–24	Hawaii	Goff *et al.* 1989
	14	15	Japan	Nishida 1984; Nishida *et al.* 1986
	7	20		
	5	25		
	5	30		
	5	35		
	5.8+	23	Hawaii	Goff *et al.* 1991
S. (*Boettcherisca*) *septentrionalis* Rohdendorf	Not specified	Not specified	Russia	Marchenko 1981, 1988, 2001
S. (*Liopygia*) *argyrostoma* (Robineau-Desvoidy)	14	20–23	Egypt	Hafez 1940
	8.3	25		
= *S. falculata* Pandellé	5.8	30		
	13–18	17	California, U.S.A.	Saunders 1972
	13.6	28.7	Egypt	Kamal 1979 (thesis), in Zohdy & Morsy 1982
	13.1	24.6		
	11.8	19.2		
	15.9	20	Egypt	Zohdy & Morsy 1982
	7.1	25		
	5.4	30		
	Development not completed	8	Austria	Grassberger & Reiter 2002
	18.3	15		
	12.3	20		
	8.1	25		
	6.3	30		
	5.4	35		
S. (*Liopygia*) *crassipalpis* Macquart	5–6	27	Maryland, U.S.A.	Smith 1933
= *S. securifera* Villeneuve	15	15	Japan	Nishida 1984; Nishida *et al.* 1986
	9	20		
	6	25		
	4	30		
	6	35		
	19.0	15	Ohio, U.S.A.	Chen et al. 1987
	10.2	20		
	6.3	25		
	4.7	30		
S. (*Liopygia*) *nodosa* Engel	6.5–8.5	25	South Africa	Aspoas 1991
S. (*Liopygia*) *ruficornis* (Fabricius)	7	29.5	Guam	Bohart & Gressitt 1951
	31.5	13	Saudi Arabia	Amoudi *et al.* 1994
	17.5	16		
	10.5	19		
	9.7	22		
	8.5	25		
	6.8	28		
	6.0	31		
	5.5	34		
	6.3	37		
	7.5–10.8	26	Hawaii	Goff *et al.* 1997

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Table 1 (*continued*)

Species	Larval duration (days)	Temperature (°C)	Location	Source
S. (*Liosarcophaga*) *dux* Thomson = *S. exuberans* Pandellé	7 4–8 8.2–10.2	29.5 23–34 25	Guam India South Africa	Bohart & Gressitt 1951 Alwar & Seshiah 1958 Aspoas 1991
S. (*Liosarcophaga*) *shermani* Parker	7.9	26.7	Washington, U.S.A.	Kamal 1958
S. (*Liosarcophaga*) *tibialis* Macquart	7.0–9.0	25	South Africa	Aspoas 1991
S. (*Neobellieria*) *bullata* Parker	8.8	26.7	Washington, U.S.A.	Kamal 1958
S. (*Neobellieria*) *cooleyi* Parker	7.8	26.7	Washington, U.S.A.	Kamal 1958
S. (*Parasarcophaga*) *taenionota* (Wiedemann) = *S. knabi* Parker	8	29.5	Guam	Bohart & Gressitt 1951
S. (*Sarcorohdendorfia*) *impatiens* Walker	8.0–10.0	25	Australia	Roberts 1976
S. (*Sarcosolomonia*) *stricklandi* Hall & Bohart	6	29.5	Guam	Bohart & Gressitt 1951
Ravinia lherminieri (Robineau-Desvoidy)	6.3	27 ± 2	Maryland U.S.A.	Pickens 1981
Wohlfahrtia pachytyli (Townsend)	5.8 4.5 2.5 2.0 Development not completed	20 25 30 35 40	South Africa	Price & Brown 2006
W. trina (Wiedemann)	26.7 8.3 6.7 5.5 4.7 4.3	16 24 27 30 32 35	Egypt	Tawfik 1962 (thesis), in Zohdy & Morsy 1982

cycle of 12:12 h light:dark. Adult flies were fed only sugar, dried skimmed milk and water *ad libitum*. Larvae were collected by placing 200 g of chicken liver in the holding cage for 12 hours for adults to oviposit. As Aspoas (1991) also observed, females usually laid eggs that hatched immediately.

Each replicate was created by placing ten larvae on 62.5 g of chopped chicken liver in a container. Such low densities of maggots avoid the effects of intraspecific competition that might stunt growth, and the effects of maggot-generated heat that might stimulate growth (Goodbrod & Goff 1990). A total of eighteen replicates (180 maggots), three per temperature treatment, were incubated at constant temperatures of 10, 15, 20, 25, 30 and 35 °C until the maggots migrated from their food. This temperature range was chosen to represent a realistic range of environmental temperatures that larvae might experience, and are likely to lie on the linear part of the temperature / growth rate curve (Pedigo 1996). The mass of every larva in each replicate was measured to 0.1 mg on a Sartorius MC5 electronic microbalance daily, yielding 1510 measurements. The length of each maggot was also measured to 0.1 mm using a geometrical gauge. Daily measurement was previously found to have no detectable effect on blowfly maggot development (Davies & Ratcliffe 1994), and the same was assumed in this study. The end of feeding and the onset of wandering (Denlinger & Žďárek 1994) was recorded when larvae left their food, and the end of the wandering phase was recorded when larvae pupariated (Denlinger & Žďárek 1994)

The developmental constants (Higley & Haskell 2001) were estimated by major axis regression using the approach described by Ikemoto & Takai (2000). The departure of developmental rate from the straight line model at extreme temperatures has been attributed to increasing physiological stress under suboptimal thermal conditions (Pedigo 1996), which should be reflected in increased mortality and decreased growth under these conditions. For this reason, statistical differences

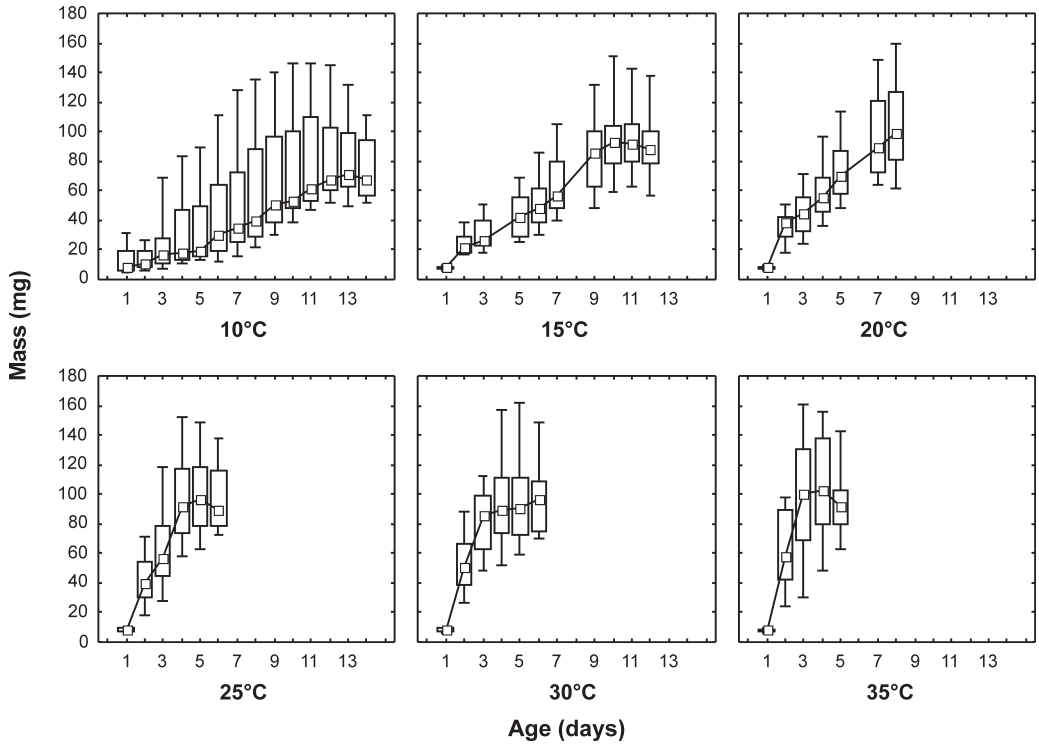

Fig. 1. Box-and-whisker plot of median, interquartile range, minimum and maximum body mass of *Sarcophaga tibialis* larvae during development at six constant temperatures.

in mass, length and mortality at each temperature were tested using ANCOVA, with age as the covariate, and Tukey's HSD test for unequal sample sizes (due to mortality) was used for assessing the origin of any significant differences.

Where even the barest minimum of reliable data (Table 1) was accumulated to allow meaningful regression analysis, the developmental constants of other species were also calculated.

RESULTS

Sarcophaga tibialis

The growth curves at any particular temperature were sigmoidal (Figs 1, 2). There were significant differences in mortality between temperature treatments ($F_{5,80}$ = 3.67, P = 0.005), and larval survival was significantly lower at the extreme temperatures (10 °C and 35 °C) compared to 20 °C (Fig. 3a). However, mortality was always less than 20 % (Fig. 3a). Larval mass differed significantly between temperature treatments ($F_{5,1224}$ = 135.3, P = 0.000), with a steady increase in mass as temperatures increased (Fig. 3b). There were significant differences in larval length between temperature treatments ($F_{5,1220}$ = 148.1, P = 0.000). Larvae reared at 10 °C were significantly shorter than those in other treatments, which did not differ (Fig. 3c). Individual variation in size, particularly mass, was significantly greater (multiple pair-wise Levene's tests with sequential Bonferroni correction; $P < 0.05$) at 10 °C than at other temperatures (Fig. 3). The longest larval developmental period observed in this study was 14 days at 10 °C, and the shortest five days at 35 °C. At 30 °C and 35 °C larvae appeared to reach the third instar about a day after hatching (Fig. 1). Eventually the larvae reached a peak of development and stopped feeding, after which they moved into their wandering phase, lost weight (Fig. 1) and began to shorten (Fig. 2) before pupariating. Because these two developmental events could be identified in all of the replicates, thermal summation models of development (Pedigo 1996; Higley & Haskell 2001) were estimated for the onset of wandering and the onset of pupariation.

Most of the data fell near the linear part of the temperature/growth rate curve and could there-

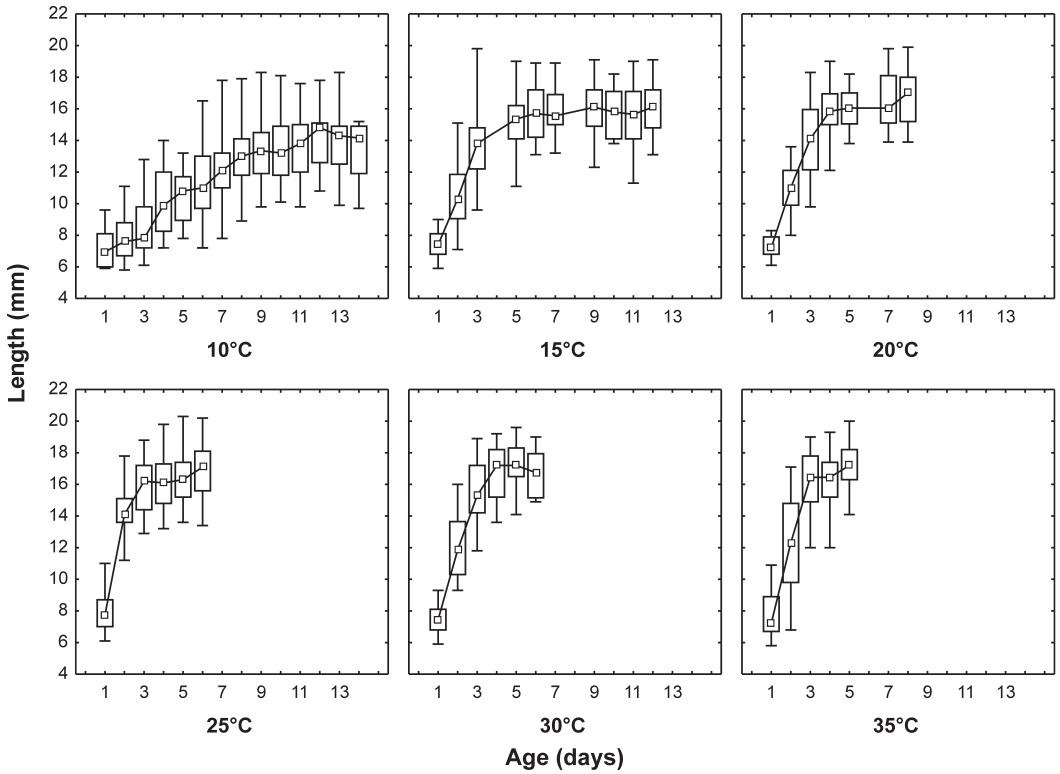

Fig. 2. Box-and-whisker plot of median, interquartile range, minimum and maximum body length of *Sarcophaga tibialis* larvae during development at six constant temperatures.

fore be used to estimate the constants of a standard thermal summation model (Fig. 4). The data from the 10 °C and one of the 30 °C replicates were excluded from the analysis of the end of the feeding phase (Fig. 4a) because they were not on the linear section, following Ikemoto & Takai (2000). Data from the 10 °C and 35 °C replicates and one of the 30 °C replicates were also excluded from analysis of the end of wandering phase (Fig. 4b). The resulting developmental zeros (D_0) and thermal summation constants (K) are given in Fig. 4. The thermal summation constants differed by 20 d°C, as can be expected because the wandering phase lasted only about a day. Estimates of the developmental zeros for the two models were not significantly different.

Other sarcophagids

Of the 19 species for which development has been recorded at constant temperatures (Table 1), only four met the minimum sample size of five observations that is a standard for linear regression (Sokal & Rohlf 1981). Nonetheless, we also analysed two further species for which there were four observations that were reasonably separated, very nearly aligned (Fig. 5) and within the thermal window usually characterized by a linear relationship (Higley & Haskell 2001). The confidence interval for the regression lines (Fig. 5) and the standard errors and confidence intervals for the estimates of the developmental constants (Table 2) provide a guide to the accuracy of the estimates derived from the smaller samples. The data for *S. argyrostoma* provided by Kamal (in Zohdy & Morsy 1982) are anomalous in that temperature and duration of development were positively correlated, and were therefore excluded from that analysis.

DISCUSSION

Musvasva *et al.* (2001) reported that the feeding stage of *S. tibialis* was about 4.5 days at 25 °C, whereas in this study it was five days. Aspoas (1991) reported that the entire larval stage of *S. tibialis* lasted about eight days at 25 °C, or about

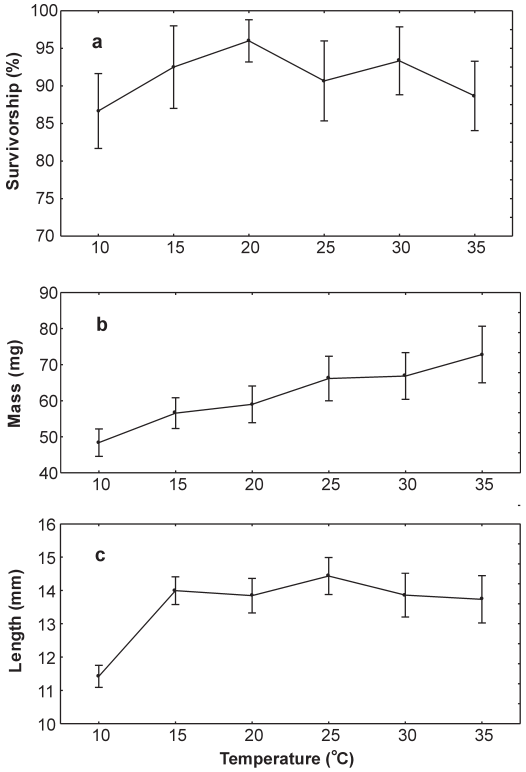

Fig. 3. Mean (with 95 % confidence intervals) response of larvae of *Sarcophaga tibialis* reared at six constant temperatures, adjusted for age using ANCOVA; **a**, mortality; **b**, mass; **c**, length.

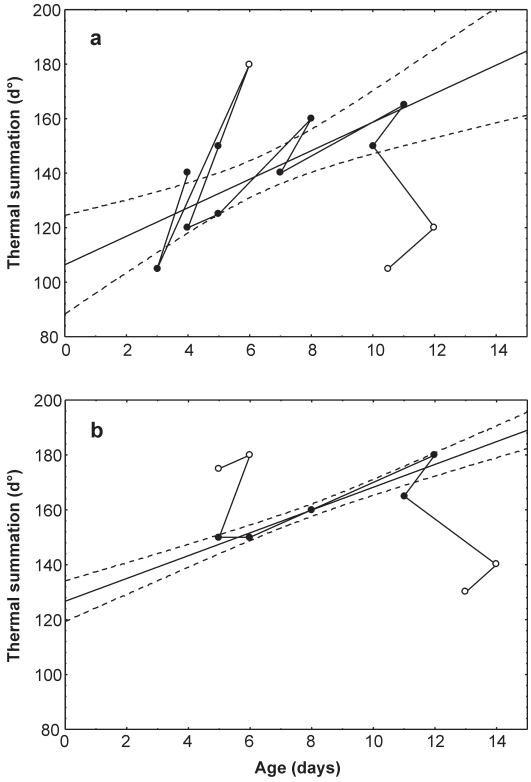

Fig. 4. Regression lines and 95 % confidence intervals (dashed lines) used to determine the developmental constants of *Sarcophaga tibialis* larvae using six constant temperatures ($n = 18$). Points not on the linear section (open symbols) were excluded from the analysis. **a**, End of feeding phase; 14 of the replicates (filled symbols, many superimposed) lay on the linear section of the graph ($R^2 = 0.607$; $D_0 = 5.2$ °C, S.E. = 1.21 °C; K = 106.4 d°C, S.E. = 8.31 d°C). **b**, End of wandering phase; 11 of the replicates lay on the linear section of the graph ($R^2 = 0.925$; $D_0 = 4.1$ °C, S.E. = 0.39 °C; K = 126.7 d°C, S.E. = 3.28 d°C).

two days longer than the results of this study. This might be due to the use of an ox heart diet in one study and chicken liver in the other (Kaneshrajah & Turner 2004). Comparable results have been reported for a variety of species of *Sarcophaga* (Table 1).

Larval mortality was lowest at 20 °C (Fig. 3a), suggesting that the optimal (as opposed to the maximal) temperature for the development of *S. tibialis* larvae is near 20 °C. However, mortality was significantly higher only at 10 °C and 35 °C (Fig. 3a), larval length was significantly shorter only at 10 °C (Fig. 3b), and increases in larval mass levelled off above 25 °C (Fig. 3c), so that the thermal window for successful development is apparently quite wide. These results support the selection of replicates at 10 °C for exclusion in the estimation of the developmental constants (Fig. 4). The analysis indicates that, while the temperatures selected spanned realistic environmental conditions, they may not have included the upper thermal limit of development, and that temperatures above 35 °C should be tested. However, since this upper limit is not on the linear part of the growth curve, its omission does not affect the quality of the thermal summation models derived here. Zohdy & Morsy (1982) reported a minimum in mortality in *S. argyrostoma* at 25 °C.

The good fit of the regression lines of the thermal summation models indicates that reliable estimates of developmental durations can be made over this temperature range. The model becomes increasingly inappropriate as temperatures progress beyond these limits and more sophisticated

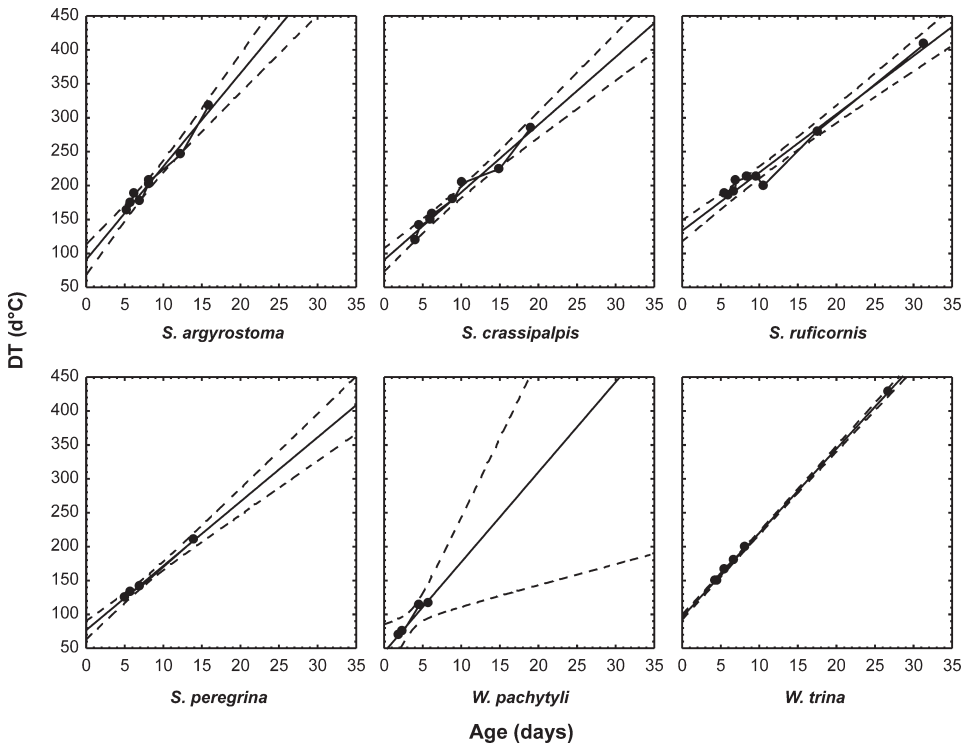

Fig. 5. Regression lines and 95 % confidence intervals (dashed lines) used to determine the developmental constants of larvae of six species of Sarcophagidae (Table 2) reared under constant temperatures. Data were obtained from published studies (Table 1).

models (*e.g.* Davidson 1944; Von Zuben *et al.* 1998; Higley & Haskell 2001) would need to be developed empirically to deal with those conditions. Pedigo (1996) and Higley & Haskell (2001) provide details of the practical application of thermal summation models for the prediction of developmental durations, *e.g.* for estimating PMIs. It should also be remembered that, at least in blowflies, groups of larger maggots may generate enough metabolic heat to warm their immediate surroundings by 28 °C or more (Deonier 1940; Payne 1965; Turner & Howard 1992). This makes it important to measure the temperature of the maggots' microhabitat as well as ambient temperature when intending to estimate a PMI.

The estimate of D_0 for *S. tibialis* is much lower than for other sarcophagids, but that for K is within the range of variation found for six other species (Table 2). Developmental constants for the period from oviposition/larviposition to puparriation have been published for *S. septentrionalis* (D_0 = 7.8 °C; K = 117 d°C: Marchenko 2001), *S. crassipalpis* (D_0 = 10.6 °C; K = 92.8 d°C: Chen *et al.* 1987), *S. ruficornis* (D_0 = 6.3 °C; K = 152 d°C: Amoudi *et al.* 1994) and *P. argyrostoma* (D_0 = 7.2 °C; K = 147.6 d°C: Grassberger & Reiter 2002). The values for the last three species differ from ours (Table 2) partly because we have combined data from different studies to yield larger sample sizes and used a more precise method of calculating the constants (Ikemoto & Takai 2000). However, the values for *S. crassipalpis* lie within the 95 % confidence interval of our estimates.

The very high coefficients of determination for our analyses (Table 2) strongly suggest that variation within species between experiments due to differences in geographical origin, diet (Kaneshrajah & Turner 2004) and photoperiod (Saunders 1972) are of relatively minor importance compared to temperature (Fig. 5). Variation in the constants between species due to phylogenetic relationship cannot be assessed from this small sample, but the very low thermal summation constant (Table 2) for *W. pachytyli* might reflect ecological adaptation to being a parasitoid of grasshoppers (Price & Brown 2006).

Table 2. Estimates of developmental constants for completion of the larval stage (hatching or larviposition to pupariation) in seven species of Sarcophagidae, derived from major axis regression analyses (Fig. 5).

Species	*n*	R^2	Developmental zero (°C)			Thermal summation constant (d°C)			Sources
			D_0	S.E.	95 % C.I.	K	S.E.	95 % C.I.	
S. tibialis	11	0.925	4.1	0.39	3.4–4 .9	126.7	3.28	120.3–133.1	This study
S. argyrostoma	8	0.970	14.0	0.99	11.3–16.2	88.8	9.22	68.0–113.2	Hafez 1940; Zohdy & Morsy 1982; Grassberger & Reiter 2002
S. crassipalpis	8	0.974	10.11	0.67	8.3–11.6	89.1	7.05	73.1–107.6	Nishida 1984; Nishida *et al.* 1986; Chen *et al.* 1987
S. ruficornis	9	0.979	8.69	0.48	7.5–9.7	132.2	6.61	117.6–148.9	Bohart & Gressitt 1951; Amoudi *et al.* 1994
S. peregrina	4	0.997	9.49	0.36	7.9–11.0	76.6	3.17	63.1–90.4	Nishida 1984; Nishida *et al.* 1986; Goff *et al.* 1991
W. pachytyli	4	0.940	13.7	2.38	3.0–23.5	42.7	9.54	3.2–85.3	Price & Brown 2006
W. trina	6	1.000	12.4	0.12	12.1–12.7	95.9	1.43	91.9–100.0	Tawfik 1962, in Zohdy & Morsy 1982

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