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ORIGINAL ARTICLE

Development of *Thanatophilus micans* (Fabricius 1794) (Coleoptera: Silphidae) at constant temperatures

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Abstract *Thanatophilus micans* is capable of finding corpses at least as quickly as most fly species and, as the most widespread species of the Silphidae in Africa, offers a useful model for estimating post-mortem interval. Larvae were reared at ten constant temperatures from 15°C to 35°C and their length measured at 4, 8, or 12-h intervals depending on their instar. Length generally increased with increased rearing temperature, but decreased at extremely high temperatures. Note was made of the age at which individuals progressed past developmental milestones. Development took longer at lower temperatures. These results are presented as a combined isomegalen and isomorphen diagram. Developmental constants were generated for each milestone using major axis regression. Developmental threshold values did not differ significantly between milestones. Development took longer than in blow flies, but was faster than in Dermestidae. The three models presented here, therefore, cover an important time frame in estimating minimum PMI once fly larvae have matured to the point of leaving a corpse, and, therefore, provide a tool that was not previously available to forensic entomologists.

Keywords Forensic entomology · *Thanatophilus micans* · Silphidae · Development · Post-mortem interval

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Introduction

The development of carrion-breeding insects is widely accepted as a useful indicator of minimum post-mortem interval (mPMI) [1, 2, 4, 18]. Most estimates of mPMI are made using the development of maggots because adult flies are capable of finding corpses within hours of death [18]. For this reason, most of the research into the development of carrion-related insects has focused on maggots (fly larvae), and beetles have been neglected.

Adults of *Thanatophilus micans* (Silphidae) and *Dermestes maculatus* (Dermestidae) have been observed on animal carcasses within 24 h of death (personal observation), and larvae of *T. micans* have been observed on these carcasses within a few days of death, but larvae of *D. maculatus* do not normally appear until the carcass has dessicated substantially. This implies that some silphids, at least, have some of the desirable forensic characteristics of blowflies. Beetles typically develop more slowly than flies, and, therefore, offer an opportunity to estimate mPMI from developmental data after maggots have left the carcass. Estimates of mPMI based on beetle development are reputedly less precise (days or weeks) than those based on maggot development (hours or days) [3], but once maggots are no longer present on a carcass, they are still suitable. In addition, using live beetle samples instead of old fly puparia collected at crime scenes is likely to reduce the contamination concerns raised by Archer et al. [21].

Given that *T. micans* is the most common and widespread silphid species in Africa [17] and that it can locate carcasses soon after death, a development model for this species is of forensic value. This study presents such a model, developed from the growth rate of *T. micans* at ten constant temperatures, to illustrate the

value of coleopteran developmental data to forensic entomology in general.

Materials and methods

A culture of *T. micans* was established by collecting adults from various dead animals in the Grahamstown district of South Africa. Pairs of adults were placed at a range of temperatures (15°C, 17°C, 18°C, 19°C, 20°C, 25°C, 28.4°C, and 35°C) with a small amount of food (Shallow-water hake, *Merluccius capensis*) and oviposition substrate (damp sand). Shallow-water hake was chosen because it is the most readily available frozen fish in South Africa, and fish or fish meal is commonly used to raise carcass beetles [5, 14]. Frozen fish was chosen as it is readily available, and freezing of food does not influence insect development [22]. Freshly hatched first instar larvae were separated into plastic Petri dishes with pupation substrate (damp sand) and food ad libitum and reared at ten constant temperatures (oviposition temperatures, plus 22.5°C and 32.5°C), ten individuals per temperature, until adults emerged from the pupation substrate. The Petri dishes were closed using elastic bands and turned 90° onto their sides. This arrangement was narrow enough to allow monitoring of the pupal chamber, allowing pupation and eclosion times to be noted, but also providing enough space for the larvae to move and feed.

Developmental milestones were identified and the period between milestones noted by checking all individuals at regular intervals based on development stage (eggs 8-hourly; 1st instar larvae 4-hourly; 2nd instar larvae 8-hourly; 3rd instar larvae, pupae and adults 12-hourly). Beetle larvae were measured using a measurement triangle [19] at the same intervals, giving a total of 3,398 measurements. The developmental milestones identified were: *Oviposition*; *Dig 1* (when 1st instar

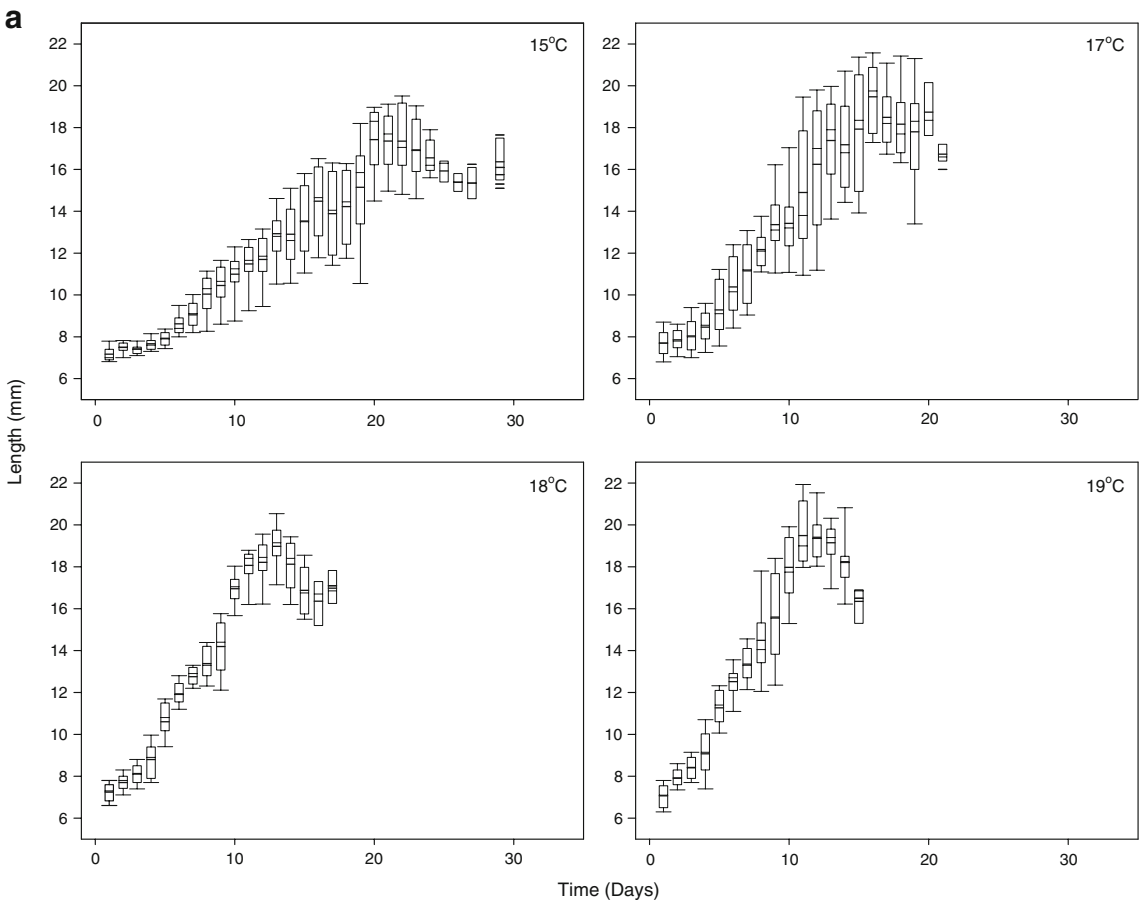


Fig. 1 Growth curves of *Thanatophilus micans* larvae at constant temperatures: **a** 15°C, 17°C, 18°C, and 19°C and **b** 20°C, 22.5°C, 25°C, 28.4°C, 32.5°C and 35°C. All curves are sigmoidal, showing slow initial growth, followed by an accelerated growth phase and tailing off as development is completed. Note the range of ages at any given length, particularly at the lowest temperatures. Data for development at 32.5°C and 35°C should be interpreted with care as development was not completed

larvae dug out of the oviposition substrate); *Ecdysis 1*; *Ecdysis 2*; *Dig 2* (when 3rd instar larvae dug into the pupation substrate); *Pupation*; *Eclosion* and *Dig 3* (when the adults dug out of the pupation substrate). Mortality was monitored at the same intervals to monitor optimum temperature for survival.

Lengths were compared between temperatures using ANCOVA with age as a covariate to control for the effect of differing time to grow. Only temperatures where development was completed were used in the analysis. An isomegalen diagram was prepared from the length and age data using spline interpolation in Statistica v.8. Develop-

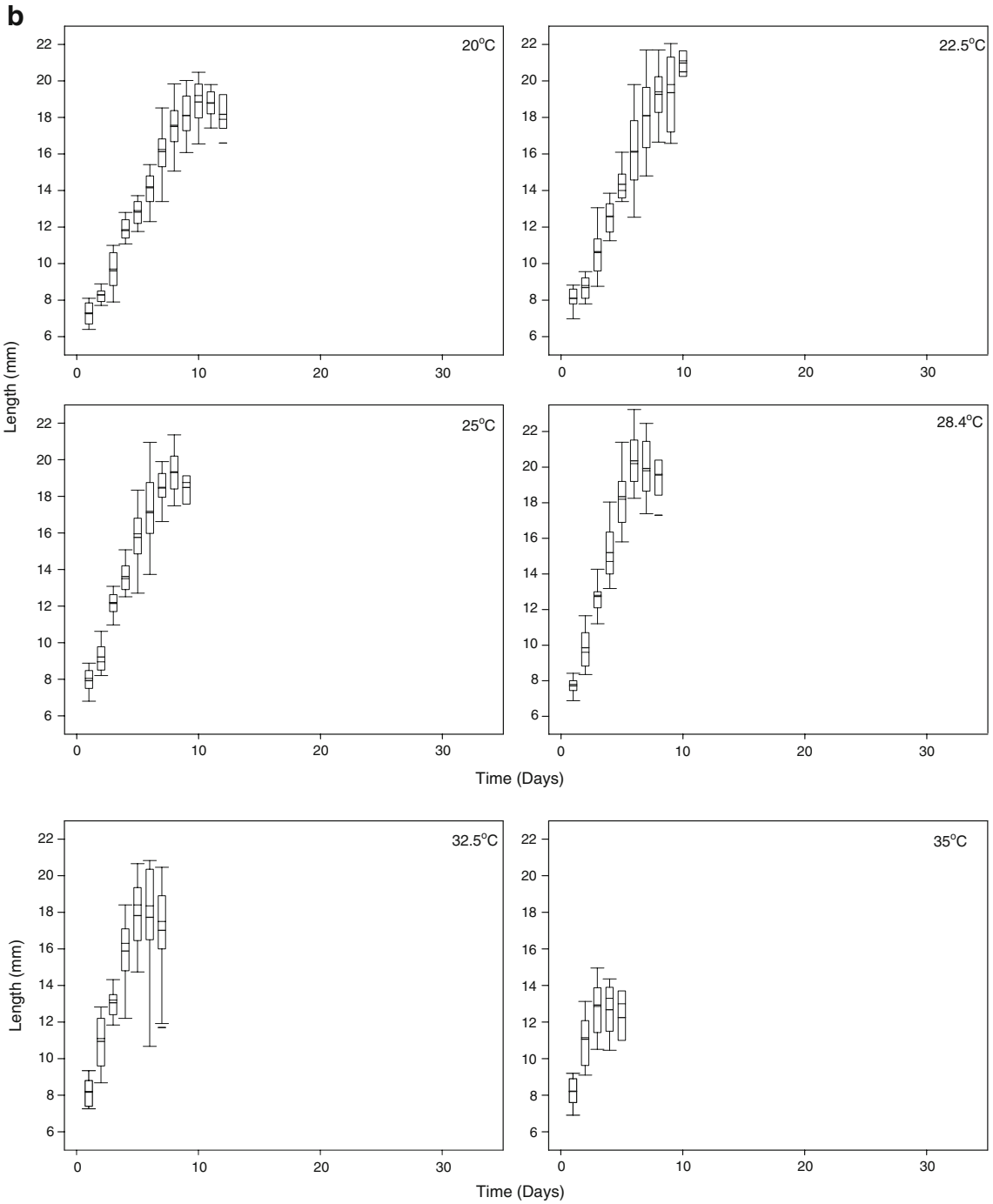


Fig. 1 (continued)

mental constants for each milestone were estimated using major axis regression as described by Ikemoto and Takai [13]. Data from the 28.4°C experiment were not used in calculations due to incubator malfunctions during the experiment, but are still presented because the malfunction occurred only once, for a short period (1 day), during the experiment.

Results

Growth curves at all temperatures were sigmoidal (Fig. 1). Mortality ranged from 20% to 100%, with the highest mortality at extreme temperatures and lower mortality at intermediate temperatures (Fig. 2). Larval length differed significantly between temperatures (F= 355.34, p<0.001), showing five distinct groups (Fig. 3). Larvae reared at low temperatures (15°C and 17°C) were significantly shorter than those reared at higher temperatures and larvae reared at 15°C were also significantly shorter than larvae reared at 17°C. The larvae reared at higher temperatures formed three overlapping groups (Table 1), showing an increase in body length with increased temperature (Fig. 3), even after taking age into account. This is seen in the isomorphen diagram, where developmental contours intersect several body length contours on the isomegalen diagram (Fig. 4). Ecdysis 1 and Ecdysis 2 have the fewest intersections (three contours) and Dig 2 the most (four contours).

Egg development took 5.33 days (128 h) at 17°C to 1.66 days (40 h) at 35°C. Development from hatching to adult took between 63.16 days (1,516 h) at 15°C and 19.33 days (464 h) at 28.4°C to be completed. Development was not completed above 28.4°C, and at 28.4°C larval mortality was high (Table 1). At 17°C and 15°C mortality was also high (Table 1).

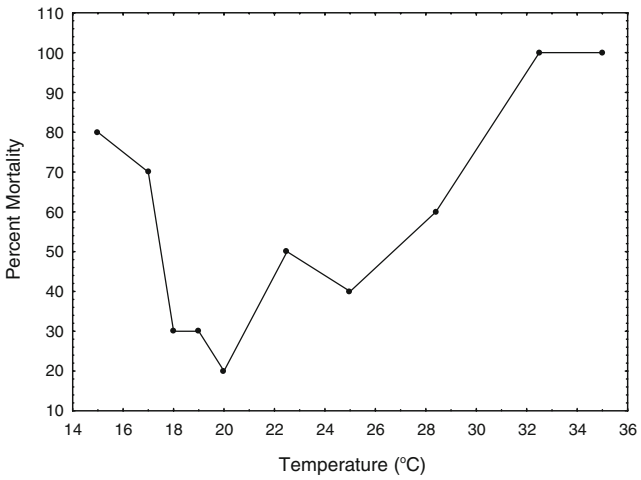


Fig. 2 Mortality rate in larvae of *Thanatophilus micans* at ten constant temperatures, 15°C, 17°C, 18°C, 19°C, 20°C, 22.5°C, 25°C, 28.4°C, 32.5°C, and 35°C

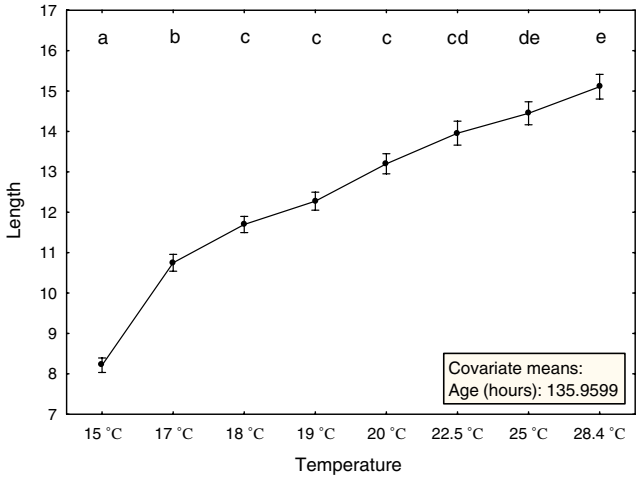


Fig. 3 Means (±95% CI) of body length of mature larvae of *T. micans* at different constant temperatures. Letters above the points (*A–E*) indicate statistically different groups (Table 1). Length at low temperatures (15°C, 17°C) was significantly shorter than all other temperatures, while higher temperatures formed overlapping groups, showing a more gradual increase in length

Thermal summation models were constructed for each previously identified landmark, using as many co-linear temperatures as possible (Table 2, Fig. 5). Because oviposition was not achieved at all temperatures, eggs were transferred between temperature conditions and development time for all other landmarks, therefore, represents the time between digging to the surface (Dig 1) and the specific landmark, and not time from oviposition to the landmark. To obtain an accurate linear regression model, only data points that fall in the near-linear relationship should be used, and points that deviate from this linear relationship should be excluded in the generation of developmental models [11, 13]. Of the 59 data points presented, 50 were suitable in the seven models, three points were rejected

Table 1 Tukey HSD test showing significant differences after ANCOVA was performed on larval mature length data

Temperature treatment (°C)	15°C	17°C	18°C	19°C	20°C	22.5°C	25°C
17.0	**0.0001**						
18.0	**0.0001**	**0.0152**					
19.0	**0.0001**	**0.0003**	0.9378				
20.0	**0.0001**	**0.0002**	0.7997	0.9999			
22.5	**0.0001**	**0.0001**	0.1845	0.8201	0.9606		
25.0	**0.0001**	**0.0001**	**0.0001**	**0.0038**	**0.0228**	0.4783	
28.4	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0004**	0.2207

Age (hours) was used as a covariate to control for the effect of differing time to grow. Significant differences (p<0.05) are shown in bold.

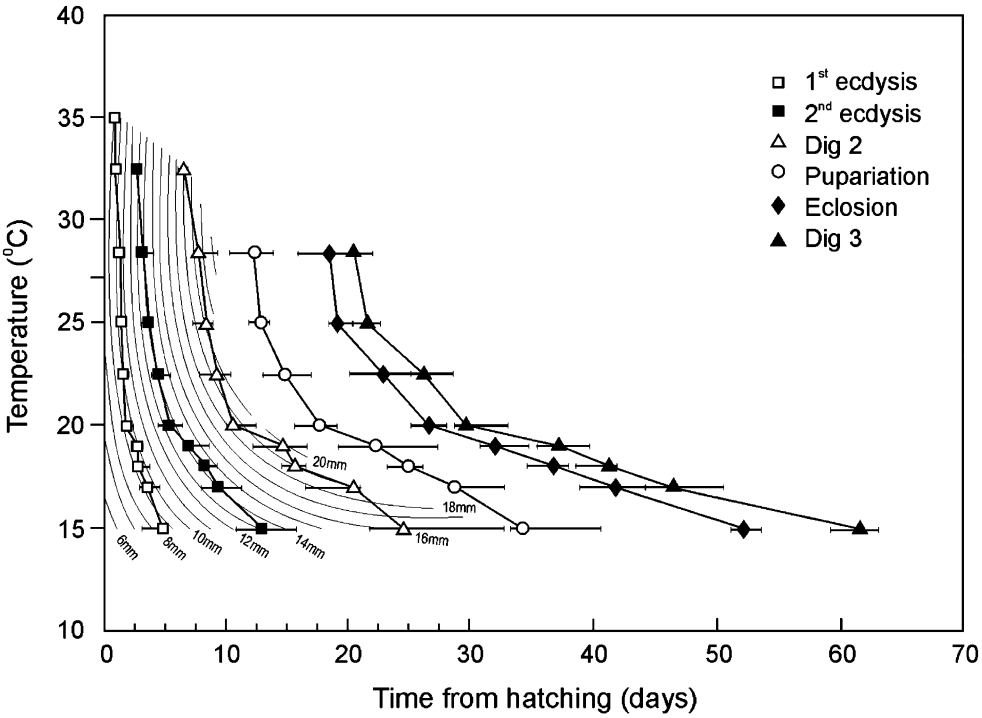


Fig. 4 Overlaid isomorphen and isomegalen diagrams showing intersections of developmental and body length contours. The isomorphen diagram was constructed using the median times to each developmental event at each temperature; *error bars* denote range of time at given event and temperature. The isomegalen contours were constructed using spine interpolation

because they did not form a linear relationship and six points were rejected because they were from the less reliable 28.4°C data set.

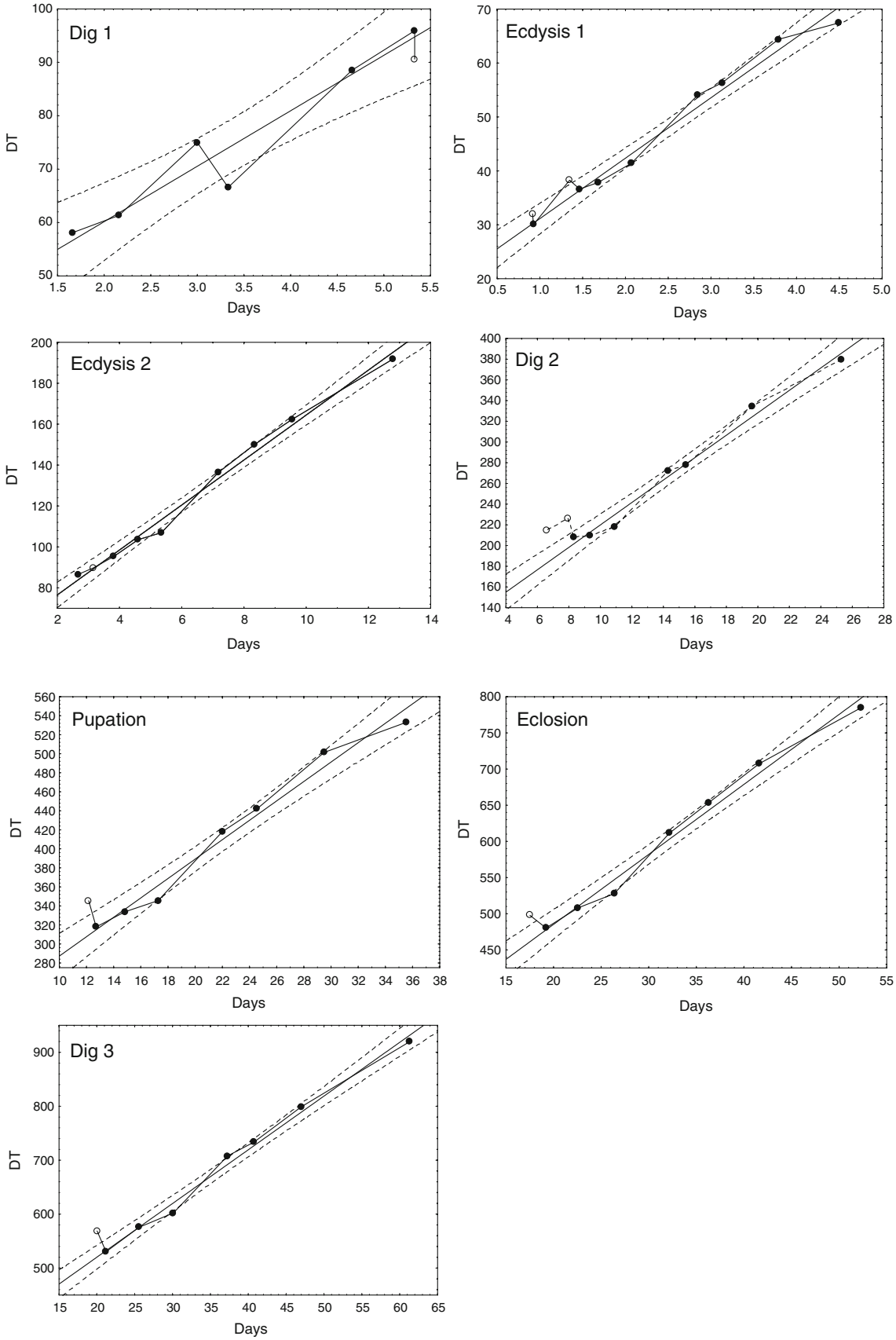
Discussion

Development of *Thanatophilus micans* from oviposition to pupation took 15.7 days at 25°C. This is longer than several forensically important Diptera, including *Sarcophaga tibialis*, which takes 4.5 days at 25°C [20], *Lucilia sericata*, which takes 6.6 days at 25°C [9] and *Protophormia terraenovae*, which takes 9.6 days at 25°C [10]. Development also took longer than *Chrysomya albiceps*, which takes 22.5 days at 17.5°C [15], while *T. micans* takes 29.5 days at 17°C. Other forensically important Coleoptera take longer to develop than *T. micans*, including *Dermestes haemorrhoidalis*, which takes 40.6 days at 25°C [5] and *Dermestes peruvianus*, which takes 52.5 days at 25°C [5]. *Dermestes maculatus* takes 59.2 days at 25°C to complete development from egg to adult [16] while *T. micans* takes 22.2 days at 25°C. The development time of Silphidae therefore fills an important gap in our ability to estimate mPMIs. In field observations around Grahamstown, *T. micans* larvae were found on carcasses within 4 days of death. This corresponds to the egg development times in this study; given that egg development took 3 days at 25°C, this implies that like blowflies, *T. micans* can lay eggs on freshly dead carcasses and corpses.

Intermediate temperatures produced lower mortality, with the exception of 22.5°C, where mortality was 50% (Table 1). This suggests a wide range of temperatures where *T. micans* can survive well. The optimal temperature for survival was 20°C, where mortality was lowest. Larval length was significantly shorter at 15°C and 17°C than at all other temperatures, but the remaining temperatures

Table 2 Summary of development constants for the seven developmental milestones defined in the text

Event	Temperature range	R^2	*df*	*K*		D_0 (SE)
				Degree days (SE)	Degree hours (SE)	
Dig 1	17.0°C–35.0°C	0.9323	5	1.59 (0.25)	38.1 (5.9)	10.76 (1.43)
Ecdysis 1	15.0°C–35.0°C	0.9828	7	0.82 (0.06)	19.7 (1.4)	11.31 (0.53)
Ecdysis 2	15.0°C–32.5°C	0.9902	7	2.26 (0.14)	54.2 (3.3)	11.05 (0.45)
Dig 2	15.0°C–32.5°C	0.9868	6	4.61 (0.49)	110.6 (11.7)	10.90 (0.78)
Pupation	15.0°C–25.0°C	0.9806	6	7.62 (0.63)	182.9 (15.1)	10.30 (0.64)
Eclosion	15.0°C–25.0°C	0.9880	6	12.08 (0.69)	289.8 (16.5)	9.73 (0.48)
Dig 3	15.0°C–25.0°C	0.9921	6	13.31 (0.65)	319.5 (15.7)	10.00 (0.39)


Dig 1
DT
Days
Ecdysis 1
DT
Days
Ecdysis 2
DT
Days
Dig 2
DT
Days
Pupation
DT
Days
Eclosion
DT
Days
Dig 3
DT
Days

◀ **Fig. 5** Major axis regression lines and 95% confidence intervals for the seven development milestones identified for *Thanatophilus micans*, Dig 1, Ecdysis 1, Ecdysis 2, Dig 2, Pupation, Eclosion and Dig 3. In the first diagram (*Dig 1*), *Days* is the time from oviposition to the developmental milestone. In all other diagrams, *Days* is the time from Dig 1 to the developmental milestone. In all diagrams, *DT* is the rearing temperature multiplied by the time to reach the milestone. Fifty of the 59 points generated were used in the analyses: *closed symbols* were used in the calculations and *open symbols* were excluded

formed three overlapping groups, showing a steady increase in larval length through the temperature range at which *T. micans* survives well. Larger female beetles tend to produce more offspring than smaller beetles [6, 12], which means that while the optimal temperature for individual survivorship is 20°C; population growth might well be higher at increased temperatures. Further work is needed to determine the optimal temperature for population growth (as opposed to individual growth) in *T. micans*.

The intersected contours on the overlaid isomorphen and isomegalen diagrams (Fig. 4) provide good evidence that length is an ambiguous indicator of physiological age in this species. This is especially true at low temperatures, where the ranges of body sizes at each developmental event were very large (Fig. 4). This has been noted in Diptera by previous authors [7, 8, 15], and the same is probably true for Coleoptera in general.

Developmental threshold (D_0) values ranged by 1.6°C between developmental landmarks, but the D_0 values for the life stages that occur above ground ranged by only 0.4°C, while the stages below ground ranged by 1.0°C (Table 2). These two groups also showed no overlap, with the epigeal stages having D_0 values 0.2°C higher than the hypogeal groups. The observed differences are not significant, as the 95% confidence intervals overlap in all cases. It is likely that the observed differences are due to the subsurface environment buffering the organisms from temperature fluctuations that occur above ground. The difference between carcass and soil temperatures needs to be taken into account when using the models presented here for estimating mPMIs.

The D_0 values are not unexpected for an African insect, but cannot be compared to other silphid species, as no relevant data are published. Richardson and Goff [16] present some developmental data for *D. maculatus*, from which a D_0 value for *D. maculatus* can be calculated. The D_0 value calculated from these data is 12.48°C, almost 2.5°C higher that the value for *T. micans*. This value is, however, not statistically robust, as only three data points fall on the linear section of the graph. Coombs [5] presents data for both *D. haemorrhoidalis* and *D. peruvianus*, from which D_0 values can also be calculated, but these are also not statistically robust, with only four and three points on the linear section, respectively. The D_0 value for *D. haemorrhoidalis* is 12.99°C, which is almost 3°C higher than *T. micans*; and 12.32°C for *D. peruvianus*, which is almost 2.4°C higher. Given that the three D_0 values calculated for the *Dermestes* species are all not statistically robust, it is not wise to compare them too rigorously because they cover a relatively small range of temperatures. Definitive data should be generated before definitive comparisons can be made.

While comparisons between many parameters of this model are possible, the most valuable aspect of this study is that it provides a model for a time period where forensic entomologists were previously unequipped to make development-based mPMI estimates.

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