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Models for the rates of pupal development, fat consumption and mortality in tsetse (*Glossina* spp)

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Abstract

Environmental temperature is an important driver of the population dynamics of tsetse (*Glossina* spp) because the fly's immature stages are particularly vulnerable to temperatures (T) outside the range $T = 16$–32°C. Laboratory experiments carried out 50 years ago provide extensive measures of temperature-dependent rates of development, fat consumption and mortality in tsetse pupae. We improve on the models originally fitted to these data, providing better parameter estimates for use in population modelling. A composite function accurately models rates of pupal development for $T = 8$–32°C. Pupal duration can be estimated by summing the temperature-dependent daily percentage of development completed. Fat consumption is modelled as a logistic function of temperature; the total fat consumed during pupal development takes a minimum for $T \approx 25$°C. Pupae experiencing constant temperatures <16°C exhaust their fat reserves before they complete development. At high temperatures, direct effects kill the pupae before fat stores are exhausted. The relationship between pupal mortality and temperature is well described by the sum of two exponential functions. Summing daily mortality rates over the whole pupal period does not reliably predict overall mortality. Mortality is more strongly correlated with the mean temperature experienced over pupal life or, for $T \leq 30$°C, the fat consumption during this period. The new results will be particularly useful in the construction of various models for tsetse population dynamics, and will have particular relevance for agent-based models where the lives of individual tsetse are simulated using a daily time step.

Keywords: tsetse, *Glossina*, pupal duration, mortality, fat consumption, models

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Introduction

The reproduction of almost all blood-feeding flies involves each female producing many eggs during her lifetime, but then abandoning them to fend for themselves as they develop through the larval and pupal stages to become new adults. This reproductive strategy, found for example in mosquitoes, tabanids and stable flies, is generally dependent on seasonal rainfall: dry periods are a problem for these insects. Tsetse flies, *Glossina* spp, vectors of trypanosomes that cause human and animal trypanosomiasis in Africa, provide an exception to this rule.

Unlike most other biting flies, but in company with families such as the Hippoboscidae, Nicteribiidae, Streblidae, both sexes of tsetse feed exclusively on blood, which is available year-round in their host animals, with no need to rely on nectar that is obtainable only during the flowering season (Glasgow, 1963). Moreover, while air moisture is important for the healthy development of tsetse pupae (Bursell, 1958), the flies have developed a reproductive strategy that makes their immature stages independent of the surface water required for breeding of blood-feeding insects such as mosquitoes. They have achieved this by abandoning the egg-laying mode of reproduction. Instead, the female tsetse matures a single egg every 8–11 days, retaining this egg in her uterus where it develops into a larva, feeding on a milk-like secretion

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produced by the mother (Tobe & Langley, 1978). The fully mature larva is deposited on friable soil in a shady spot and rapidly burrows into the ground to pupate. Typically, the pupa is provisioned with fat reserves sufficient to complete pupal development and then sustain the emergent teneral in the first day or so of adult life, to allow it to find a host and feed. The unusually slow method of reproduction means that tsetse populations can only survive if mean mortality rates are lower than for other flies. On the other hand, the reproductive strategy allows tsetse to breed throughout the dry season and, while tsetse abundance can vary seasonally, the flies are essentially non-seasonal (Jackson, 1949). Tsetse cannot aestivate to avoid unfavourable weather and, indeed, their populations can survive only where temperature profiles allow them to reproduce throughout the year (Leak, 1998).

Temperature takes centre stage in relation to various aspects of the fly's life, affecting rates of mortality and of fat metabolism in adults and pupae, and rates of larviposition and pupal development. These rates provide important inputs for an increasing number of models of tsetse population dynamics (Vale & Torr, 2005; Torr & Vale, 2011; Hargrove *et al*., 2012; Moore *et al*., 2012; Ackley & Hargrove, 2017; Lord *et al*., 2018), using techniques varying from spreadsheet models to differential equations. Recently, there has also been a growing interest in using agent-based models – where the lives of individual tsetse are followed at short discrete time intervals (Alderton *et al*., 2013, 2016, 2018; Lin *et al*., 2015; Grébaut *et al*., 2016). All of the above modelling approaches can be useful for the prediction of future changes in the distribution and abundance of tsetse, and the diseases they transmit, under various intervention protocols, and in the face of possible climatic changes. In order for these models to be calibrated accurately, however, it is imperative to have good prior information about tsetse birth, development and death rates and how these rates are affected by temperature. In this regard, the immature stages of tsetse are of prime interest, particularly because it is becoming increasingly clear that these stages are the most vulnerable to extremes of temperature (Hargrove & Ackley, 2015; Ackley & Hargrove, 2017).

The only studies which provide useful information on the quantitative aspects of pupal development, metabolism and survival were carried out in the 1960s and 1970s. At this time, Phelps carried out a number of studies of fundamental importance for the foundations of tsetse population dynamics. He measured the effects of temperature on development rates in tsetse pupae, both in the laboratory (Phelps & Burrows, 1969*a*) and in the field (Jackson & Phelps, 1967; Phelps & Burrows, 1969*b*), on rates of fat consumption (Phelps, 1973) and on mortality in these immature stages (Phelps & Burrows, 1969*c*; Phelps & Clarke, 1974). The studies are remarkable both for the scope and precision of the measurements, and for the indications they provide about the importance of temperature in tsetse biology. Over the range of temperatures used in the studies, pupal development rates, and thus the duration of the pupal period, changed by an order of magnitude. Moreover, the results showed that there are extremely sharp temperature thresholds, above and below which pupal survival probabilities go rapidly to zero.

The original data deserve re-examination for a number of reasons. Firstly, in the absence of efficient routines for fitting non-linear functions to data, Phelps & Burrows (1969*a*) used linear approximations or simply fitted data by eye. Modern computers and programmes facilitate better fits to the data and more informative models. Secondly, the authors noted that the model they fitted did not provide good agreement to the data on development rates outside the range 20–30°C. We suggest a composite function that provides good fits to all data for temperatures between 8 and 32°C. Thirdly, while the authors provided crucial data on pupal mortality, they did not produce a functional model for changes in mortality with temperature. Finally, we can now use Phelps' data to suggest quantitative links between pupal mortality and temperature-dependent rates of fat consumption.

In modelling the population dynamics of tsetse, it is essential that we take into account the above-mentioned effects of temperature on pupal biology – along with analogous effects on the rates of pupal production by adult females (Hargrove, 1995) and on adult mortality (Hargrove, 2001). This is not a straightforward procedure, however, because temperatures can change continuously, and sometimes rapidly, during the biological events we are trying to model – and rates of pupal development and fat catabolism then also change with the short-term temperature variations. Ideally, if we want to construct the most accurate models of tsetse population dynamics, we need to track temperature changes continuously during the various life processes we are modelling, and integrate the *rates* of development during each period over which temperature has been averaged. In practice, this is not possible in the field, because we never have continuous measures of temperature: there are thus questions about the time scales over which temperatures should be averaged in the models. In this paper, we use Phelps' data to provide improved estimates of the temperature-dependent rates for various biological processes occurring in the pupae of *Glossina morsitans morsitans* Westwood.

Materials and methods

The majority of the paper consists of a re-analysis of the data referred to above. The experiments were all carried out in Zimbabwe on *G. m. morsitans* Westwood (= *G. morsitans orientalis* Vanderplank). The laboratory work was done in Harare and the field work at the following locations in the Zambezi Valley: Lusulu Field Station, Latitude 18°05′S, Longitude 27°50′E, Altitude 988 m; Kariba Field Station, Latitude 16°32′S, Longitude 28°48′E, Altitude 549 m; Makuti Field Station, Latitude 16°18′S, Longitude 29°15′E, Altitude 1006 m.

All data analyses, involving linear and non-linear regressions, were carried out using Microsoft Excel and Statacorp Stata, Version 14.2. All error limits indicate 95% confidence intervals.

Results

Pupal durations as a function of temperature

(i) $T = 16$–32°C: The data are selected from Phelps & Burrows (1969*a*) and Phelps (1973) who measured, directly, the mean pupal duration, $I_P(T)$, for *G. m. morsitans* incubated at constant temperatures between $T = 16$ and 32°C. The data were well described by the function:

$$I_P(T) = (1 + \exp(a + bT)) / k$$

where a, b and k are all constants (Phelps & Burrows, 1969*a*). Here we incorporate the constant in the denominator into

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the other constants, using the equivalent form:

$$I_P(T) = A + B\exp(C(T - T_1)) \quad (1)$$

with the constants A, B and C suitably redefined. T_1 is an arbitrary constant, which does not affect the goodness of fit, but simply ensures that the value of B falls in a convenient range: we take $T_1 = 16$°C. The best fits to the data obtained with this model are shown in fig. 1a, using the parameters shown in table SM1. All tables, providing full details of each analysis, are available in the Supplementary Material: parameter estimates, with 95% confidence intervals, are presented in the legends of each figure. Observed and predicted values differed by a maximum of 0.04 days for all temperatures, except for males kept at 16°C, and even here the difference was only 0.24 days (0.24% of the observed mean duration of 101.8 days). The original raw data are not available so it is not possible to assign confidence intervals to the mean values – but the extremely good fit to the data in fig. 1a suggests that the variation in the data must have been very small.

Males and females are treated separately because, although there is overlap, for each parameter in table SM1, between the 95% confidence intervals for males and females, the observed pupal durations for males are higher at each of the 12 temperatures tested. This would happen by chance with probability $P = 0.00024$. The sexual dimorphism in the pupal period is thus a real effect, although the differences in pupal durations are small. The absolute differences between the sexes decrease with temperature over the range $T = 17$–31°C (fig. 2a), though the difference expressed as a percentage of the male pupal duration increases linearly with temperature over this range (fig. 2b). At 16 and 32°C, however, both the absolute and percentage differences lie below the general trend in the data. This may be related to the fact that, at these low and high limits, mortalities were much higher (52.2 and 45.8%) than at intermediate temperatures.

(ii) $T < 16$°C: For temperatures <16°C, it was not possible to measure pupal duration directly, because all pupae kept at these low temperatures died within the puparial case. Phelps & Burrows (1969*a*) did find, however, that if pupae were incubated at some low test temperature ($T = \tau$ in the region 8–14°C) and at 25°C, for alternating 24 h periods, survival probabilities were in excess of 78%. They were then able to infer the rates, $r(\tau)$, of development that would have occurred at a given low test temperature ($T = \tau$), on the assumption that the rate of development over the whole pupal period was the mean of the rates for the times spent at τ and at 25°C, respectively. We follow these workers in treating $I_P(T)$ and $r(T)$ as reciprocals of each other so that, with temperatures alternated over equal periods, we have:

$$I_P(\tau, 25) = 1/[(r(\tau) + r(25))/2] = 2/(r(\tau) + r(25))$$

or, equivalently,

$$r(\tau) = (2/I_P(\tau, 25)) - r(25) \quad (2)$$

and we can then estimate the pupal duration appropriate for temperature τ from $I(\tau) = 1/r(\tau)$. The implicit, and as yet untested, assumption is that this procedure would work if the low temperatures were alternated with some temperature other than 25°C, or if the periods of alternating temperature were not 25°C – assuming only that the chosen temperatures and periods of exposure were such that they did not cause severely elevated pupal mortality.

Phelps & Burrows (1969*a*) tested the procedure summarized in equation (2) by keeping pupae at alternating temperatures of 20 and 25°C, for 24 h at each temperature. At these two temperatures, the observed pupal durations were 47.9 and 26.4 days, respectively, and rates of development, therefore, took values of $r(20) = 1/47.9 = 0.02087$ days^{-1} and $r(25) = 1/26.4 = 0.03786$ days^{-1}, respectively. The predicted pupal duration for the alternating regime is then:

$$I_P(20, 25) = 2/(0.02087 + 0.03786) = 34.1\text{days}$$

which differed from the observed pupal duration of 34.4 days by <1%.

If this result holds more generally, for arbitrary temperature profiles, then we could estimate the proportion, $d(t)$, of pupal development that has been completed by time t post-larviposition by evaluating the integral:

$$d(t) = \int_0^t r(s)\,ds \quad (3)$$

where $r(s)$ is the instantaneous rate of development at time s. The function in equation (3) simply adds up these rates over each instant in time: the pupal duration is then given by the value of t for which $d(t) = 1$. In practice, we never have continuous measures of temperature, but one can approximate the integral by summing mean rates of development over a convenient time period, such as 1 day. If the mean temperature on day j is $T(j)$ and the development completed on that day is $r(T(j))$ then the proportion of development completed by the end of i days post-larviposition is given by:

$$D(i) = \sum_{j=1}^{j=i} r(T(j)) \quad (4)$$

and the pupal duration is given by the value of i for which $D(i)$ first exceeds 1.0. Again, the function in equation (4) sums rates over time – but, now, the rates are daily averages calculated from the mean daily temperature.

Pupal developmental rates as a function of temperature

As explained above, pupal durations cannot be measured directly at temperatures <16°C, but must be estimated instead as the inverse of the development rate at these low temperatures. Phelps & Burrows (1969*a*) modelled the relationship between the rate of pupal development, $r(T)$, and temperature using the function:

$$r(T) = k/(1 + \exp(a + bT)) \quad (5)$$

In the absence of the computer software required to fit the data, they used an iterative procedure due to Aitchison & Silvey (1960): a slightly better fit (i.e., with a lower residual sums of squares) was achieved using Stata's non-linear fitting routine (fig. SI1, table SI1; Supplementary Materials). As will become apparent, however, using a single function to describe the data over the entire range of temperature leads to problems.

Phelps & Burrows (1969*a*) found that their fit to the data was poor for T outside the range 20–30°C. We found that the measured rates for $T = 16$–32°C were very well fitted by equation (5) but, when data for $T < 16$°C were also included, the fit was not as good. At temperatures <16°C, the rate of development was, however, well described by a simple exponential function of temperature:

$$r(T) = K_1\exp(K_2T) \quad (6)$$

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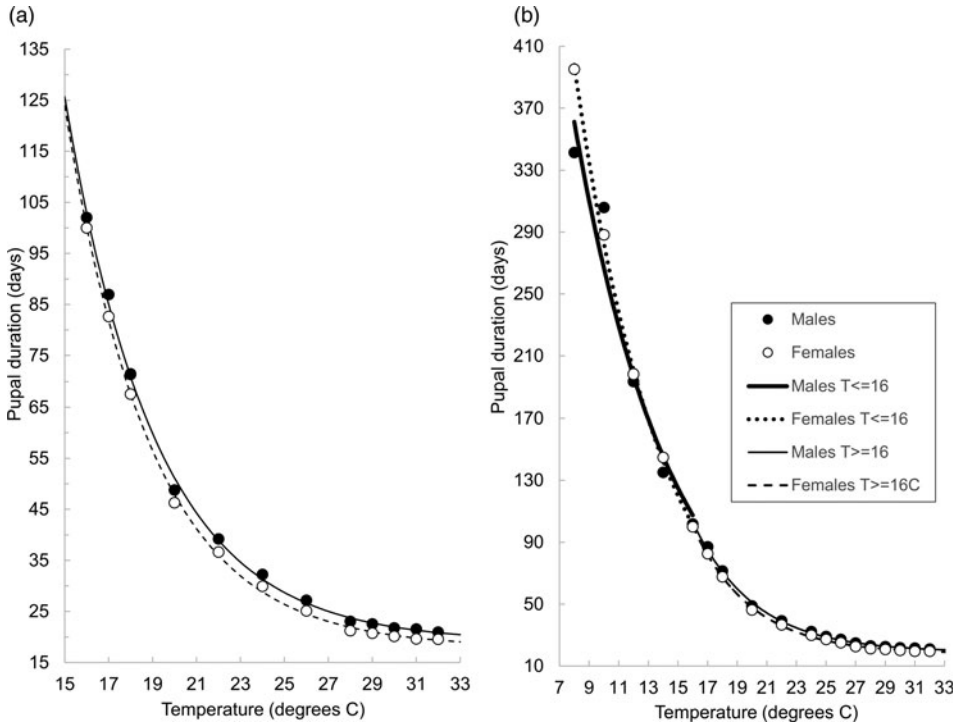

Fig. 1. Pupal duration in male and female *G. m. morsitans* as a function of the constant temperature at which the pupae were maintained in the laboratory. (a) Data for temperatures in the range 16–32°C, where pupal durations were measured directly. Predicted values calculated using equation (1). Parameter estimates: Males $A = 19.1$ (17.4–20.8), $B = 84.0$ (81.7–86.4), $C = -0.24$ (−0.26 to −0.22). Females $A = 17.9$ (16.8–19.1), $B = 82.3$ (80.7–84.0), $C = -0.25$ (−0.27 to −0.24). Further details in table SM1. (b) Data as in (a) but with additional data for pupae where the development rates at 8, 10, 12 and 14°C have been inferred by maintaining pupae at constant temperatures for 24 h periods, the periods alternating between the test temperature and 25°C. Predicted values calculated using equation (7). Parameter estimates: Males $B = 107.4$ (75.1–139.8), $C = -0.15$ (−0.20 to −0.11). Females $B = 101.3$ (96.1–106.4), $C = -0.17$ (−0.18 to −0.16). Further details in table SM3. At 16°C the predicted values using the two equations are sufficiently similar that the difference between them cannot easily be separated visually.

where K_1 and K_2 are constants. The fit to the data of this composite function is nearly perfect and provides a good practical means of calculating the rate of pupal development at any temperature between 8 and 32°C (fig. 3): the parameters required for these fits are provided in table SM2. For the equivalent relationship between pupal duration and temperatures <16°C, we fitted the data using:

$$I_P(T) = B\exp(C(T - T_1)) \quad (7)$$

Parameter values for the best fits for $T < 16$°C are shown in table SM3 and the combined fits for data across the whole temperature range $T = 8$–32°C are shown in fig. 1b. As with the development rates, using a single function to model the pupal duration over the whole temperature range led to unacceptable errors in the predicted duration at higher temperatures in the range (Supplementary Materials, fig. SI2, table SI2).

Fat consumption by tsetse pupae maintained at constant temperature in the laboratory

Having maintained tsetse pupae at constant temperatures in the laboratory until they emerged, thereby estimating pupal duration and mortality, Phelps (1973) measured the amount of fat in the newly emerged teneral flies. He then used linear regression to estimate the 'size-specific' fat content adjusted using the fat content for a fly of fat-free (or residual) dry weight (RDW) 4 mg – this being close to the mean RDW for flies in his study. With knowledge of the fat content of a newly deposited pupa of this RDW, it was then possible to estimate the total amount of fat used during pupal development. The daily rate of fat consumption was calculated by dividing this total by the pupal duration.

Phelps (1973) modelled increases in the logarithms of the daily fat consumption as a two-part linear function, with a discontinuity in the slope of the function somewhere between 20 and 22°C (fig. 4a). Such a discontinuity is not biologically plausible and, indeed, the untransformed fat consumption data are well fitted by a linear model with no evidence of a discontinuity (Hargrove, 2004). The latter model is, however, not a good one because it predicts negative rates of fat consumption for sufficiently low temperatures. Fitting a logistic function of the form:

$$F(T) = a/(1 + \exp(-b(T - c))) \quad (8)$$

where $F(T)$ is the daily rate of fat consumption at temperature T and a, b and c are constants, addresses the above problems and provides a good fit to the data for male and female pupae (fig. 4b; table SM4). Notice that, for large values of T in equation (8), the exponential term goes rapidly to zero and the rate of fat consumption converges to a value of a. Conversely, as T declines below values of about 20°C, the exponential term – and thus the denominator – becomes large and the rate of fat consumption converges to zero.

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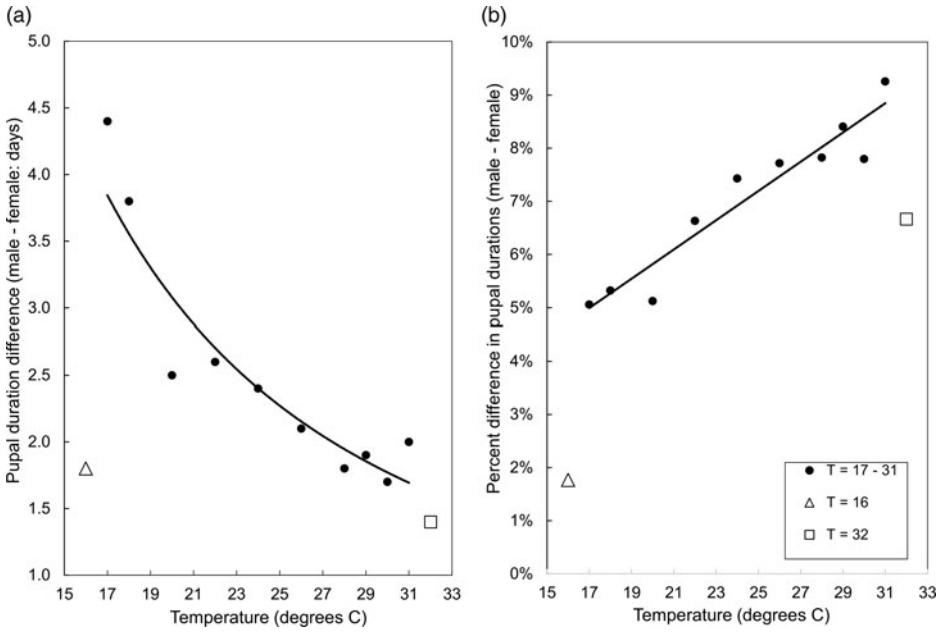

Fig. 2. Observed mean pupal durations for *G. m. morsitans* incubated at constant temperature (T): differences between males and females. (a) Absolute differences. (b) Differences as a percentage of the duration in males. A power function in (a), and linear function in (b), fitted to the data for $T = 17$–31°C indicate the decrease in the absolute value of the sex difference, and the increase in the percentage difference, with increasing temperature. The values at $T = 16$ and 32°C have been excluded from the fitting exercise.

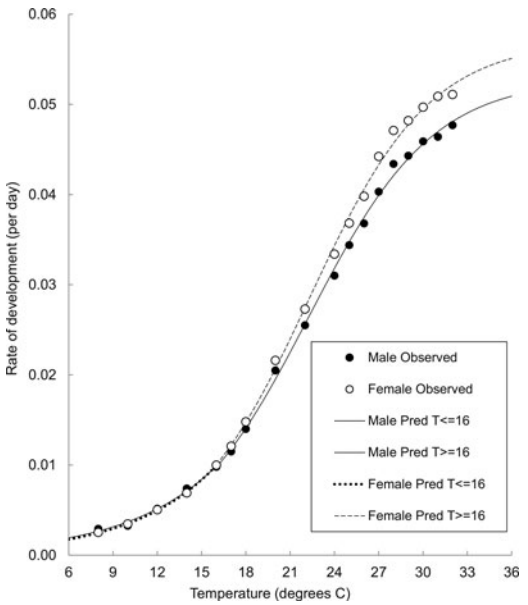

Fig. 3. Development rates for pupae of *G. m. morsitans* incubated in the laboratory at constant temperatures (T). Data for $T \geq 16$°C fitted using equation (5). Parameter estimates: Males $k = 0.053$ (0.051–0.055), $a = 5.3$ (4.9–5.7), $b = -0.24$ (−0.26 to −0.22). Females, $k = 0.057$ (0.054–0.060), $a = 5.5$ (5.0–5.9), $b = -0.25$ (−0.27 to −0.22). Further details in table SM2A. Data for $T < 16$°C fitted using equation (6). Parameter estimates: Males $K_1 = 0.00064$ (−0.00006 to 0.0013), $K_2 = 0.18$ (0.09–0.26). Females $K_1 = 0.00066$ (0.00050–0.00081), $K_2 = 0.17$ (0.15–0.19). Further details in table SM2B. At 16°C the predicted values using the two equations are sufficiently similar that the difference between them cannot easily be separated visually.

The estimates of daily fat consumption, $F(T)$, and pupal duration, $I_P(T)$, can then be used to estimate the total fat consumed during a pupal period for a given incubation temperature T (fig. 5a). Total fat consumed takes a minimum in the region of $T = 25$°C, increasing monotonically as temperatures increase or decrease from this optimum level.

The predicted level of fat in the emerging teneral adult is calculated by subtracting the fat consumed from the estimated level of fat in a newly deposited pupa (fig. 5b). The amount of fat available to an emerging fly declines as temperatures move away from 25°C. In particular, as temperatures decline below 16°C, it is predicted that fat reserves may be exhausted before development is complete (fig. 5b). If that were to happen the pupa would then, of course, starve and the adult would not emerge. This suggests the possibility, to be addressed in the following sections, that total fat consumption during development might be used to predict pupal survival.

Pupal mortality

Tsetse pupal mortality can be due to biological factors such as predation (Rogers, 1974; Rogers & Randolph, 1984) and parasitism (Buxton, 1955; Heaversedge, 1969). It can also be due to physical factors such as desiccation (Bursell, 1958), but we will be concerned here only with deaths due to the effects of temperature. These can take different forms: for sufficient extremes of temperature, death will obviously occur more or less instantaneously. Pupae can also be killed rapidly even at temperatures that could conceivably be found in nature: Potts (1933) found that a 30 min exposure to temperatures of 45–50°C killed all pupae of *G. m. morsitans*. Even at less extreme temperatures, commonly experienced in the hot seasons in areas where tsetse are abundant, such as the Zambezi Valley of Zimbabwe, Phelps & Burrows

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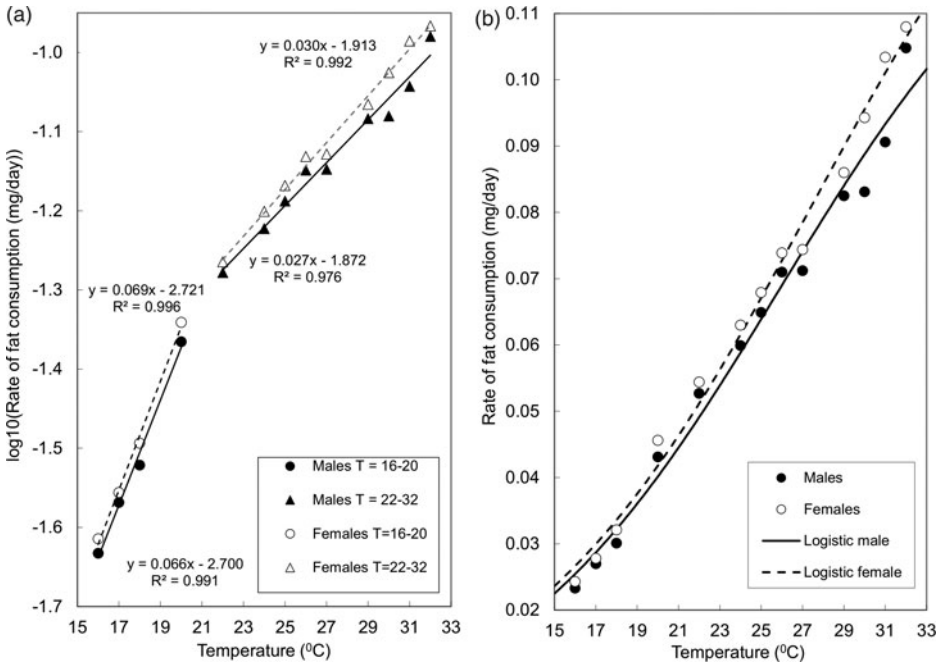

Fig. 4. Models for fat consumption by male and female pupae of *G. m. morsitans* maintained at a constant temperature in the laboratory. (a) Logarithm of fat vs. temperature with a discontinuity in the slope between 20 and 22°C (redrawn from Phelps, 1973). (b) All data fitted using a logistic function (equation 8). Parameter estimates: Males $a = 0.14$ (0.09–0.19), $b = 0.15$ (0.10–0.20), $c = 25.9$ (20.5–31.3). Females $a = 0.16$ (0.11–0.20), $b = 0.14$ (0.11–0.18), $c = 27.4$ (22.6–32.3). Further details in table SM4. $R^2 = 0.997$ for males, 0.998 for females.

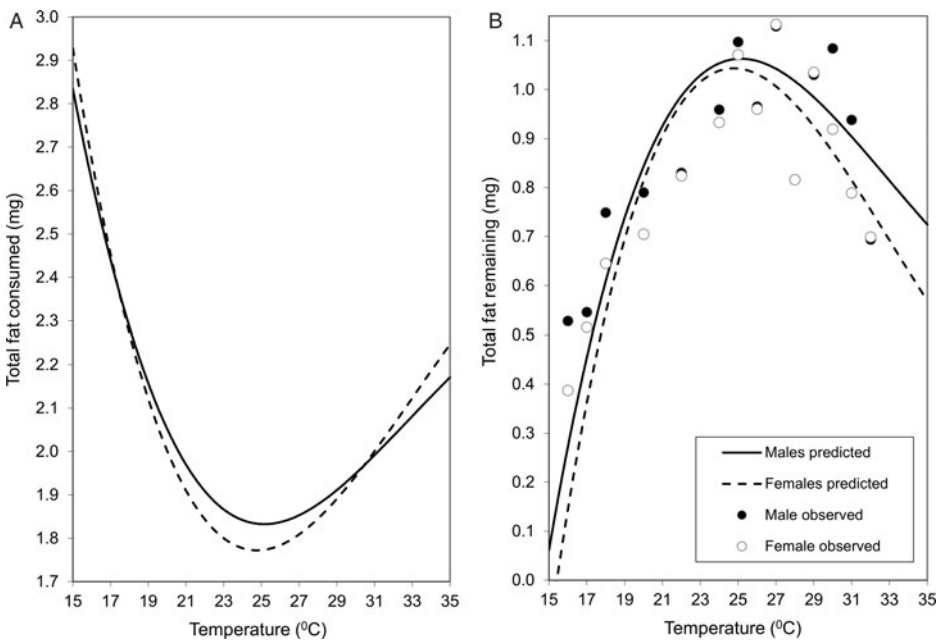

Fig. 5. Fat metabolism during the pupal period of male and female *G. m. morsitans* maintained at a constant temperature in the laboratory. (a) Total fat consumed during the pupal period. Figures estimated using the functions for pupal duration (table SM1) and daily fat consumption (table SM4). (b) Fat remaining at adult emergence, for a teneral fly of 4 mg residual dry weight.

(1969*c*) found that virtually all pupae died if exposed repeatedly to temperatures of 38°C for 6 h a day. They concluded that the high temperature interfered directly with normal physiological development processes. As indicated previously, pupal mortality can also result from indirect effects of temperature, whereby fat reserves are exhausted before pupal development is complete. In the following sections, we investigate the extent to which mortality is due to

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this indirect effect of temperature, as opposed to the more direct effects of extreme temperatures.

Mortalities of tsetse pupae maintained at constant temperature in the laboratory

Phelps & Burrows (1969*a*) and Phelps (1973) provide data on the survival of the pupae of *G. m. morsitans* maintained at a constant temperature in the laboratory (fig. 6a). There was a problem with the calculation of the daily mortality rates for pupae because, while Phelps knew the sex of the flies that emerged, he did not always know the sex of the pupae that died. He was thus unable to disaggregate mortality by sex. From the combined data of Phelps & Burrows (1969*a*) and Phelps (1973), we can see that, in their combined experiments, a total of 496 male and 479 female adult flies emerged. Using the quoted mortality figures (pooled on sex), it is then possible to estimate, very roughly, that the total number of pupae incubated was 1212. If we assume that the sex ratio among the pupae at the start of the incubation was 0.5, we can estimate survival probabilities of 0.818 (95% CI 0.785–0.848) for males and 0.790 (95% CI 0.756–0.822) for females. The complete overlap of the 95% confidence intervals indicates that there is no statistically significant difference between the survival probabilities for males and females.

The pooled mortality estimates suggest a model where mortality might increase exponentially, both at high and low temperatures. Before fitting such a model, however, it is necessary to transform the original data – since the percentage mortality is constrained between 0 and 100%, whereas the suggested exponential functions tend to infinity as temperatures increase or decrease to +/– infinity, respectively.

If we define $m\,(I_P(T))$ as the probability that a pupa, maintained in the laboratory at a constant temperature T°C, dies prior to emergence after $I_P\,(T)$ days, then the probability, $\Phi\,(I_P(T))$, that the pupa survives the whole pupal period until adult emergence is given by

$\Phi\,(I_P(T)) = 1 - m\,(I_P(T))$. We now define $v\,(I_P(T))$ as the instantaneous mortality over the period $I_P\,(T)$. The survival and the instantaneous mortality rates over this period are related by:

$$\Phi\,(I_P(T)) \;=\; \exp\,(-v\,(I_P\,(T))) \tag{9}$$

or, equivalently, taking logs of both sides in equation (9), and re-arranging,

$$v\,(I_P\,(T)) \;=\; -\ln\,(\Phi(I_P\,(T))) \;=\; -\ln\,(1 \;-\; m\,(I_P\,(T))) \tag{10}$$

where $v\,(I_P\,(T))$ can take any value in $(0, \infty)$ and has units $1/I_P(T)$ – i.e., the inverse of the pupal duration.

Figure 6b shows how $v\,(I_P\,(T))$ varied with temperature for the Phelps (1973) data. The data were well fitted using the sum of two exponential functions, with an additional constant term:

$$v(I_P(T)) = K_0 + K_1 \exp\,(-\,K_2(T - T_1)) + K_3 \exp(K_4(T \;-\; T_2)) \tag{11}$$

with the parameter values shown in table SM5. Using the values of K_0–K_4 we can then also evaluate $m\,(I_P\,(T))$, defined above as the probability that the pupa dies prior to emergence. As expected, $m\,(I_P\,(T))$ converges rapidly to unity as temperatures decrease, or increase, to the neighbourhoods of 16 or 32°C, respectively (fig. 6c).

Pupal mortality rates: instantaneous daily rates as a function of temperature

Figure 7 shows that the first exponential term in equation (11) only has any visible effect for $T < 21$°C, and the second exponential only for $T > 28$°C. Between these temperatures, the instantaneous mortality rate is effectively constant at $K_0 = 0.05$–0.06, with units of the reciprocal of pupal duration $(I_P(T))$ – which, itself, also changes with the temperature (T). When T is constant over the whole pupal period, we can, alternatively, define $\mu\,(T)$ as the daily mortality rate and we have:

$$(I_P(T)) \;=\; \mu\,(T)\,I_P\,(T)$$

or

$$\mu\,(T) \;=\; (I_P(T)) \,/\, I_P\,(T) \tag{12}$$

Using equation (12) with equations (1) and (11) we have:

$$\mu\,(T) \;=\; v\,(I_P(T)) \,/\, I_P\,(T) \;=\; [K_0 \;+\; K_1 \exp\,(-K_2\,(T - T_1) \;+\; K_3 \exp\,(K_4(T - T_2))] \,/\, [A \;+\; B \exp\,(C(T - 16))] \tag{13}$$

There is no obvious way to simplify this equation. Instead we evaluate $\mu(T)$ for T in the range 16–32°C using equations (1) and (11) and the parameter values in tables SM1 and SM5. The form of the graph of the data (fig. 8) again suggests fitting the sum of two exponentials, using a function of the form:

$$\mu\,(T) = k_0 \;+\; k_1 \exp\,(\,-\,k_2(T - T_1)) \;+\; k_3 \exp\,(k_4(T - T_2)) \tag{14}$$

with the parameter values shown in table SM6.

Modelling pupal mortality under conditions of varying temperature

We saw above that the time taken to complete pupal development, under conditions of varying temperature, could be estimated by summing over time the daily, temperature-dependent, rates of development (equation 4). It would be convenient if we could, similarly, estimate the probability of surviving all of the pupal development as the product of the temperature-dependent survival probabilities of surviving for each day. If this were true and if $\phi(T(j))$ were the survival probability on day j of pupal development, and $\Phi\,(I_P)$ the survival over the whole pupal period, then we could calculate the survival over the whole pupal period from:

$$(I_P) \;=\; \prod_{j=1}^{j=I_P} (T(j)) \tag{15}$$

Here the symbol Π means that the specified survival terms, $\phi(T(j))$, in equation (15) should all be multiplied together – with the implication that all of the survival events are independent.

For temperatures in the range 20–30°C, this works reasonably well. For example, when pupae were kept for alternate days at 20 and 25°C, the pupal duration was 34 days and, from equation (14), the predicted probability of surviving 17 days at each temperature was 0.966 in each case, giving an overall predicted survival of $0.966 \times 0.966 = 0.933$, close to the observed value of 0.911.

When, however, temperatures <16°C were alternated with 25°C, the approximation was poor. For example, when the pupae were kept at 14 and 25°C on alternate days, the pupal duration was 52 days: the predicted survival probability, from equation (15), of surviving 26 days was 0.949 at 25°C, but only 0.043 were predicted to survive this period at 14°C – giving an overall predicted survival probability of $0.949 \times 0.043 = 0.041$. In practice, however, when pupae were kept at these alternating temperatures, the probability of surviving to adulthood was up to 20 times the predicted value. Discrepancies were even larger for pupae kept at temperatures alternating between $T = 25$°C and $T < 14$°C: in all these cases, it is predicted that no pupae

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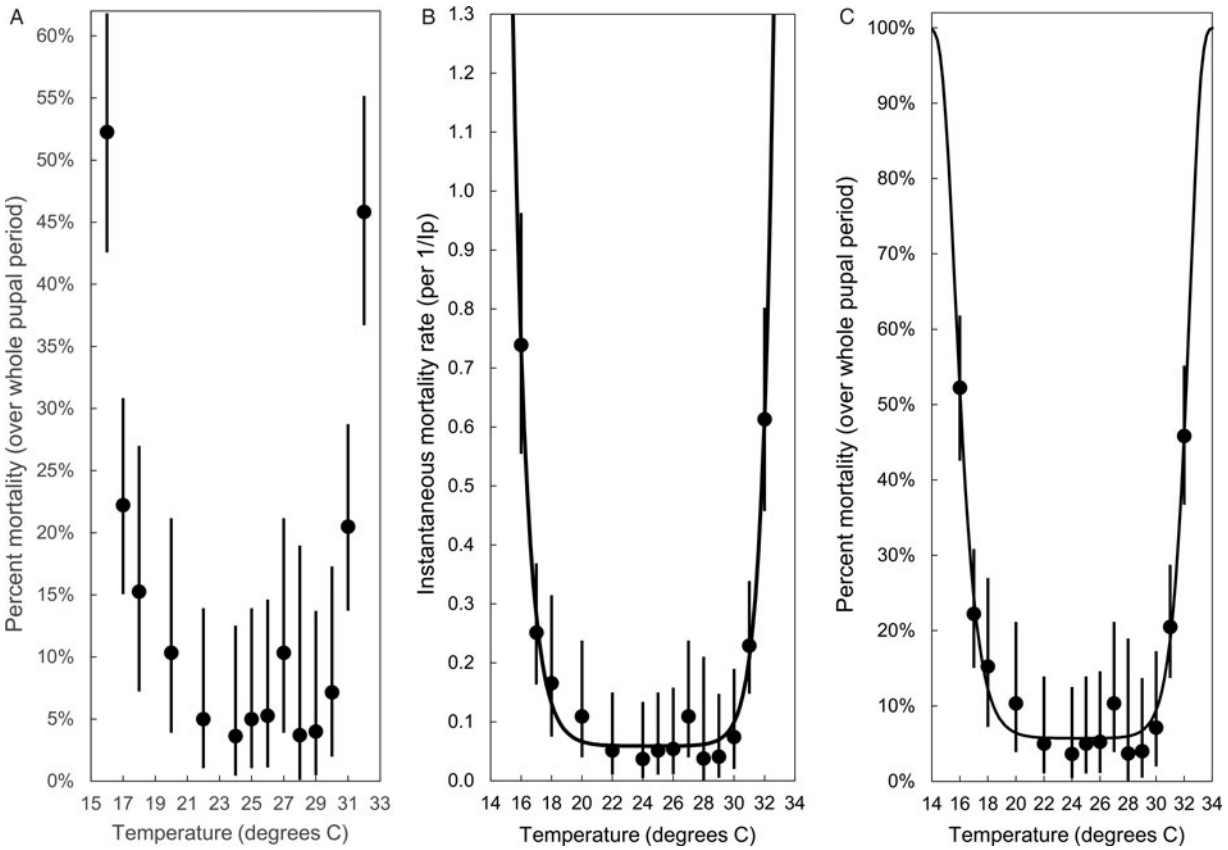

Fig. 6. Mortality among pupae of *G. m. morsitan*s maintained at a constant temperature in the laboratory. (a) Raw data from Phelps & Burrows (1969a) and Phelps (1973). The 95% confidence intervals calculated assuming proportions dying are binomially distributed. (b) Instantaneous mortality, $\nu(I_P(T))$: units $1/I_P(T)$. Data fitted using the model given in equation (11). Parameter estimates: $K_0 = 0.059$ (0.032–0.086), $K_1 = 0.68$ (0.60–0.75), $K_2 = 1.12$ (0.81–1.43), $K_3 = 0.56$ (0.48–0.63), $K_4 = 1.31$ (0.83–1.80). Further details in table SM5. (c) Percentage mortality $m(I_P(T))$: the fitted line is calculated from the function for $\nu(I_P(T))$ in (a), using the relationship between $\nu(I_P(T))$ and $m(I_P(T))$ given by equation (10).

survive until emergence – whereas the observed survival probabilities at 12, 10 and 8°C ranged between 79 and 87%.

It thus appears that we cannot use equation (15) to calculate overall survival probability in real-world environments where temperatures are changing from day-to-day. Slightly better agreement with the observed survival probabilities resulted if we predicted survival using the mean temperature that pupae experienced. For example, flies kept at alternating temperatures of 20 and 25°C are assumed to be living at a mean temperature of 22.5°C, at which temperature the predicted survival is 93.5%, in good agreement with the observed value of 91.1%. When temperatures were alternated between 25°C and either 14, 12, 10 or 8°C, the mean temperatures were 19.5, 18.5, 17.5 and 16.5°C with predicted and observed survivals of 90 vs. 85%, 88 vs. 79%, 84 vs. 87% and 69 vs. 84%. While the agreement is not perfect, the use of a mean temperature should provide a good first approximation for situations where temperatures do not dip below 10°C.

Pupal mortality as a function of total fat consumption during development

Phelps & Burrows (1969*a*) found that pupae incubated at any constant temperature <16°C do not survive to adulthood – but that 79–87% of pupae incubated on alternate days at temperatures <16°C and at 25°C did survive. This is consistent with the idea that incubation at the low temperature does no direct harm to the pupa: the pupa simply completes a very small percentage of its development, and uses little fat. As temperatures fall, the daily rates of fat consumption decline at a quasi-linear rate (fig. 4b), but there is an exponential increase in the pupal period (fig. 1b). Consequently, while pupae can even survive 24 h at 0°C (Phelps & Burrows, 1969*c*), the probability of surviving the much-extended pupal period at low temperatures, and emerging as an adult, is much reduced. Indeed, as is obvious from fig. 5b, pupae incubated at any temperature below 15°C exhaust their fat reserves before completing development. Notice, however, that high temperatures are not predicted to result in the exhaustion of fat reserves in the same fashion. Predicted levels of remnant fat are always in excess of 0.6 mg even at a constant 35°C (fig. 5b) when we know that tsetse pupae kept at this constant temperatures do not survive even a single day.

The amount of fat consumed on a given day of pupal life, at a given temperature, can be calculated from the logistic function (equation 8) using the parameter values from table SM4. The total amount of fat consumed during pupal life is then the sum of the daily consumption values – and these totals can be

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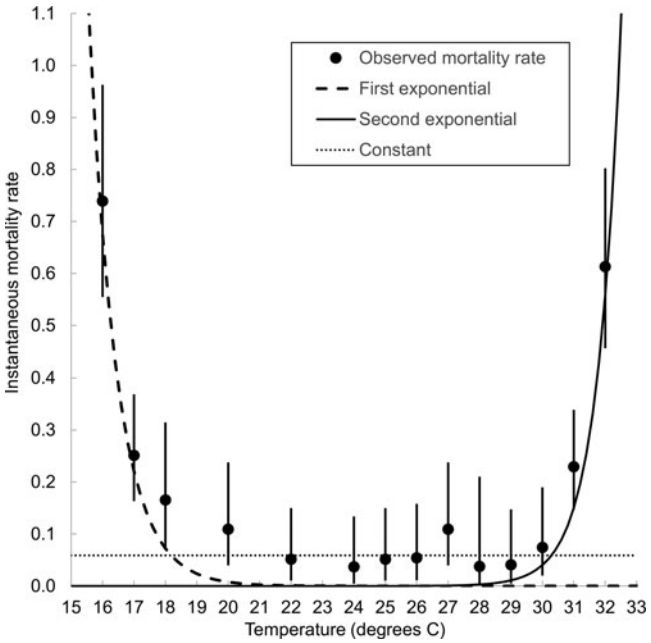

Fig. 7. Instantaneous mortality rates (units $1/I_P(T)$ – i.e., per pupal period) among pupae of *G. m. morsitans* maintained at a constant temperature in the laboratory. The fitted function is given in equation (11) with parameters specified in table SM5. The plot as in fig. 6b but showing the function decomposed into the two exponential functions and the constant term. Raw data from Phelps & Burrows (1969a) and Phelps (1973).

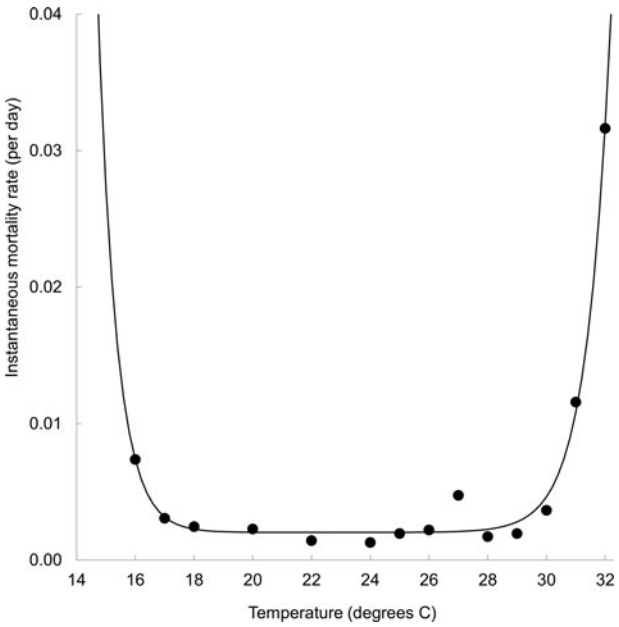

Fig. 8. Instantaneous daily mortality rate for female *G. m. morsitans* pupae as a function of the constant temperature at which the pupae were maintained in the laboratory. Raw data from Phelps & Burrows (1969a) and Phelps (1973). Data fitted using equation (14). Parameter estimates: $k_0 = 0.0020$ (0.0011–0.0029), $k_1 = 0.0053$ (0.0027–0.0080), $k_2 = 1.55$ (−0.65 to 3.76), $k_3 = 0.030$ (0.027–0.032), $k_4 = 1.22$ (0.95–1.49). Further details in table SM6.

calculated both for the flies kept at constant, and at alternating, temperatures. There is a strong correlation between this estimated total fat consumption during pupal life and observed pupal mortality, for all flies incubated at temperatures ≤30° C (fig. 9).

For flies kept at constant temperatures of 31 or 32°C, however, mortality is much higher than expected given the fat consumption. These results are consistent with the idea that, as T exceeds 30°C, direct effects of temperature come into play that interfere with normal pupal development, generally resulting in pupal death before fat reserves have been exhausted. Phelps & Burrows (1969*a*) noted this effect: for example, when they kept flies at a constant temperature of 36°C, the small numbers of flies that did emerge exhibited puparial durations about 10 days longer than predicted values. This phenomenon has never been fully explained: Phelps & Burrows (1969*c*) suggested that high temperatures interfered directly with normal physiological development but it is not known how this interference might be mediated.

Discussion

Results presented here confirm Phelps' findings, highlighting the central importance of temperature in the pupal development of tsetse. The high quality of his data, combined with the use of better models for fitting them, facilitate the improved estimation of the effect of temperature on pupal mortality rates, integrated levels of development completed, fat metabolized and of the interactions between these rates. The present study thus contributes towards a better understanding of the underlying causes of the effects of temperature on tsetse

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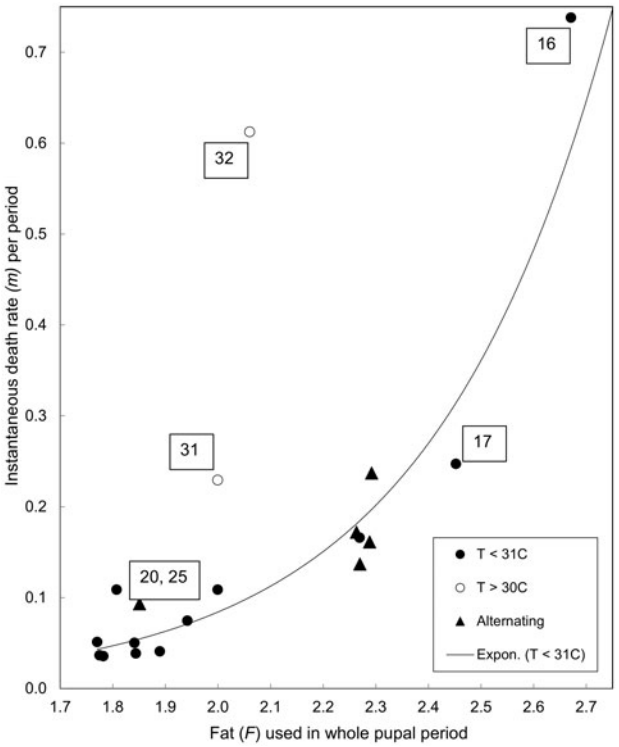

Fig. 9. Fat consumption as a predictor of death in pupae of *G. m. morsitans*. Total fat consumption estimated using the functions for pupal duration (table SM2) and daily fat consumption (table SM4). Death rates calculated from figures in table 1 of Phelps (1973). Function fitted to pupae incubated at constant temperatures $T < 31$°C: $m = 2.49 \exp(2.91 F)$. $R^2 = 0.87$. Figures in boxes indicate the incubation temperatures for selected results.

population dynamics, and will allow improved predictions of the effects of natural and anthropogenic changes in temperature.

Using the results in modelling tsetse population dynamics

An example of the specific way in which the new results could be used in agent-based population modelling is summarized as follows. With knowledge of time variation in temperature, equations (4), (6) and (7) can be used to accumulate the pupal development completed after each chosen time step. Simultaneously, equation (8) can be used to calculate the amount of fat used over this time. When the pupal duration is judged complete, the mean temperature over this period and the total fat used will then be immediately available. Where the mean temperature over the pupal period is in the region of 20–30°C, the pupal mortality can be estimated using the predictive function in fig. 9. Where the temperatures are higher, or lower, than the above limits, mortality can be estimated using equation (11).

We acknowledge that the above procedure handles only the 'demand' side of pupal development. The 'supply' side of the equation depends on the experiences of the pupa's mother, particularly during her pregnancy. The physiological input from the mother depends on, among other things, the balance between the amount of fat the mother produces through feeding, and the amount of fat she catabolizes during each pregnancy. The adult female's ability to provision her pupa with an optimal quota of fat and raw materials for development is a function of the availability of vertebrate host on which she can feed (Lord *et al*., 2017). It is also a function of the meteorological conditions she experiences. When, for example, temperatures in the Zambezi Valley of Zimbabwe increase above 32°C, tsetse seek shelter in dark, cool spaces, sometimes for the majority of daylight hours, thereby much reducing the time available for feeding (Vale, 1971; Torr & Hargrove, 1999). Not surprisingly, the size of pupae produced decreases as temperatures increase in the hot season (English *et al*., 2016; Hargrove *et al*., 2018).

This high-temperature effect is exacerbated by the fact that small pupae not only have less fat in absolute terms, but also less as a proportion of pupal RDW (Phelps, 1973). Since, also, the fat endowment received by a deposited larva is a random variable, we may anticipate that, when mothers are stressed at high temperatures, some small pupae will be in danger either of exhausting their fat reserves prior to emergence, or of having insufficient stores to sustain them until they are able to find their first meal as adults. In support of this idea, Phelps & Clarke (1974) found that, at extremes of temperature, there were significant losses of under-sized male flies in the first few days of adult life and they attributed these losses, largely, to the low fat reserves in the emerging teneral flies.

The supply side situation is further complicated by the fact that the duration of pregnancy also changes with temperature. The functional form of that change has been estimated for field populations (Hargrove, 1995): it is thus possible, in principle, to accumulate daily pregnancy completion rates in an analogous fashion to the approach used here to follow pupal

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development. The authors are actively involved in developing models that take into account all of the above temperature-dependent factors affecting the various aspects of pupal and adult life.

Particular geographical areas in which such modelling may be pertinent are in much of West Africa, where the northern limits to tsetse distribution are set by the hot climate of the Sahara. If global warming near these limits pushes the mean temperature experienced by pupae to above 32°C, then figs 6–8 suggest that the distribution of tsetse will recede southwards. Conversely, in the KwaZulu-Natal region of South Africa, the lower limits of temperature are important, and global warming will tend to increase the range of tsetse. It is thus important that we have given special attention to the potentially crucial detail of the way pupal mortality is affected by temperature change at the cooler end. We do acknowledge, however, that the changing status of tsetse populations depends on many factors (such as land-use, active tsetse control measures and wild host reductions), of which meteorological status is just one.

Caveats and concerns

What temperatures do tsetse actually experience?

Our ability to model confidently the effect of temperature on wild populations of tsetse is hindered by poor knowledge of the temperatures that flies actually experience in the field, it being known that such temperatures often differ from the ambient values measured in a Stevenson screen. For example, when temperatures exceed 32°C in the hot dry season in the Zambezi Valley of Zimbabwe, adult tsetse take refuge in dark places (Vale, 1971) where temperatures can be 3–4°C cooler than ambient at the hottest time of the day (Torr & Hargrove, 1999). Similarly, at these times, females often deposit larvae in sites such as warthog burrows where the mean temperatures can be 6–7°C cooler at the hottest time of the day and >2°C cooler, on average, than ambient over a 24 h period (Muzari & Hargrove, 2005). These behaviour patterns complicate matters for workers intent on modelling tsetse population dynamics. Since, in general, the temperatures being experienced by the flies are not known, where models of tsetse population dynamics do allow for temperature effects, the temperatures used are those measured at a standard meteorological station (Ackley & Hargrove, 2017; Lord *et al*., 2018). Land Surface Temperatures, measured using satellites (Li *et al*., 2013), provide estimates that may approximate more closely the mean temperatures experienced by tsetse than the air temperatures measured in a Stevenson screen. Present results suggest the need, however, to attempt to measure how either set of temperature measures translate into those experienced by the flies in the various microhabitats used by adults and pupae under different meteorological conditions.

Paucity of information on tsetse species other than G. m. morsitans

We consider here only results for a single species of tsetse. This is because there is so little information on pupal life for any other species. For *G. tachinoides* Westwood raised in a laboratory at 25°C, Gruvel (1975) cites pupal durations of 31 and 28 days for males and females, respectively, 2 and 1 day longer than for *G. m. morsitans* of the same sex. He provides no information, however, for pupal durations at other temperatures. Challier (1973) and Mellanby (1936) found that mean pupal durations for *G. palpalis* incubated at 24°C were 33.1 and 31.1 days for males and females, respectively. These were 1.2 and 0.8 days longer than the durations measured by Phelps & Burrows (1969*a*) for male and female *G. m. morsitans*. At 30°C *G. palpalis* pupal durations were 22 days, with insufficient numbers emerging to establish a difference between males and females: the corresponding durations for *G. m. morsitans* were 21.8 and 20.1 days, respectively. This evidence suggests only small variations between species in temperature-specific pupal duration. For *G. palpalis* pupae incubated at 20°C, however, Mellanby (1936) reports pupal durations of 64 days for all flies that emerged, markedly longer than the 47.9 and 46.3 days reported here for *G. m. morsitans* males and females incubated at the same temperature. Harley (1968), likewise, found pupal durations that were up to about 10% longer in *G. m. morsitans* than Phelps' estimates. Moreover, the pupal durations were up to 14, 17 and 33% longer in *G. pallidipes*, *G. fuscipes* and *G. brevipalpis*, respectively, with the percentage difference increasing as incubation temperatures decreased. None of the above studies provided any information on the numbers of male and female flies emerging, or any estimates of the variance of the mean pupal durations. There is even less information about the effects of temperature on fat consumption and mortality in pupae. Further basic work is thus still needed to establish a sound platform for the modelling of the population dynamics of tsetse species other than *G. m. morsitans*.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485319000233.

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Author contributions

Both authors contributed to the data analysis and manuscript preparation, and read and approved the final version.

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Conflict of interest

The authors declare that they have no competing interests.

Ethical standards

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data used in this paper are available in the papers referenced.

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