EFFECT OF TEMPERATURE ON THE OVARIAN DEVELOPMENT IN THE PUPA OF *GLOSSINA PALLIDIPES* AUSTEN I: ESTIMATION OF THE ENVIRONMENTAL TEMPERATURE EXPOSURE FROM THE SIZE OF THE FIRST EGG FOLLICLE

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Abstract—Newly-deposited pupae of *Glossina pallidipes* were incubated until emergence at constant temperature (20.5, 22.5, 25.0, 27.5, and 29.5°C) and compared with pupae held at ambient conditions (19 to 31°C). Egg follicles and enclosed oocytes of newly-emerged females were dissected and their lengths measured together with the lengths of the cutting blade of the hatchet cell of the right and left wings. Linear discriminant functions analysis showed that the mean length of egg follicle A accounted for 80% of the temperature-related variability. We therefore used a simple predictive equation for temperature experience based on mean follicle length in small batches of newly-emerged females. The equation predicted with reasonable accuracy the temperature experience of 19 monthly samples of newly-emerged *G. pallidipes* reared in an insectary under ambient conditions. Mean sizes of egg follicle A of the newly-emerged tsetse may therefore be of utility in estimating temperature experience of pupae.

Key Words: Glossina pallidipes, pupae, egg follicles, wings, temperature

Résumé—Des pupes nouvellement pondues de *Glossina pallidipes* ont été incubées jusqu'à l'émergence à des températures constantes (20,5, 22,5, 25,0, 27,5 et 29,5°C) puis comparées aux pupes exposées aux conditions ambiantes (19 à 31°C). Les follicules d'oeufs et les oocytes y inclus des femelles nouvellement émergées ont été disséqués et leurs longueurs mesurées en relation avec celles du côté tranchant de la cellule en forme de hâche des ailes droite et gauche. L'analyse du composant majeur a montré que la longuer moyenne du follicule d'oeuf A était responsable pour 80% de la variabilité induite par la température. Nous avons par conséquent utilisé une simple équation prédictive pour la température d'exposition basée sur la longueur moyenne du follicule sur un petit groupe de femelles nouvellement écloses. L'équation a prédit avec relativement d'exactitude la température d'exposition de 19 échantillons mensuels de *G. pallidipes* nouvellement émergées élevées dans un Insectarium aux conditions ambiantes. La taille moyenne du follicule A du tsé tsé nouvellement éclos peut par conséquent être utile pour l'estimation de la température d'exposition des pupes.

Mots Clés: Glossina pallidipes, pupe, follicules d'oeufs, ailes, température

INTRODUCTION

Little precise information is available on the optimal temperature for reproduction of tsetse (*Glossina* spp.) under natural conditions, although Bursell (1960) considered the absolute temperature limits for *G. morsitans morsitans* to lie between 16 and 32°C. Mellanby (1937) found that *G. fuscipes fuscipes* is sterilised when reared at 30°C and Nash (1955) observed that *G. palpalis gambiensis* does not

reproduce well below 22°C. Glasgow (1970) concluded that the optimum reproduction rate in Glossina occurs at about 26°C, with some variation among species.

Previous studies on the effects of temperature on tsetse were concerned with topics such as: rates of development of flies (EATRO, 1955; Phelps and Burrows, 1969 a, b; Harley, 1968); lethal limits (Glasgow, 1970); metabolic rates (Bursell, 1960; Rajagopal and Bursell, 1966); laboratory colonisation (Leegwater van Der Linden, 1984); and susceptibility to trypanosome infection (Taylor, 1932; Buxton, 1955; Harley, 1966). Effects of temperature on ovarian development have received little attention. Saunders (1961) observed variation in egg follicle size in G. morsitans emerging from wild pupae collected in different seasons. He suggested that the size difference arose from seasonal temperature variations, but he never verified these observations in the laboratory, though he reported a link between the size of a female and the size of the first egg follicle. Van der Vloedt (1972) noted that G. palpalis maintained at 22–24°C had the best reproductive performance, and the largest egg follicles.

This study is an empirical endeavour to substantiate earlier observations on the relationship between egg-follicle size and seasonal temperature (Saunders, 1961). The objective was to develop a simple model for estimation of mean microclimate temperature at pupal sites and in bush or laboratory colonies reared at ambient conditions for sterile male release or other experimental purposes.

It is suggested that only a rough idea of actual environmental temperature exposure of tsetse can be obtained from Stevenson screens and indeed automatic weather stations as these equipment are often placed (for convenience) some distance away from the tsetse-infested biotopes. In colonies reared at ambient conditions, on the other hand, temperature records are usually obtained from minimum and maximum thermometers at a few fixed times of the day, which may not reflect the actual heat experience of the pupae or the adults.

MATERIALS AND METHODS

Pupae

Monthly batches of newly-deposited pupae of G. pallidipes (1–3 h old) were obtained from a colony reared at ambient conditions (19 to 31°C) in a thatched hut insectary (Ochieng et al., 1987) at the Mbita Point Field Station (MPFS), International Centre of Insect Physiology and Ecology (ICIPE), Kenya. Pupal weights ranged from 18 to 50 mg, but only those above 24 mg were used for the treatments. Batches of

50 pupae were held in clean plastic petri dishes and kept until emergence in the colony or dark incubators at 20.5 ± 1, 22.5 ±1, 25.0 + 0.5, 27.5 ± 1 or 29.5 + 0.5°C. Each controlled-temperature treatment was replicated four to six times (6–22 females per replicate) from February to December 1990. Relative humidity (R.H.) was not controlled inside the incubators; however, due to the proximity of MPFS to Lake Victoria, ambient humidity never fell below 60%. Relative humidity and temperature in the insectary were measured daily at 0800 and 1700 h using dry and wet bulb thermometers and minimum and maximum thermometers, respectively.

Measurements of follicle, oocyte and wing lengths

Ovaries were dissected within 1–3 h of emergence and egg follicles separated in 0.9% saline. Flies which had emerged during the night were not dissected. The length (in µm) of each follicle and its enclosed oocyte were measured using a pre-calibrated eyepiece (x250). The measurement was standardised using the points of attachment to the germarium and the follicular tube. The oocyte length was taken as the distance between the midpoint of the oocyte/nurse cells interface and the posterior follicular cells. Egg follicles were named according to Saunders (1961): ovarioles in the right ovary were designated as A (internal) and B (external) and those in the left as C (internal) and D (external). Only flies with normal sequential ovarian development were included in the data. For each fly, the dorsal aspect of the length of the cutting blade of the hatchet-shaped cell of either wing was measured as an index of body size (Jackson, 1946).

Statistical analysis

Data were analysed using one- or two-way analysis of variance (ANOVA) and linear regression analysis (Basica, Epistat System). Before performing linear regression analyses, classification of replicates mean sizes to each temperature was carried out using linear discriminant function analysis (LDFA) (SAS System).

RESULTS

Relationship of temperature to follicle, oocyte and wing vein lengths

Only egg follicles A and C had completely descended from their respective germaria in the newly-emerged females. Mean lengths of follicles A and C, their oocytes and wing veins at each temperature are shown in Table 1. Mean lengths of follicle A and oocyte A diminished with rising temperature

Table 1. Mean lengths (±S.E.) of egg follicles, their oocytes and wing veins of newly-emerged *G. pallidipes* kept as pupae at five constant temperatures

Temp. (°C)	Mean* length ± S.E. (μm)					
	Ovariole A		Ovariole C		Wing vein	
	Follicle	Oocyte	Follicle	Oocyte	Right	Left
20.5	507.3 ± 10.1 a	136.7 ± 9.7 c	228.0 ± 6.8	51.7 ± 0.9	1934.1 ± 30.9	1939.1 ± 33.4
22.5	525.5 ± 18.7 a	160.7 ± 16.3 c	236.0 ± 10.5	53.3 ± 3.6	1935.6 ± 25.6	1934.3 ± 28.0
25.0	486.5 ± 6.5 a	150.7 ± 13.5 c	216.1 ± 3.0	52.3 ± 6.3	1912.2 ± 12.2	1911.2 ± 12.2
27.5	448.0 ± 14.2 b	116.2 ± 4.3 d	216.7 ± 3.1	47.0 ± 1.7	1925.9 ± 11.2	1927.0 ± 9.7
29.5	387.3 ± 12.5 b	105.1 ± 12.5 d	229.4 ± 22.4	46.2 ± 1.2	1938.7 ± 11.3	1930.5 ± 13.6

*Means are based on 4–6 replicates with 6–22 females per replicate. Different letters denote significantly different subsets at $P < 0.05$.

(ANOVA's F = 18.6, d.f. = 20, $P<0.001$ and F = 5.3, d.f. = 20, $P<0.004$, respectively). There were no significant univariate trends in the lengths of follicle C, oocyte C and wing veins.

To see if multivariate trends could be used as predictors of temperature experience, we performed LDFA on the data of five temperature groups using all six measurements. Classification functions derived from this analysis classified 80% of the females into their respective temperature groupings. As most of the discriminating power was related to the length of follicle A, we proceeded to examine a simpler method for estimating temperature experience based on this variable alone.

Estimation of temperature experience of pupae

Although there was considerable variation in the relationship between the length of follicle A and temperature experience for individual flies, means of each replicate (N = 6 to 22 females) were highly significantly correlated with temperature ($r = -0.82$, $P<0.01$). This simple negative relationship suggested that it should be possible to estimate the temperature experience of newly-emerged females based on the mean length of follicle A using the following equation, where *y* is the mean temperature (in °C) and *x* is the mean length of follicle A (in μm):

$$y = 46.92 - 0.05x \qquad (1)$$

We tested the latter equation using laboratory data for monthly samples of newly-emerged *G. pallidipes* from the insectary (19 to 31°C). The flies were dissected monthly from April 1990 to October 1991. Follicle A was measured in small groups of flies (15–30) and monthly mean lengths (Fig. 1) were used to estimate the mean temperature for the month in question. Estimated temperatures are compared in Fig. 2 with the actual absolute maxima and minima
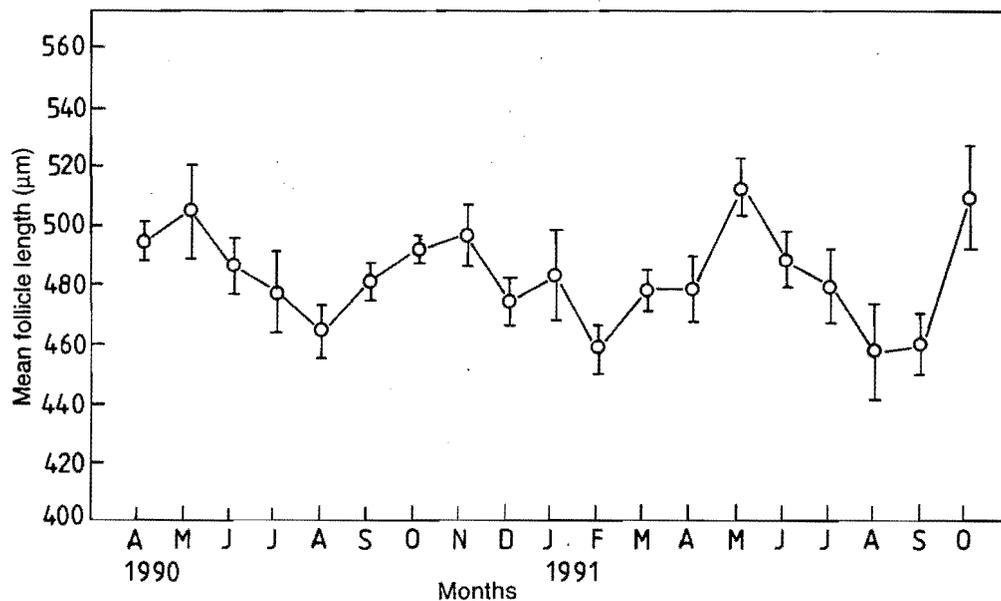

Fig. 1. Monthly mean follicle A length (± S.E.) of newly-emerged *G. pallidipes* reared at ambient conditions in MPFS insectary
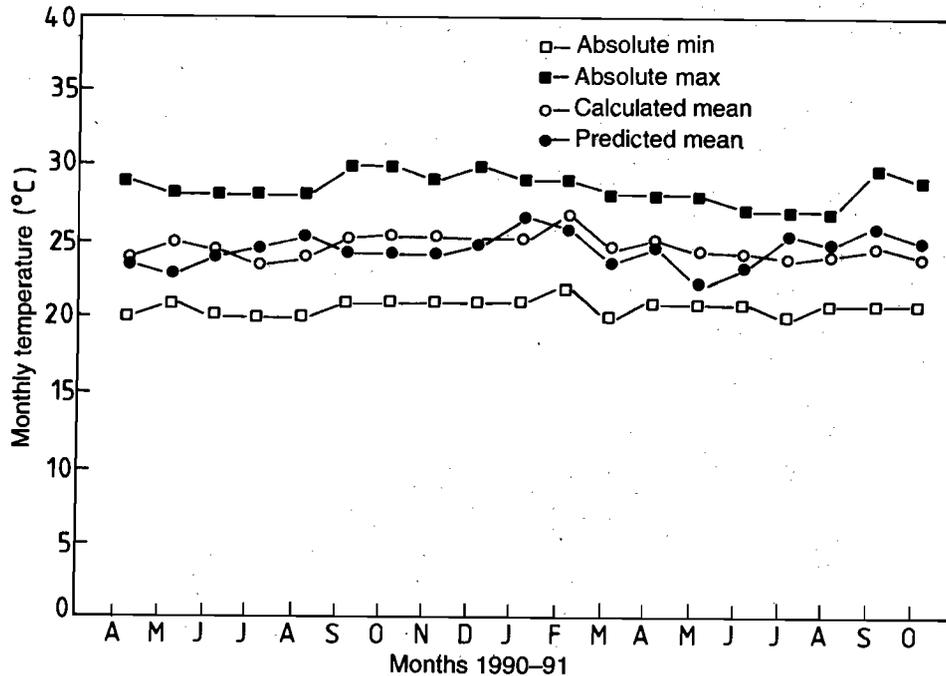
Fig. 2. Monthly absolute minimum, maximum, actual mean and estimated mean temperatures (°C) experienced by the pupae of *G. pallidipes* in MPFS insectary

and mean temperatures. Overall, there was an excellent agreement.

DISCUSSION

Our results show that there is a general negative relationship between the mean size of the first egg follicle of newly-emerged *G. pallidipes* and to a lesser extent, the size of its oocyte, and the temperature at which the pupae had been incubated in the range 19.5–30.0°C. This result confirms earlier unverified observations (Saunders, 1961) on seasonal variation in the size of the first follicle of *G. morsitans* with temperature.

The lack of a similar relationship for follicle C or oocyte C could be due to the alternating pattern of egg development in *Glossina*. At 25°C, follicle A shows active vitellogenic growth on day 21 of puparium formation (Saunders, 1961; Tobe and Langley, 1978). Follicle C, on the other hand, assumes this vitellogenic growth phase only sometime after emergence. Since follicle C is not actively growing in the pupa, its size at emergence is presumed to be constant and independent of temperature.

In many studies the length of the middle part of their fourth longitudinal vein of the wing is used as an index of size in *Glossina* (Jackson, 1946; Dransfield et al., 1989; Wall, 1990). The mean wing vein lengths reported here (Table 1) were much higher than those reported for *G. pallidipes* in Tanzania (Jackson, 1953), Uganda (Glasgow, 1963) and Kenya

(Dransfield et al., 1989). There is no immediate explanation for this discrepancy. However, we suspect that larger adults with large wings may have evolved in the particularly benign environmental conditions of the Lambwe Valley of Kenya from which the pupae in our laboratory colony had originated. In the Lambwe valley, *G. pallidipes* thrive in an exceptionally ideal environment (Turner and Brightwell, 1986). The climate is equable with no marked seasonal changes: mean monthly temperatures and saturation deficits rarely fluctuate by more than 2–3°C and 4 mm Hg from the annual means of 23°C and 11 mm Hg, and are thus close to the predicted optima for savanna tsetse (Rogers, 1979). Moreover, Ruma National Park in the area provides a stable habitat of uniform evergreen thickets harbouring abundant protected wildlife which guarantees a constant food supply for tsetse in all seasons.

Furthermore, Dransfield et al. (1989) found a significant association between wing vein size of *G. pallidipes* and minimum relative humidity in the preceding 2 months at Nguruman, Kenya. In our experiments, mean monthly relative humidity never fell below 60%, which is more than twice the critical 25% threshold required for *G. pallidipes* (Dransfield et al., 1989) and hence the larger flies in the present work.

The regression equation (1) derived from the experimental data on the relationship between temperature and the size of the first egg follicle

proved reasonably successful in estimating the thermal experience of *G. pallidipes* emerging from the MPFS insectary over 19 consecutive months (Fig. 2). Thus within the range of the temperatures encountered, this simple equation has proved highly reliable. It is, therefore, suggested that the technique be investigated for its reliability in estimating the temperature experience of pupae of other species of tsetse under diverse conditions. If validated, the equation would thus provide a simple method for estimating the mean temperatures experienced by pupae under laboratory and field conditions.

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