

## EFFECT OF TEMPERATURE ON THE OVARIAN DEVELOPMENT IN THE PUPA OF *GLOSSINA PALLIDIPES* AUSTEN II: INFLUENCE ON THE REVERSED SEQUENCE OF EGG FOLLICLE MATURATION

MOHAMED M. MOHAMED-AHMED, LEONARD H. OTIENO and JOSEPH MUCHIRI  
International Centre of Insect Physiology and Ecology  
P.O. Box 30772, Nairobi, Kenya

(Received 22 March 1994; accepted 31 August 1994)

**Abstract**—The egg follicle C of the left ovary was developing first, indicating reversed ovarian sequences in 5.8, 4.8, 4.2, 2.2, 1.1 and 2.0% newly-emerged female *G. pallidipes* from pupae kept at  $29.5 \pm 0.5$ ,  $27.5 \pm 1.0$ ,  $25.0 \pm 0.5$ ,  $22.5 \pm 0.5$ ,  $20.5 \pm 1.0^\circ\text{C}$  and then maintained at ambient temperature (19 to  $31^\circ\text{C}$ ) in the local colony. There was a highly significant positive correlation between the frequency of reversed ovarian sequences in flies and temperature in the range 19.5– $30.0^\circ\text{C}$ . The incidence of the abnormality was very low (0.2%) in wild-caught females from the Lambwe Valley, probably reflecting the constancy of favourable temperatures in that location. Old females with altered ovarian sequence had apparently both normal pregnancy and alternate maturation of eggs between the ovaries. This appears to be the first report suggestive of a temperature-induced reversed sequential ovarian development in *Glossina*.

**Key Words:** *Glossina pallidipes*, pupa, reversed sequential ovarian development, temperature

**Résumé**—Le follicule C de l'ovaire gauche de la mouche tsé-tsé *Glossina pallidipes* est le premier à se développer, indiquant ainsi un développement séquentiel anormal dans 5,8, 4,8, 4,2, 2,2, 1,1 et 2,0% de femelles émergeant des pupes maintenues à  $29,5 \pm 0,5$ ,  $27,5 \pm 1,0$ ,  $22,5 \pm 0,5$ ,  $20,5 \pm 1,0^\circ\text{C}$  et puis à la température ambiante (19 à  $31^\circ\text{C}$  à l'insectarium). Une corrélation significativement positive entre la fréquence de développement ovarien anormal chez les insectes et les températures de 19,5 à  $30,0^\circ\text{C}$  a été observée. La fréquence d'anomalies chez les femelles capturées à Lambwe Valley était très basse (0,2%), reflétant sans doute la stabilité des températures favorables dans cette localité. Les femelles âgées ayant un développement ovarien séquentiel anormal ont à la fois une gestation normale et une maturation alternée entre les deux ovaires. Il est ainsi démontré pour la première fois que la température induit un développement ovarien séquentiel anormal chez *Glossina*.

**Mots Clés:** *Glossina pallidipes*, pupa, développement ovarien anormal, température

### INTRODUCTION

The polytrophic ovaries of tsetse (*Glossina* spp.) contain only four ovarioles, two in each of the right and left ovaries (Saunders, 1960a). Only one egg follicle descends from the germarium of an ovariole at a time and normally there are never two ovarioles at the same stage of development (Saunders, 1960b). Development always commences in ovariole A of the right ovary and then in a regular alternate pattern

between the right and the left ovaries throughout the female reproductive life. Such cycle culminates in the maturation and ovulation of a single egg at regular intervals known as the interlarval periods. By noting the relative position of the largest egg follicle and the follicular relic left by the preceding ovulation, it has been possible to age females with considerable accuracy up to 80 days (Challier, 1965). This paper describes, for the first time, a temperature-induced irregular pattern of ovarian development in newly-

emerged adults of *G. pallidipes* obtained from pupae kept at constant temperatures in the range 19.5–30.0°C. Although cases of irregular ovarian sequence have been reported in wild *G. pallidipes* (Turner and Snow, 1984; Van Sickle and Phelps, 1988) the causes of such an anomaly are not known.

## 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 (19–31°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 in dark incubators at 20.5 ± 1.0°C, 22.5 ± 1.0°C, 25.0 ± 0.5°C, 27.5 ± 1.0°C or 29.5 ± 0.5°C. Each treatment was replicated two 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.

### Dissections and measurements of egg follicle

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 (x 250). The measurement was standardised using the points of attachment to the germarium and

the follicular tube. 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).

## RESULTS

The egg follicle A in the right ovary developed first in the majority of newly-emerged females kept at any one temperature in the range 19.5–30.0°C. However, in some females, this normal pattern was reversed and follicle C in the left ovary was the most developed at emergence followed by follicles A, D and B, respectively (Table 1). Such a reversed ovarian development pattern increased with rising temperature from 19.5 to 30.0°C. There was thus, a highly significant positive correlation between the frequency of reversed sequential ovarian development in flies and the temperature at which the pupae had been incubated in the range 19.5–30.0°C (Fig. 1,  $R = 0.91$ ). Moreover, follicles A and C were observed to have nearly equal sizes in 1.1, 1.4 and 3.8% of newly-emerged females from pupae kept at 22.5, 25.0 and 29.5°C, respectively (Table 1). In the latter flies, the

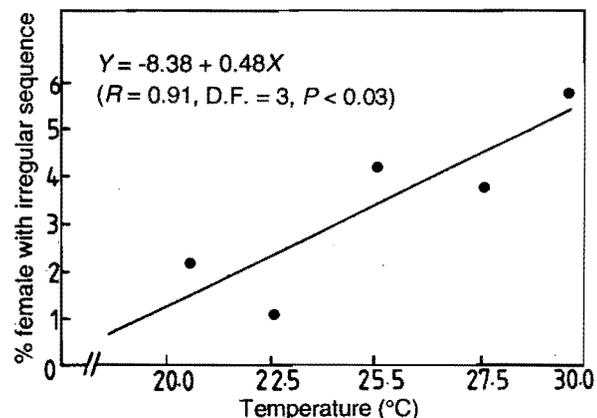


Fig. 1. Relationship between temperature and reversed ovarian sequence in newly-emerged *G. pallidipes*

Table 1. Effect of constant temperature on sequence of ovarian growth in *G. pallidipes*

Temperature (°C)	No. of batches used	No. of females	Follicle C first (%)	Follicle C approx. = follicle A (%)	Total abnormality (%)
20.5	3	45	2.2	0.0	2.2
22.5	5	95	1.1	1.1	2.2
25.0	5	71	4.2	1.4	5.6
27.5	2	41	4.8	0.0	4.8
29.5	5	104	5.8	3.8	9.6
Colony*	6	101	2.0	0.0	2.0
Lambwe Valley	12	2287	0.2	0.0	0.2

\* Colony maintained at ambient conditions 19 to 31°C.

ratio of mean lengths of the follicles, C/A, was 0.83:1 as opposed to 0.59:1 in normal flies.

In the laboratory, 15 12-day-old female *G. pallidipes* from the local colony (Ochieng et al., 1987) were mated with 15 12-day-old virgin males which had originated from pupae maintained at  $29.5 \pm 0.5^\circ\text{C}$ . It was suspected later that the mating was unsuccessful. Consequently, all surviving 14 females were dissected when 30 days old and none had been found inseminated. By then, however, most of these females had ovulated at least two eggs. Yet in one of the females, follicle D was the most developed while follicle B contained no relic (i.e. had not yet ovulated an egg). Since in tsetse, eggs generally mature in the order A...C...B...D (Saunders, 1960a), it appeared that in this particular fly, ovariole C had matured the first egg, followed by A, then D and B would have been last. Thus, although the normal alternate pattern of maturation of eggs between the right and the left ovaries was preserved in that fly, the normal sequence of growth of the ovarioles was altered. Similarly, four pregnant females out of 2287 obtained from the Lambwe Valley during the period from January to December 1990 showed evidence of follicle C occupying the first reproductive cycle (Table 1).

#### DISCUSSION

The present work shows, for the first time, the occurrence of reversed ovarian development in newly-emerged *Glossina pallidipes* obtained at any one of the five constant temperatures in the range  $19.5\text{--}30.0^\circ\text{C}$  and under ambient conditions in the local colony (Ochieng et al., 1987). The high, significant positive correlation between the frequency of these abnormal sequences and temperature (Fig. 1) suggests that the anomaly was promoted by exposure of pupae to high temperatures. This was in spite of the fact that the constant temperatures used were all well within  $16\text{--}32^\circ\text{C}$ , the natural reproductive range of tsetse (Bursell, 1960). However, it is well documented (Glasgow, 1970) that incubation of tsetse puparia at the higher limits of this range results in some smaller adults, paler adults, adults with abnormal wing venation and adults more susceptible to trypanosome infections; to this list, may now be added females with abnormal ovarian sequence. The observed approximation in size between follicles A and C (Table 1) probably suggests an intermediate effect of high temperature on the sequence of maturation of egg follicle in *G. pallidipes*.

On the other hand, the incidence of a reversed ovarian development appears to be absent (Saunders, 1962; Madubunyi, 1978; Ryan and Molyneux, 1982) or rare in wild *Glossina* (Turner and Snow, 1984;

Van Sickle and Phelps, 1988). In the present work, however, four females out of 2287 *G. pallidipes* from the Lambwe Valley were found with reversed ovarian sequences (Table 1). Similarly, Turner and Snow (1984) working in Kenya, found three females with an altered pattern of ovarian development in approximately 10,000 *G. pallidipes*. In Zimbabwe, Van Sickle and Phelps (1988) discovered 13 *G. pallidipes* with altered sequential follicular development out of an unspecified number of females.

The causes and advantages for *Glossina* of commencement of egg follicle development in the right or the left ovary are not yet known (Tobe and Langley, 1978). However, the four females from the Lambwe Valley with reversed sequential ovarian development (Table 1) were all inseminated and pregnant. This probably indicates that an irregular ovarian sequence has no effect on the fecundity of the affected individual, and hence, the tsetse population as a whole. Indeed, Turner and Snow (1984) excluded females with irregular ovarian patterns when estimating the natural loss in the population of *G. pallidipes* in Kenya.

The present study has demonstrated an increasing frequency of reversed sequential ovarian development in newly-emerged *G. pallidipes* as the temperature at which the pupae are incubated rises in the range  $19.5\text{--}30.0^\circ\text{C}$ . The absence or low frequency of such abnormal pattern in wild flies probably reflects, particularly in the Lambwe Valley, the favourability of the entire habitat or the avoidance of high temperatures through the excellent choice of microclimate by flies. 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  $2\text{--}3^\circ\text{C}$  and 4 mm Hg from the annual means of  $23^\circ\text{C}$  and 11 mm Hg, and are thus close to the predicted optima for savanna tsetse (Rogers, 1979). It is suggested that in the area, females showing reversed ovarian development probably had come from pupae deposited accidentally in locations where high temperatures prevailed. This, however, does not completely rule out other possible causes of the abnormality, including genetic and endogenous physiological factors such as hormonal imbalance and metamorphic aberration acting alone or after prolonged exposure of female pupae to elevated temperatures beyond a certain threshold.

*Acknowledgements*—We thank the staff of the Insect Mass Rearing Unit, MPFS, ICIPE, for the supply of tsetse pupae, and the Director General of ICIPE for the permission to publish this paper. This work was

supported by grants from the European Economic Community and the United Nations Development Programme.

#### REFERENCES

- Bursell E. (1960) The effect of temperature on the consumption of fat during pupal development in *Glossina*. *Bull. Ent. Res.* 51, 583–598.
- Challier A. (1965) Amelioration de la methode de determination de l'age physiologique des glossines. Etudes faits sur *Glossina palpalis*. *Bull. soc. exot.* 58, 250–259.
- Glasgow J. P. (1970) In Mulligan, H. W. (ed). *The African Trypanosomiases*, ed, 905 pp. George Allen and Unwin/ Ministry of Overseas Development, London.
- Madubunyi L. C. (1978) Relative frequency of reproductive abnormalities in a natural population of *Glossina morsitans morsitans* (Diptera Glossinidae) in Zambia. *Bull. Ent. Res.* 68, 437–442.
- Ochieng R. S., Otieno L. H. and Banda H. K. (1987) Performance of the tsetse fly *Glossina pallidipes* reared under simple laboratory conditions. *Entomol. Exp. Appl.* 45, 265–270.
- Rogers D. (1979) Tsetse population dynamics and distribution: A new analytical approach. *J. Anim. Ecol.* 48, 825–849.
- Ryan L. and Molyneux D. H. (1982) Reproductive statistics of a natural population of *Glossina morsitans centralis*. *Ann. Trop. Med. Parasitol.* 76, 215–218.
- Saunders D. S. (1960a) Ovaries of *Glossina morsitans*. *Nature* 185, 121–122.
- Saunders D. S. (1960b) The ovulation cycle in *Glossina morsitans* Westwood and a possible method of age determination of female tsetse by the examination of their ovaries. *Trans R. Soc. Lond.* 112, 221–238.
- Saunders D. S. (1962) Age determination for female tsetse flies and the age compositions of samples of *G. pallidipes*, *G. p. fuscipes* and *G. brevipalpis*. *Bull. Ent. Res.* 53, 221–238.
- Tobe S. S. and Langley P. A. (1978) Reproductive physiology of *Glossina*. *Annu. Rev. Ent.* 23, 283–308.
- Turner D. A. and Snow W. F. (1984) Reproductive abnormality and loss in natural population of *Glossina pallidipes* (Diptera: Glossinidae) in Kenya. *Bull. Ent. Res.* 74, 299–309.
- Turner D.A. and Brightwell R. (1986) An evaluation of a sequential aerial spraying against *Glossina pallidipes* Austen (Diptera: Glossinidae) in the Lambwe Valley of Kenya: Aspects of post-spray recovery and evidence of natural population regulation. *Bull. Ent. Res.* 76, 331–349.
- Van Sickle J. and Phelps R. J. (1988) Age distribution and reproductive status of declining and stationary populations of *Glossina pallidipes* (Diptera: Glossinidae) in Zimbabwe. *Bull. Ent. Res.* 78, 51–61.