Medical and Veterinary Entomology (1992) **6**, 127–130

Effect of puparia incubation temperature: increased infection rates of *Trypanosoma congolense* in *Glossina morsitans centralis*, *G.fuscipes fuscipes* and *G.brevipalpis*

PAUL N. NDEGWA, LUCY W. IRUNGU and SHAMSHUDEEN K. MOLOO* University of Nairobi, Zoology Department, and
*International Laboratories for Research on Animal Diseases (ILRAD), Nairobi, Kenya

Abstract. Puparia of *Glossina morsitans centralis* (Machado), *G.fuscipes fuscipes* (Newstead) and *G.brevipalpis* (Newstead) were incubated at 25 ± 1°C, 28 ± 1:25 ± 1°C, day:night or 29 ± 1°C throughout the puparial period, and maintained at 70–80% relative humidity. Puparial mortality was higher at 29 than at 25°C (optimum temperature) in all three species, particularly in *G.f.fuscipes* and *G.brevipalpis*. Adults of *G.m.centralis* from puparia incubated at 29°C, and those of this subspecies, *G.f.fuscipes* and *G.brevipalpis* from puparia incubated at 28:25°C, day:night or 25°C throughout, were infected as tenerals (27 h old) by feeding them at the same time on goats infected with *Trypanosoma congolense* (Broden) IL 1180 after the parasites were detected in the wet blood film. Infection rates on day 25 post-infected feed were higher in *G.m.centralis* from puparia incubated at 29°C and in adults of the three different tsetse species from puparia incubated at 28:25°C, day:night, than in those from puparia incubated at 25°C. However, in *G.f.fuscipes* the labral and hypopharyngeal infection rates were not significantly different from those of the tsetse produced by puparia kept at 25°C.

Key words. *G.m.centralis*, *G.f.fuscipes*, *G.brevipalpis*, puparia, temperature, *T.congolense*, infection rates.

Introduction

Temperature has been considered to exert marked influence on the infection rates of Salivarian trypanosomes in tsetse (Ford & Leggate, 1961), and may have important epidemiological implications. The relationship between trypanosome infection in tsetse and temperature under field conditions was first reported by Kinghorn *et al.* (1913) who found that in northern Rhodesia (now Zambia) *G.morsitans* was readily infected in the hotter of the two areas; a relationship also reported for other tsetse belts (Buxton, 1955; Ford & Leggate, 1961). Burtt (1946) and Fairbairn & Culwick (1950) showed that the percentage of *G.morsitans* infected with *T.rhodesiense* was influenced by the incubation temperature of puparia in the case of male flies, and by both puparial- and fly-maintenance temperatures in the case of female flies. Fairbairn & Watson (1955) found that *T.vivax* infection rate in *G.palpalis* increased with increasing incubation temperature of puparia but decreased with increasing fly-maintenance temperature.

The present paper investigates the effect of incubation temperature of puparia of *G.m.centralis*, *G.f.fuscipes* and *G.brevipalpis* on *T.congolense* infection rates in the emergent flies, with observations on emergence rates.

Correspondence: Dr P. N. Ndegwa, Zoology Department, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.

Materials and Methods

Puparia. Puparia of *G.m.centralis*, *G.f.fuscipes* and *G.brevipalpis* were obtained, within 24 h of deposition as larvae, from colonies at International Laboratories for Research on Animal Diseases (ILRAD). The *G.m.centralis*

colony was started in 1969 at the former East African Trypanosomiasis Research Organisations EATRO (now UTRO), Tororo, Uganda, with adults which had emerged from puparia collected in the field in Singida, Tanzania. The parental stock of the ILRAD colony was obtained from the EATRO colony in 1979 (Moloo *et al.*, 1985). *G.f.fuscipes* originated from Central African Republic with the parental stock from France (Moloo *et al.*, 1985). The *G.brevipalpis* colony was started at ILRAD with puparia which had been obtained from wild-caught tsetse in the Kibwezi forest, Kenya (Moloo & Kutuza, 1988b).

Tsetse colonies. The three different tsetse colonies were maintained at 25 ± 1°C and fed on rabbits. The *G.m.centralis* colony was kept at 70 ± 2.0% relative humidity whilst *G.f.fuscipes* and *G.brevipalpis* colonies were kept at 80 ± 3.5% relative humidity.

Trypanosome reservoir host. Male goats were used as the reservoir hosts for the trypanosomes for infecting the experimental tsetse. The goats were infected by injecting them intramuscularly (i.m.) with *T.congolense* diluted in 3 ml of phosphate-buffered saline–glucose (PBS), pH 8.0 (Lanham & Godfrey, 1970). The infection was monitored by bleeding the goats from the ear daily except weekends.

Experimental techniques. Batches of puparia of *G.m.centralis*, *G.f.fuscipes* and *G.brevipalpis* were obtained from the respective tsetse colonies within 24 h of deposition as larvae. Each batch was divided into two equal groups; one group of each species was kept at 25 ± 1°C, the other at 29 ± 1°C, both at 70–80% relative humidity, until emergence. The mean puparial period and emergence rate of each species was determined at both incubation temperatures. Another batch of puparia of each tsetse species was similarly divided but incubated at 25 ± 1°C or 28 ± 1°C during the day and 25 ± 1°C at night. On emergence, the teneral tsetse of each species were sexed, mated and allowed to feed at the same time on the goats infected with *T.congolense* after the parasites were detected in a wet blood film. All the flies were thereafter maintained on uninfected lop-eared rabbits.

On day 25 after the infected feed, all the surviving tsetse in each group were dissected and their midguts, labra and hypopharynges were examined for trypanosomes by phase contrast microscopy to determine infection rates.

Results

Data in Table 1 show that, at 25 ± 1°C (optimum temperature), the emergence rate of the three tsetse species was very good (100%) whereas at 29 ± 1°C, puparial mortality of *G.brevipalpis* and *G.f.fuscipes* was 70.0% and 80.0%, respectively. In these two tsetse species the few flies that emerged were weak, fed very poorly and all died before day 25. Puparia of *G.m.centralis* had a comparatively lower mortality rate (6.7%) at 29 ± 1°C and the survival of the flies that emerged was good. When puparia of all the three tsetse species that failed to eclode were dissected, it was noted that death had occurred both at early or late stages in development. The puparial period of all three different tsetse was longer at 25°C than at 29°C by 11.87 and 11.32 days, 10.66 and 13.17 days and 12.85 and 12.94 days in the male and female *G.m.centralis*, *G.f.fuscipes* and *G.brevipalpis*, respectively. In addition, the puparial period (male and female) at both temperatures was always shortest in *G.m.centralis*, intermediate in *G.f.fuscipes* and longest in *G.brevipalpis*.

Table 2 shows infection rates of *T.congolense* in the three tsetse species from puparia incubated at various temperatures. Infection rates in all surviving flies of each tsetse species from the two batches incubated at 25°C and fed on different infected goats were pooled together for analysis. Adult *G.m.centralis* from puparia incubated at 29°C throughout, or 28:25°C day:night, had significantly higher *T.congolense* infection rates (midgut, labral and hypopharyngeal) ($P < 0.001$) than those from puparia kept at 25°C throughout. The hypopharyngeal infection rate was highest in tsetse from puparia incubated at 29°C (67%), intermediate in tsetse from puparia incubated at 28:25°C, day:night (58%) and lowest in tsetse from puparia kept at 25°C (34%). However, the differences between the midgut ($\chi^2 = 2.00$), labral ($\chi^2 = 1.99$) and hypopharyngeal ($\chi^2 = 2.26$) infection rates in tsetse from puparia kept at 29°C, and the alternating temperature of 28:25°C day:night were not significant ($P > 0.05$). In this subspecies the adult mortality rate by day 25, in tsetse emerged from puparia kept at 29°C, was 50.8% compared to a mere 6.7% in tsetse from puparia kept at 25°C throughout.

In *G.f.fuscipes* there was no significant difference between labral and hypopharyngeal infection rates in tsetse from puparia incubated at 28:25°C day:night and 25°C throughout but the midgut infection rate was significantly higher ($\chi^2 = 34.7$, $P < 0.001$) at the alternating incubation temperature of puparia. In *G.brevipalpis*, midgut and mature infection rates were significantly higher in tsetse from the puparia incubated at 28:25°C day:night ($\chi^2 = 24.81$, $P < 0.001$ and $\chi^2 = 4.32$, $P < 0.05$, respectively).

The hypopharyngeal infection level in *G.f.fuscipes* emerged from puparia incubated at 28:25°C day:night (4.8%), and 25°C throughout (2.1%) were lower than in either *G.brevipalpis* (16.4% and 8.6%, respectively) or *G.m.centralis* (58.2% and 33.6%, respectively). Although the hypopharyngeal infection rates were much lower in *G.brevipalpis*, there was no significant difference in the midgut infection rates between this species and *G.m.centralis* ($P > 0.05$).

Discussion

Increased susceptibility to *T.congolense* IL 1180 infection was evident in tsetse emerged from puparia incubated at higher than optimum temperature (29°C), and in those emerged from puparia kept at 28:25°C day:night. However, this phenomenon was only apparent in *G.m.centralis* and in *G.brevipalpis* whilst in *G.f.fuscipes* only the midgut infection rate appeared to be raised by incubating the puparia at 28:25°C day:night. The relatively poor vectorial capacity of *palpalis* group flies has long been known.

Table 1. The mean puparial period and survival of puparia of *Glossina morsitans centralis* (Gmc), *Glossina fuscipes fuscipes* (Gff) and *Glossina brevipalpis* (Gbre) at 25 ± 1°C and 29 ± 1°C.

Temp. (°C)	Tsetse species	No. of puparia	Sex	Mean puparial period (day ±SE)	Range	Coefficent of variation
25 ± 1	Gmc	30	M	34.95 ± 0.17	33–36	2.2
			F	32.20 ± 0.13	32–33	1.3
	Gff	30	M	37.33 ± 0.19	36–38	1.7
			F	35.17 ± 0.09	35–36	1.1
	Gbre	30	M	39.25 ± 0.17	38–40	1.8
			F	36.54 ± 0.35	33–38	3.5
29 ± 1	Gmc	30	M	23.08 ± 0.28	22–24	3.4
			F	20.88 ± 0.18	20–23	3.4
	Gff	30	M	26.67 ± 0.28	26–27	2.2
			F	22.00 ± 0.58	21–23	4.5
	Gbre	30	M	26.40 ± 0.40	26–28	3.4
			F	23.60 ± 0.25	23–24	2.3

Table 2. *Trypanosoma congolense* IL 1180 infection rates in *Glossina morsitans centralis* (Gmc), *Glossina fuscipes* (GFF) and *Glossina brevipalpis* (Gbre) from incubated puparia.

Puparia incubation temp. (°C)	Tsetse species	No. of tsetse examined	Infection rates (%)		
			Midgut	Labrum	Hypopharynx
29 ± 1	Gmc	115	84.35 (97)	69.57 (80)	66.96 (77)
28 ± 1:25 ± 1*	Gmc	182	78.02 (142)	61.54 (112)	58.24 (106)
	Gff	125	32.00 (40)	5.60 (7)	4.80 (6)
	Gbre	140	71.43 (100)	18.57 (26)	16.43 (23)
25 ± 1	Gmc	223	52.47 (117)	39.01 (87)	33.63 (75)
	Gff	146	4.80 (7)	2.06 (3)	2.06 (3)
	Gbre	163	42.95 (70)	11.04 (18)	8.59 (14)

Numbers in parentheses indicate number infected.

* Puparia were incubated at 28 ± 1:25 ± 1°C, day:night.

Duke (1933) compared infection rates in *G.morsitans* and *G.palpalis*. Harley & Wilson (1968) compared *G.morsitans*, *G.palidipes* and *G.fusicpes* as vectors of *T.congolense* and found *G.fuscipes* to be a significantly poor vector. Moloo & Kutuza (1988a) also reported that *G.f.fuscipes* is less susceptible to *T.congolense* infection compared with *G.brevipalpis*, a *fusca* group tsetse, and *G.m.centralis*, a *morsitans* group tsetse, the latter subspecies being very susceptible. It seems, therefore, that higher than optimum temperatures for incubating puparia only raises the existing innate susceptibility of the tsetse. Fairbain & Culwick (1950) suggested that this increased susceptibility could result from either a physiological change involving intestinal secretions or an anatomical change possibly affecting the peritrophic membrane, or both such that trypanosomes establish themselves more easily and complete cyclical, development.

Maudlin & Welburn (1987) proposed that susceptibility of *G.m.centralis* to trypanosome infection was related to the activity of rickettsial symbionts in the larval–pupal stages which result in a build up of D-glucosamine in the emergent tsetse. D-Glucosamine has been shown to inhibit the tsetse midgut lectins (Ibrahim *et al.*, 1974) which have a trypanocidal property. It is therefore possible that high puparial temperature influences the activity of these rickettsial symbionts.

Lower emergence rates at 29°C were associated with high proportions of non-viable flies, particularly in *G.f. fuscipes* and *G.brevipalpis*, which were either very small with unfolded wings or normal but with seemingly impaired feeding and very low survival rate. However, in the field the puparial environment of *G.f.fuscipes* and *G.brevipalpis* are relatively cooler compared to that of *G.m.centralis* for most of the year and are probably not adapted to tem-

peratures as high as 29°C. Similar observations were made earlier by Roberts & Gray (1972), on puparia collected from the field during the dry season.

Acknowledgments

This work was supported by a grant from the University of Nairobi, and International Laboratories for Research on Animal Diseases (ILRAD) where the work was carried out. This work forms a part of a M.Sc. thesis submitted by P. N. Ndegwa to the University of Nairobi.

References

Burtt, E. (1946) Incubation of tsetse: increased transmission-rate of *Trypanosoma rhodesiense* in *Glossina morsitans*. *Annals of Tropical Medicine and Parasitology*, **40**, 18–28.

Buxton, P.A. (1955) The natural history of tsetse flies: an account of the biology of the genus *Glossina* (Diptera). Memoir of the London School of Hygiene and Tropical Medicine, number 10. H. K. Lewis, London.

Duke, H.L. (1933) Studies on the factors that may influence the transmission of polymorphic trypanosomes by tsetse II. *Glossina morsitans* versus *Glossina palpalis* as a transmitter of the polymorphic group of trypanosomes. *Annals of Tropical Medicine and Hygiene*, **27**, 123–130.

Fairbairn, H. & Culwick, A.T. (1950) The transmission of polymorphic trypanosomes. *Acta Tropica*, **7**, 19–47.

Fairbairn, H. & Watson, H.J.C. (1955) The transmission of *Trypanosoma vivax* by *Glossina palpalis*. *Annals of Tropical Medicine and Parasitology*, **49**, 250–259.

Ford, J. & Leggate, B.M. (1961) The geographical and climatic distribution of trypanosome infection rates in the *G.morsitans* group of tsetse flies (*Glossina* Wied., Diptera). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **55**, 383–397.

Harley, J.M.B. & Wilson, A.J. (1968) Comparison between *Glossina moritans*, *G.pallidipes* and *G.fuscipes* as vectors of trypanosomes of the *Trypanosoma congolense* group: the proportions infected experimentally and the numbers of infected organisms extruded during feeding. *Annals of Tropical Medicine and Parasitology*, **62**, 178–187.

Ibrahim, E.A.R., Ingram, G.A. & Molyneux, D.H. (1984) Haemagglutinins and parasite agglutinins in haemolymph and gut of *Glossina*. *Tropical Medicine and Parasitology*, **35**, 151–156.

Kinghorn, P.A., York, W. & Lloyd, L.L. (1913) Final report of the Luangwa sleeping sickness commission of British South Africa Company, 1911–1912. *Annals of Tropical Medicine and Parasitology*, **7**, 183–302.

Lanham, S.M. & Godfrey, D.G. (1970) Isolation of Salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology*, **28**, 521–534.

Maudlin, I. & Welburn, S.C. (1987) Lectin mediated establishment of midgut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans*. *Tropical Medicine and Parasitology*, **38**, 167–170.

Moloo, S.K. & Kutuza, S.B. (1988a) Comparative study on the infection rates of different laboratory of *Glossina* species by *Trypanosoma congolense*. *Medical and Veterinary Entomology*, **2**, 201–202.

Moloo, S.K. & Kutuza, S.B. (1988b) Large-scale rearing of *Glossina brevipalpis* in the laboratory. *Medical and Veterinary Entomology*, **2**, 1–5.

Moloo, S.K., Kutuza, S.B., Bakakimpa, I., Kamunya, G.W., Desai, J. & Pereira, M. (1985) Colonisation of *Glossina* species and studies on some aspects of their vector role. Proceedings of the 18th meeting of the International Scientific Council for Trypanosomiasis Research and Control, Harare (Zimbabwe), OAU/STRC. Publication No. 113, pp. 314–321.

Roberts, C.J. & Gray, A.R. (1972) A comparison of *Glossina morsitans morsitans* Newst. and *G.tachinoides* Westw. collected and maintained under similar conditions as vectors of *Trypanosoma (Nannomonas) congolense*, *T.(N)simiae* and *T.(Duttonella)vivax*. *Annals of Tropical Medicine and Parasitology*, **66**, 41–58.

Accepted 11 October 1991