

# Artificial larviposition sites for field collections of the puparia of tsetse flies *Glossina pallidipes* and *G. m. morsitans* (Diptera: Glossinidae)

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## Abstract

Tsetse flies *Glossina pallidipes* Austen and *G. morsitans morsitans* Westwood deposit their larvae in warthog burrows, in August–November, at Rekomitjie Research Station, Zambezi Valley, Zimbabwe. Artificial burrows, made from 200-l steel drums, were used to sample these flies and to collect their puparia. Sand-filled plastic trays in the burrows served as a substrate for larval deposition. The sand was covered with c. 2 cm of leaf litter after it was shown that only 3% of larvae were deposited on bare sand if both substrates were available. Other burrow modifications – artificially shading the burrow entrance, increasing the relative humidity inside the burrow, or reducing the size of the burrow entrance – significantly decreased deposition rates. The use of burrows in the hot season results in a reduction in the temperature experienced by the puparium towards an assumed optimum level of 26°C. Artificial burrows maintained a mean temperature of 28.5°C during October–November 1998, c. 2.5°C cooler than ambient; earlier work has shown that natural burrows can be c. 5°C cooler than ambient at these times. This may explain why natural burrows in full sunlight were used for larviposition, whereas artificial burrows were used only when they were in deep shade, and why significantly higher proportions of *G. pallidipes* were found in natural (66%) than in artificial burrows (34%). Better-insulated artificial burrows might produce more puparia with higher proportions of *G. pallidipes*. Burrows become waterlogged during the rains and may be too cool for optimum puparial development during the rest of the year. The percentages of *G. m. morsitans* in catches of females from artificial burrows, refuges and odour-baited traps were 34, 26 and <10% respectively. Traps are biased in favour of *G. pallidipes*; artificial burrows may show a bias in favour of *G. m. morsitans* that is a function of temperature. Artificial warthog burrows provide a convenient way of studying the puparial stage in tsetse and for the first time facilitate the capture of females as they deposit their larvae.

**Keywords:** Tsetse, *Glossina pallidipes*, *Glossina morsitans*, larviposition, puparia, warthog, burrows, Zimbabwe

## Introduction

Tsetse flies (Diptera: Glossinidae) have a highly specialized form of reproduction in which a single fertilized egg at a time is retained in the uterus. When the egg hatches, the mother nourishes three instars of the larval stage by

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way of the so-called 'milk gland' (Buxton, 1955). The late third instar larva is typically deposited in soft soil into which it burrows and rapidly forms around itself a hard, waterproof case, inside which occurs all further development required to produce the adult fly. Included in this process are larval, pre-pupal, pupal, pharate adult and, briefly, adult stages. Accordingly a special term (puparium—plural puparia) has been coined to denote the case and its contents.

The adult stages of the tsetse flies have been extensively studied for more than a century. Relatively little work has been done on the puparial stages, however, partly because the commonly used method of finding puparia in the field involves methodical search of deposition sites. Given that larvae are often deposited at low densities this is a tedious, labour-intensive task.

Tsetse puparia are found in shady places on the ground, in dry river-beds, in loose soil or in sand covered with leaf litter (Phelps *et al.*, 1966) and, less frequently, under fallen logs and rocks (Phelps & Burrows, 1969). Puparial yields from various sites vary with season. In Zimbabwe, during the hot, dry season of August to November, tsetse use 'cavity sites' (Phelps *et al.*, 1966), such as rot holes in trees, rodent burrows, broken down termite mound and holes in riverbanks. Particularly favoured sites are the burrows, dug by aardvark *Orycteropus afer* Pallas and later taken over by warthog *Phacochoerus aethiopicus* Pallas. These are often referred to as 'warthog burrows' (Vale, 1971) and this terminology is followed here.

Given the use of warthog burrows as deposition sites it seemed that it might be possible to simplify the study, and collection, of tsetse puparia by constructing artificial burrows. Regular sampling of these sites should facilitate efficient puparial collection. The present paper describes the practical development of this idea.

## Materials and methods

### Study area

Experiments were carried out at sites near three seasonal rivers, the Matupa, Chiuyi, and Rukomechi, within a 7-km radius of Rekomitjie Research Station (16°10'S, 29°25'E) in the Zambezi Valley of Zimbabwe, where *Glossina m. morsitans* Westwood and *G. pallidipes* Austen are abundant.

### Construction of artificial burrows

Artificial burrows were made from 200-l steel drums cut and welded as indicated in fig. 1. The device was buried in the ground with the open-end flush with the ground. The base of the horizontal subterranean portion was covered with sand to a depth of c. 17 cm to form a flat base inside the burrow. Six plastic trays (36 × 26 cm, 5 cm deep) were placed on this base, with their long axes parallel to that of the burrow, in three rows and two columns (fig. 1). The trays were filled with sand to a depth of c. 3 cm, and leaf litter to a depth of c. 2 cm, forming a substrate for larviposition.

Puparia were collected by removing the trays, scooping off the leaf litter, and sieving the sand through wire gauze. Species were identified from the shape of the polypneustic lobes (Phelps *et al.*, 1967). Identification was checked during the first weeks of the study by keeping puparia at c. 25°C in the laboratory until the more easily identified adults emerged.

A set of four burrows was generally sited under a single tree with their blind ends touching each other and their openings facing, approximately, north, south, east and west. This arrangement is referred to as the 'standard configuration'. The most appropriate trees identified for the sites were large specimens of the evergreen Natal mahogany *Trichelia emetica* Vahl (Meliaceae) with dense canopies.

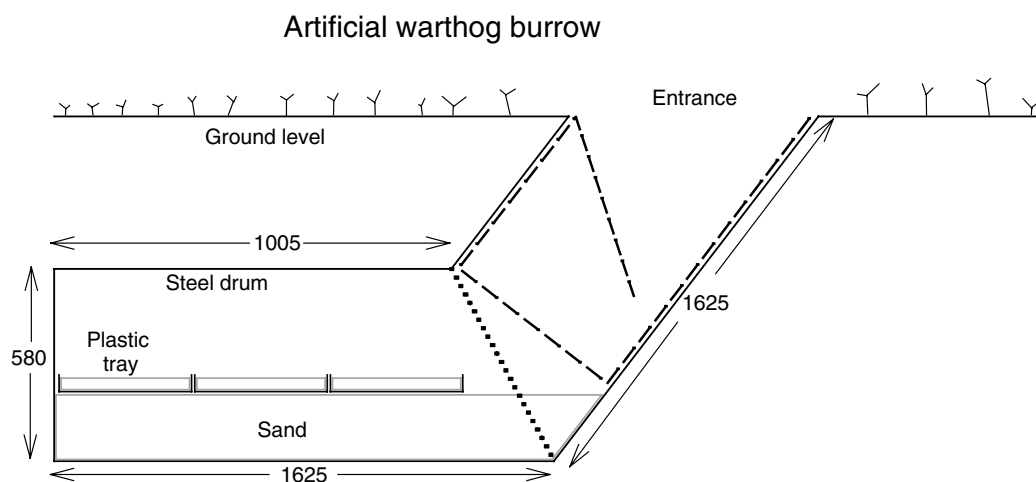


Fig. 1. Longitudinal section through an artificial warthog burrow; dimensions in millimetres. The plastic trays were filled with sand, covered with leaf litter. The dotted line indicates where the drums were cut, and then welded together. Dashed lines indicate a trap, consisting of a frame of 8-gauge wire covered with a fine netting of black fabric, which could be inserted into the mouth of the burrow in order to capture female flies as they came to deposit a larva. The flies made their way under the vertical shelf and were then captured in the body of the trap when they flew towards the light, after depositing their larva inside the cage. When the trap was not in place, flies deposited their larvae in the trays and were then free to leave.

### Natural burrows

Tsetse puparia were collected from four natural warthog burrows near the Chiuyi River. Collection involved scooping out loose soil from the burrows and sieving it as described above. At the start of the experiment, all natural burrows were cleared of puparia using this method. Sieved soil was then poured back into the burrows and spread to cover as much of the lower surface as possible. Any leaf litter scooped out with the loose soil was also replaced in the burrow. All burrows were checked for puparia at two-weekly intervals.

### Seasonal effects

Three sites, each with four artificial burrows in the standard configuration, were checked at least fortnightly from January 1996 to January 1997. When it became clear that virtually no puparia were ever found in either natural or artificial burrows between January and June, checking was stopped until July 1997. The burrows were then checked for puparia every five days in July–December 1997 and July–December 1998.

### Burrow modifications

Investigations were carried out to see whether the presence of leaf litter in trays in an artificial burrow was an important factor in the choice of a larviposition site within a given burrow. Two well-shaded sites were selected, each with four artificial burrows in the standard configuration. Of the six trays in each burrow, three were randomly selected and the surface of the sand covered with leaf litter. The trays were checked for puparia nine days later and the experiment repeated for a new randomly selected set of trays.

Previous studies have suggested that the moistening of the deposition substrate can lead to increases in larval deposition rates in tsetse (Saini *et al.*, 1996). The moisture content of the deposition substrate was increased by sprinkling 100 ml of water over the sand in each tray in the artificial burrows. As a variant in the same study, the size of the entrance could be varied from 'large' to 'small' using a metal lid, covering about two-thirds of the normal entrance.

One site, with four artificial burrows in the standard configuration, was used for this study. Two burrows were randomly selected for the addition of water to the sand. In each of these treatments, one burrow had its entrance partly covered with the lid; the other one was left open. The burrows were then checked for puparia at five-day intervals. After each interval of five days, another random allocation of the same set of treatments was carried out between the four burrows. Each treatment was replicated 16 times. The analysis was carried out on the total numbers of puparia obtained for each treatment.

When it was found that tsetse only deposited larvae in artificial burrows located under shady trees, an effort was made to improve the burrows by intensifying the shade over them. A square frame (side 90 cm) with legs of length 30 cm, all fabricated in steel, was placed over each burrow without obstructing the entrance. Covering the top of the frame with black cloth, blue cloth or thatch then provided additional shading. Each of the four burrows in the standard configuration was randomly allocated a shade of black, blue, thatch or nil. In addition, two of the burrows were partly closed with lids as previously described, in order to reduce the size of the burrow entrance. The burrows were checked

at five-day intervals between late August and early November 1996. After each interval of five days, the allocation of the different colours of shade was randomized again, while in each replicate two burrows were partly closed with lids while the other two were left open.

### Catches from odour-baited traps and artificial refuges

For comparison with other sampling methods, tsetse were caught in an epsilon trap (Hargrove & Langley, 1990; Muzari & Hargrove, 1996) baited with acetone, 1-octen-3-ol (octenol), 3-n-propyl-phenol and 4-methyl phenol released at c. 200, 0.4, 0.05 and 0.8 mg h<sup>-1</sup> respectively. The trap was used at a site within 500 m of the artificial burrows on the Matupa River, and c. 5 km away from the main concentration of artificial burrows on the Chiuyi River. On up to 20 days each month, the trap was set for the last two hours each afternoon, the period when tsetse are generally most active (Hargrove & Brady, 1992; Torr & Mangwiro, 2000). Mean daily catches were calculated for comparison with the time course and species composition of larviposition in the artificial burrows.

The species composition of tsetse entering artificial burrows was also compared with the species of adult female tsetse caught in artificial refuges (Vale, 1971). Five sites with the standard four-burrow configuration were chosen, and each burrow was fitted with a trap that allowed tsetse to enter the burrows but captured them before they reached the sand-filled trays (fig. 1). From 22–30 September 1998, total daily catches from these traps were compared with those from two artificial refuges made from thatch, drum and soil (Vale, 1971) set up in the same area and cleared daily once a day at c. 1400 h (Hargrove, 1999a).

### Meteorological measurements

Records of daily maximum and minimum temperatures and rainfall readings were made from instruments located at the research station. Measurements were also made of shade temperature and relative humidity using an automatic weather station (type WS01, Delta-T devices, Newmarket, UK) at an open site adjacent to deciduous woodland dominated by 'mopane' trees *Colophospermum mopane* (Fabaceae) c. 300 m from Rekomitjie Research Station. The logger was programmed to record hourly means. The same measurements were made at a second weather station in riverine woodland within 100 m of the refuges (Torr & Hargrove, 1999). In addition, in October and November 1998, hourly measurements of temperature and relative humidity were made in one of the refuges and one artificial burrow using a soil probe (ST1, accurate to 0.2°C) lying on the soil.

## Results

### Deposition rates in natural and artificial burrows. August–December 1993

In a preliminary experiment to judge the efficacy of the artificial burrows, four were deployed in areas where four natural warthog burrows had been identified. In this experiment, artificial burrows were deployed singly, rather than as a cluster of four in the standard configuration. Each artificial burrow was deployed at 20–100 m from the natural burrow with which it was being compared. The artificial and

natural burrows produced 179 and 215 puparia respectively; only 34% of the puparia from the former burrows were *G. pallidipes*, compared to 66% of those from the latter ( $P < 0.001$ ,  $\chi^2$ ,  $df = 1$ ).

Adding leaf litter to the deposition trays in artificial burrows was clearly effective. Of 153 *G. m. morsitans* and 8 *G. pallidipes* larvae deposited, 148 (97%) of the *G. m. morsitans* and all the *G. pallidipes* were deposited in the trays containing sand covered with leaf litter. Accordingly, leaf litter was used for all further experiments.

#### Larviposition in artificial burrows as related to season, temperature and rainfall

Deposition of larvae in artificial burrows showed a high level of seasonal variation (fig. 2a) with both *G. m. morsitans* and *G. pallidipes* puparia being found in the burrows only from August–November, prior to the main rainy season (fig. 2b). October and November were the hottest months with average maximum temperatures of 34.5–37.1°C in the three years studied (fig. 2c).

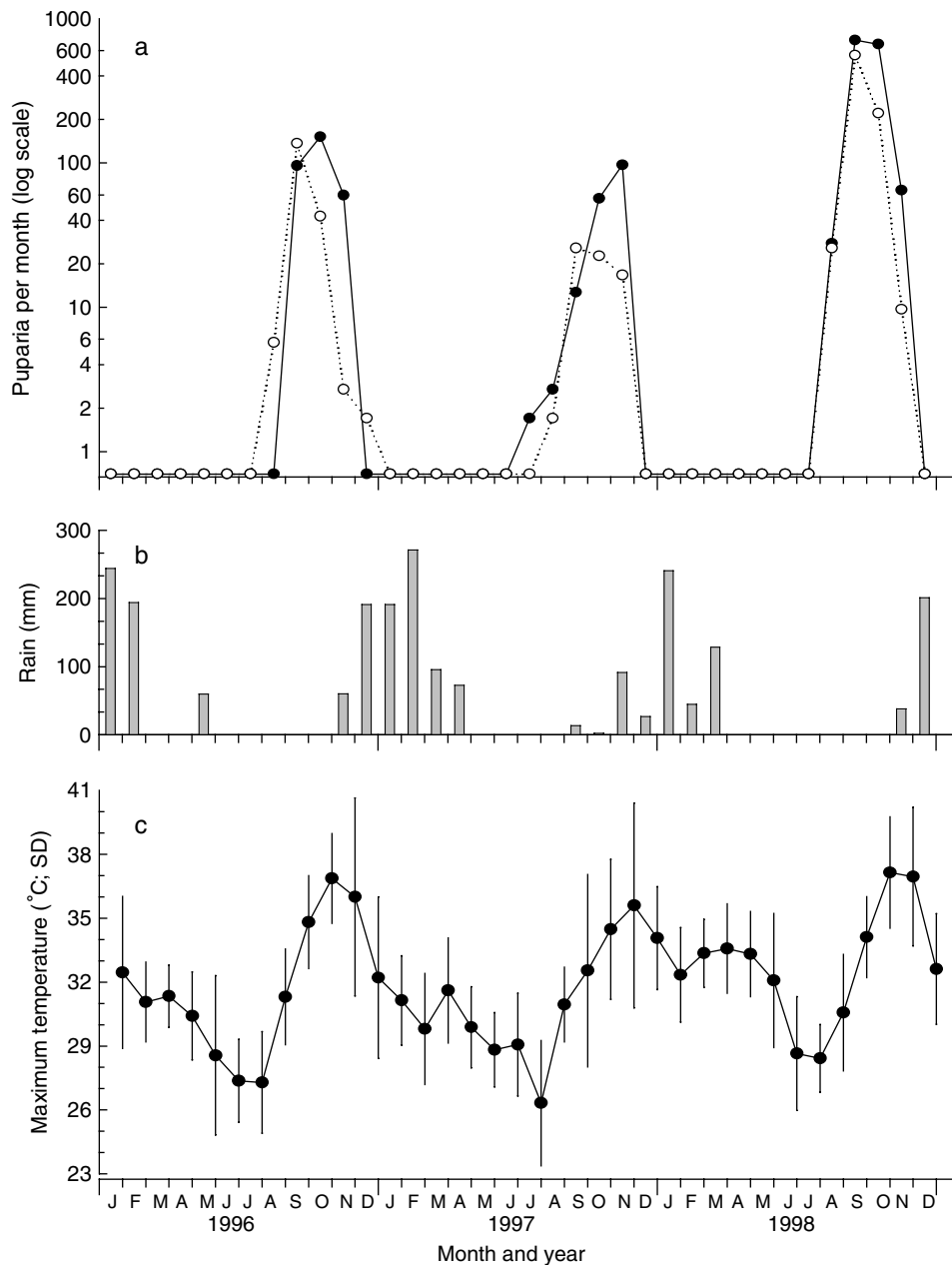


Fig. 2. Collections of *Glossina m. morsitans* (—●—) and *G. pallidipes* (· · · · ·) puparia at Rekomitjie Research Station, 1996–1998: (a) total puparia per month from 12 artificial burrows; (b) total monthly rainfall; (c) maximum daily temperature, calculated as the mean over each month.

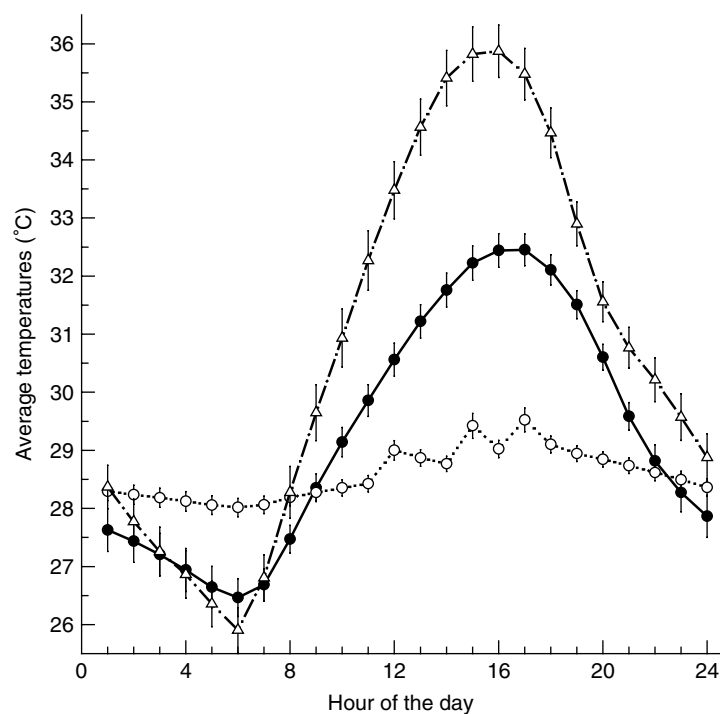


Fig. 3. Diurnal temperature variations in artificial warthog burrows ( $\cdots\circ\cdots$ ), artificial refuges ( $\text{---}\bullet\text{---}$ ) and meteorological screens ( $\text{---}\triangle\text{---}$ ) during the hot season in the study area. Mean temperatures at each hour were calculated for the period October–November 1998. Error bars indicate the standard errors of the means.

Temperatures, as measured in a single artificial burrow, were remarkably stable, varying on average by  $<0.8^{\circ}\text{C}$  over a 24 h period about a mean of  $28.6^{\circ}\text{C}$  (fig. 3). Burrow temperatures were lower than those in a meteorological screen except between midnight and 0800 h, and were  $2.25^{\circ}\text{C}$  lower on average. Temperatures in artificial refuges were intermediate between these extremes.

Jackson & Phelps (1967) measured the mean temperatures in natural warthog burrows in the Kariba area, Zimbabwe, about 100 km from Rekomitjie and found that these were up to  $5^{\circ}\text{C}$  lower than meteorological screen temperatures in October (fig. 4).

#### *Effect of the position of artificial burrows on larviposition*

In September–October 1995, a comparison was made between artificial burrows located under a shady tree and those located in the open. A set of four burrows was deployed in the standard configuration under a large leafy *T. emetica*. Another four burrows, similarly arranged, were deployed on a more open site c. 20 m away, exposed to direct sunlight for almost the whole day. All the burrows were checked and cleared of puparia nine and 18 days after the start of the trial. While the burrows in shade provided 153 *G. m. morsitans* and 8 *G. pallidipes* puparia, those in the sun produced no puparia at all. Accordingly all artificial burrows were sited thereafter in deeply shaded sites.

#### *Effects of modifications to artificial burrows*

No burrow modifications, other than the use of leaf litter, produced any increase in puparial yields – and in some cases

the modifications resulted in significant decreases. Thus, while puparial numbers were low in the experiment where water was sprinkled on the sand trays, for *G. m. morsitans*, the addition of water significantly reduced the numbers of larvae deposited in these burrows (table 1;  $P < 0.05$  for the large entrance;  $P < 0.001$  for the small,  $\chi^2$ ,  $df = 1$  in each case). For *G. pallidipes* there was no significant difference between the treatments ( $P > 0.05$ ,  $\chi^2$ ,  $df = 1$ ). Reducing the size of the burrow entrance also generally resulted in a decrease in the numbers of larvae deposited, but this difference was only significant for *G. m. morsitans* in the case where water was added to the substrate. For *G. pallidipes*, since the addition of water had no effect (see above), the results were pooled and statistical analysis showed that there was no effect of entrance size.

For *G. m. morsitans*, the addition of any extra artificial shade significantly reduced the number of larvae deposited in that burrow (table 2), for the large ( $P < 0.001$ ,  $\chi^2$ ,  $df = 3$ ) or small entrance size ( $P < 0.05$ ,  $\chi^2$ ,  $df = 3$ ). If additional shade was provided the form of this shade made no significant difference to the numbers of larvae deposited. Whether shade was added or not, reducing the size of burrow entrance significantly reduced the number of larvae deposited ( $P < 0.05$ ,  $\chi^2$ ,  $df = 1$ ) in each case. There was no significant interaction between shade and entrance size ( $P > 0.05$ ,  $\chi^2$ ,  $df = 1$ ).

For *G. pallidipes* there was no significant effect of added shade. When data were pooled across shade treatments it was seen that, as for *G. m. morsitans*, reducing the size of burrow entrance significantly reduced the number of larvae deposited ( $P < 0.05$ ,  $\chi^2$ ,  $df = 1$ ).

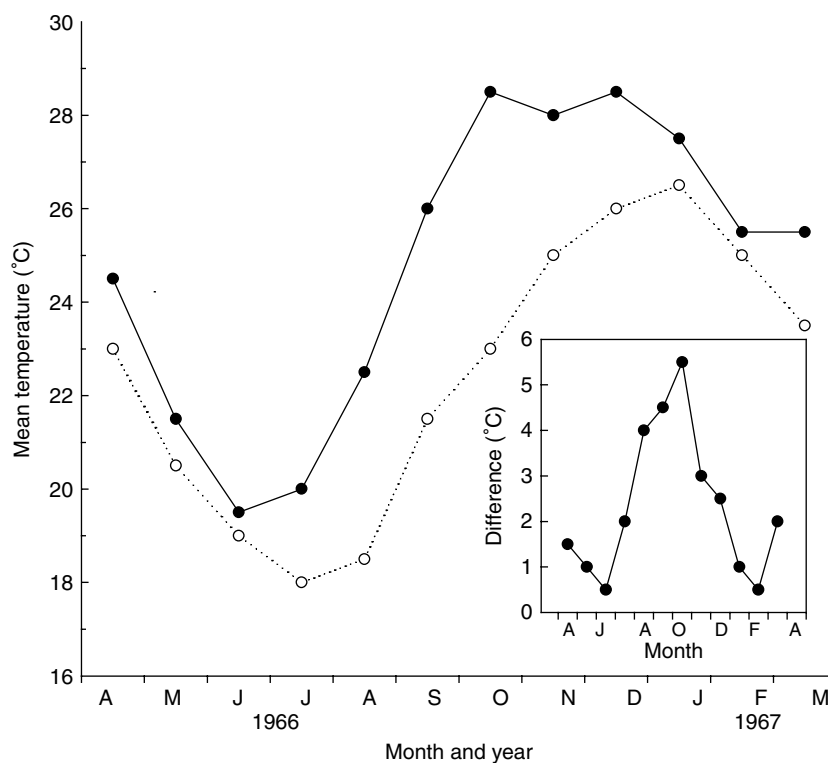


Fig. 4. Monthly mean temperatures in natural warthog burrows (· · · · ·) and in meteorological screens (—●—) near Kariba, Zimbabwe, April 1966–March 1967. Redrawn from Jackson & Phelps (1967). Inset: Difference between monthly mean screen and burrow temperatures.

Table 1. Total numbers of *Glossina m. morsitans* and *G. pallidipes* puparia collected from artificial burrows where substrate moisture content and burrow entrance size were varied.

Substrate	<i>G. m. morsitans</i> Entrance size		<i>G. pallidipes</i> Entrance size	
	Large	Small	Large	Small
Wet	9 (30.0)	1 (7.7)	6 (85.7)	1 (33.3)
Dry	21 (70.0)	12 (92.3)	1 (14.3)	2 (66.7)
Total	30	13	7	3

The percentages of the pupae deposited in wet and dry sand are shown in parentheses.

Table 2. Total *Glossina m. morsitans* and *G. pallidipes* puparia collected from artificial burrows over which different additional shade had been placed, with varying burrow entrance sizes.

Shading	<i>G. m. morsitans</i> Entrance size		<i>G. pallidipes</i> Entrance size	
	Large	Small	Large	Small
Black	9 (14.8)	4 (25.0)	6 (27.3)	2 (33.3)
Thatch	10 (16.4)	3 (18.8)	7 (31.8)	1 (16.7)
Blue	8 (13.1)	0 (0.0)	5 (22.7)	0 (0.0)
Nil	34 (55.7)	9 (56.2)	4 (18.2)	3 (50.0)
Total	61	16	22	6

The percentages of the pupae deposited in burrows with various shading are shown in parentheses.

#### Comparison of species composition of larvae deposited in artificial burrows with adult flies caught in odour-baited traps and in artificial refuges

As expected, the abundance of puparia of both species, collected from artificial burrows between September and November, increased relative to the adult females caught in the epsilon trap (fig. 5). More interestingly, the proportion of *G. m. morsitans* among puparia collected was much higher than the proportion of this species in trap catches.

Among female flies, there was a significantly smaller proportion of *G. m. morsitans* (26% of 299 flies) in refuge catches than in artificial burrows (34% of 766) ( $P < 0.05$ ,  $\chi^2$ ,  $df = 1$ ). Both proportions were higher than the proportions of *G. m. morsitans* seen in odour-baited traps in the area, in which *G. pallidipes* females generally outnumbered *G. m. morsitans* females by about an order of magnitude (fig. 5).

## Discussion

### Factors affecting the use of natural and artificial burrows

#### Temperature

At temperatures  $> c. 32^\circ\text{C}$  tsetse concentrate in dark, relatively cool microhabitats, such as rot-holes in trees and warthog burrows (Pilson & Pilson, 1967; Vale, 1971). This behaviour by adult flies results in the avoidance of



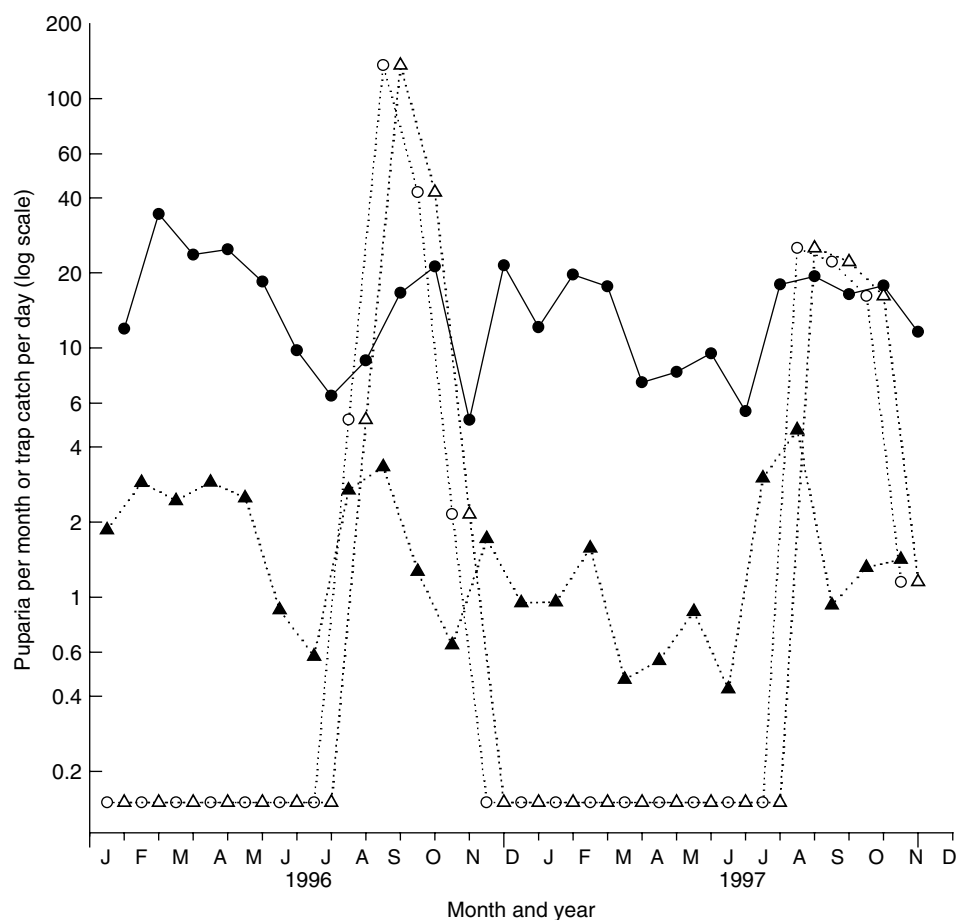


Fig. 5. Numbers of puparia collected per month from artificial warthog burrows (open symbols) at Rekomitjie Research Station in 1996 and 1997, compared with mean daily catches of adult tsetse from an odour-baited epsilon trap (solid symbols). ●○, *Glossina pallidipes*; ▲△, *G. morsitans morsitans*.

potentially fatal high temperatures. The present study shows that burrows also serve as larval deposition sites, but only for a few months each year. This behaviour, similarly, results in more favourable temperature conditions for the pupa she deposits.

Rates of puparial development, and of fat consumption by the pupa, increase monotonically with temperature (Phelps & Burrows, 1969; Phelps, 1973). The interplay between the two rates results in a quadratic relationship, between temperature and total fat consumption during development, with a well-defined minimum at *c.* 26°C (Phelps, 1973; Hargrove, 2004). In October–November temperatures in artificial burrows maintain a nearly constant value *c.* 2.5°C above this ideal. Larvae deposited in ground level sites at this time of the year would experience markedly higher, sub-optimal, mean temperatures. The use of burrows in the cool, dry season, by contrast, would not be optimal from a temperature point of view when ambient temperatures are  $\leq 26^\circ\text{C}$  and when burrow temperatures are also cooler than ambient (fig. 4).

While the use of burrows for larviposition is highly seasonal, it is not as strictly dependent on temperature as the use of artificial refuges. Thus, while puparia were found in

the burrows in August–November (fig. 2a), warm to hot months in the Zambezi Valley, no puparia were found in either natural or artificial burrows in December–April, when temperatures were often just as high as in late August and early September (fig. 2c).

#### Saturation deficit

Temperature may not be the only, or even the most important, microclimatic feature for puparia. Saturation deficit has been singled out as a more important determinant of density-independent mortality in tsetse (Rogers, 1990) and Hargrove (2001, 2004) argued that, if saturation deficit was important, it must act on stages other than the post-teneral adult, pointing out that the only times when the exoskeleton is soft, and therefore particularly prone to water loss, are between larviposition and the formation of the puparial shell, and immediately after eclosion of the teneral adult. Totally shaded areas, such as burrows lined with sand and leaf litter, provide a micro-habitat that will not only be cooler but also at a lower saturation deficit than areas at ground level under leafless trees.

### Rain and vegetation cover

Burrows can become waterlogged during the rainy seasons. Since the puparial period, even at 26°C, is about one month, and since the main rains may be expected to have started by mid-December, it will thus be adaptive for the flies to stop using the burrows by mid-November. It is not clear, however, whether the threat of flooding is the ultimate cause for the observed change, nor what proximal cue, or cues, lead the female flies to abandon burrow larviposition sites in November.

Vegetation cover provides one possible answer. From August to October the vast majority of the trees and shrubs in Rekomitjie area shed their leaves, and there is very little undergrowth. Once the first showers fall, generally in October to November, trees and shrubs begin to flush and there is more widespread cover providing shaded areas as potential larviposition sites.

### Density-dependent effects

One effect of a preferential use of warthog burrows, and similar sites, at certain times of the year is a marked, highly localized increase in puparial density. Professor R.J. Phelps (personal communication) reports finding, in 1966 near Charara, Kariba, more than 600 puparia of *G. m. morsitans*, and a similar number of *G. pallidipes*, in a single warthog burrow. If density-dependent mortality is important during the puparial phase (Rogers, 1974; Rogers & Randolph, 1990) the use of burrows may result in decreased pupal mortality due to a more favourable climatic regime, but increased mortality associated with high puparial density. Thus, as soon as alternative, widely spaced deposition sites become available with the first flush of new growth, it may be adaptive for tsetse to stop using the burrows even if climatic conditions outside are not yet optimal.

Phelps *et al.* (1966) found that the collection of puparia in the wet season (December–April) was extremely difficult and there appeared to be no marked preference for particular deposition sites by the flies. By implication, puparial density, and hence puparial density-dependent mortality, is thus at a minimum during this season.

### Temperatures in natural and artificial burrows; species preferences

The measured difference between screen and artificial burrow temperature in this study was *c.* 2.5°C. In the same months of the year Jackson & Phelps (1967) found that natural burrows were *c.* 5°C cooler than an ambient of 28.5°C. The smaller reduction observed here might simply be due to the local microclimate of the single burrow where the temperatures were measured. The design of the artificial burrows might also be a factor; these were made of steel and had rather a wider entrance than the average natural burrow. Temperature differences between the two burrow types might be responsible for the fact that, whereas artificial burrows only yielded puparia when sited under deep shade, puparia are routinely found in natural burrows when these are in full sunlight.

If the two tsetse species studied here also had different temperature preferences in selecting larviposition sites, this might explain the significant difference in species composition between puparial collections from the two types of

burrow. Leegwater van der Linden (1983) has reported that if pupae of East African *G. pallidipes* are maintained in the laboratory at 28°C the resulting adults are sterile. Whereas this result has never been confirmed for *G. pallidipes* in Zimbabwe, nor anywhere in the field, and is not known to occur in *G. m. morsitans*, it is noteworthy that this temperature is actually slightly lower than the mean temperature recorded in the artificial burrows here. If *G. pallidipes* do require lower temperatures for puparial development than *G. m. morsitans* this would be consistent with the higher proportions of *G. pallidipes* found in the natural burrows than in the relatively warmer artificial burrows. A greater sensitivity to temperature on the part of *G. pallidipes*, linked to variation in temperature between artificial burrows, might also explain the variability in species composition in different artificial burrows and in different seasons.

An encouraging possibility suggested by these results is that artificial burrows made of a non-metallic, insulating material and/or buried deeper in the soil, might be made at least as cool as natural burrows and might then provide larger samples of pupae, with a higher proportion of *G. pallidipes*.

### Species biases in larviposition sites

For burrows, both natural and artificial, the species ratio was generally heavily in favour of *G. m. morsitans*; whereas *G. pallidipes* always predominated in trap catches. In part this is due to a marked bias in catches from odour-baited traps in favour of the latter species. Estimates of tsetse populations in one area at Rekomitjie in 1973 suggested a female ratio of *G. pallidipes* to *G. m. morsitans* of <3:1 (Hargrove, 1981), close to the ratio for females caught from natural refuge sites (Vale & Phelps, 1978), whereas odour-baited electric nets recorded a ratio >10:1 (Phelps & Vale, 1978), as did the odour-baited traps in this study.

A higher proportion of *G. m. morsitans* was found in artificial burrows than in artificial refuges, though the difference was smaller than that between the former and trap catches. The possibility cannot be discounted that artificial burrow samples are biased in favour of *G. m. morsitans*, particularly since natural burrows produced significantly higher proportions of *G. pallidipes*. A definitive statement to this effect would, however, require a reliable estimate of the true species ratio in the area at the time of these studies.

The three sampling systems are, of course, sampling tsetse in quite different phases of behaviour. Flies caught in odour-baited traps, particularly females, have lower fat and haematin levels than those found in artificial refuges (Hargrove, 1999a,b) and are assumed to be flying in search of food, though males may also mate with virgin females around host animals. Flies in refuges, by contrast, appear to be responding to increased temperatures and refuges accordingly are thought to produce a more representative sample of the population, in terms of species, sex and nutritional status, than odour-baited traps (Vale & Phelps, 1978). Burrows are quite different from any sampling system previously considered since, particularly in early September before maximum temperatures exceed 32°C, they regularly produce samples that consist almost exclusively of females on the point of depositing larvae. What is not clear is whether the burrows, artificial or natural, are used with equal probability for larviposition by *G. m. morsitans* and *G. pallidipes*.



Whatever their species bias, however, it is clear that the artificial warthog burrow can be a useful tool in field-based studies of the immature stages of *G. m. morsitans* and *G. pallidipes* in savannah areas during the hot dry season. A particularly exciting feature of the study was the finding that female flies could, reliably for the first time, be captured immediately after they had deposited a larva. It was thus possible to identify the particular pupa produced by a given fly. Future papers will describe the physiological analyses of mother and offspring pairs.

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