*Bulletin of Entomological Research* (1997) **87**, 547–549



## SHORT COMMUNICATION

# Size of blood meals taken by tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) correlates with fat reserves

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The control of feeding in tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) is based on the two major variables which characterize the nutritional state of a fly, stored fat and haematin, the latter being the residue of the last blood meal. Bursell (1961a,b) stated that 'the feeding response starts at fat contents of about 24% [0.24]', and Randolph & Rogers (1981) came to the conclusion that 'both haematin and fat reserves influence fly behaviour'. Brady (1972a,b; 1973a) investigated the activity, visual responsiveness and probing responsiveness of flies with increased starvation and conjectured that '... increased sensory input from the systems monitoring the fly's state of starvation (Brady, 1973b) lowers the thresholds of a whole array of responses, particularly to host-associated stimuli.' He continued to examine the role of various physiological factors (including fat and haematin) in the mediation of the increase in activity seen as flies become 'hungrier' (Brady, 1975), as would be required for a fly to intercept the odour plume of a potential host in the field. He found, however, that the changes in weight since emergence of mature flies fed three times correlated better with the activity on the day before death than did either fat or haematin. Baylis & Nambiro (1993) showed that male *G. pallidipes* Austen feeding from an ox had significantly lower levels of absolute fat than flies simultaneously sampled approaching the ox. Loke & Randolph (1995) investigated the reciprocal effects of fat content and flight activity in male *G. palpalis* Robineau-Desvoidy held in an actograph in the laboratory. Groups of flies were fed at different intervals (two, three or four days) to produce different fat levels, and then their activity was recorded. The authors found that greater mean daily blood intake caused higher fat content in the flies and this resulted in more subsequent spontaneous flight activity.

However, as Brady (1975) points out, 'hunger' is an anthropomorphic concept and difficult to quantify. The present study examines a new aspect of this behavioural complex: the relationship between stored fat and the size of blood meals taken by individual flies. Langley (1977) suggested that the physiological cessation of feeding may be controlled by stretch receptors in the abdomen of a fly.

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*Glossina morsitans morsitans* Westwood were obtained as pupae from the Tsetse Research Laboratory, Langford, Bristol. The pupae were kept at 25°C and 70% relative humidity and the emerging adults at 25 ± 1°C in an LD 12:12 h cycle at 35% relative humidity. Flies were fed on the ears of half-lop rabbits three times before their final feeds, which were synchronized for each experiment. The flies were kept in cages of 20–25 individuals until, between the penultimate and final feeds, they were sexed and each male was placed in a separate 7.5 × 2.5 cm tube with a plastic mesh base to allow feeding. Only male flies were used since the metabolism of fat in female flies is much affected by changes involved in reproduction. Before and after the final feed, each male was weighed in its tube to an accuracy of 0.1 mg to allow the mass of the blood meal ingested to be calculated.

The field experiments were conducted on mature male *G. pallidipes* at Rekomitjie Research Station in the Zambezi valley of Zimbabwe during late October and November 1991 (hot, dry season). A bait ox was tethered in a clearing in riverine woodland from 06.00–07.45 and 15.00–18.00 h each day; attracted flies which engorged were caught with hand nets from both sides of the ox by experienced field assistants. The ox's own odour was supplemented by a sachet of 4-methyl phenol:1-octen-3-ol:3-n-propyl phenol (8:4:1) and a separate bottle of acetone. Once caught, flies were transferred to 7.5 × 2.5 cm holding tubes and then weighed immediately. If any fly had to be kept for more than 5 min, such that some primary excretion had occurred, it was released without being used.

Each fly was weighed on a field microbalance to ±0.1 mg, then immediately disabled by crushing the thorax for later determination of the haematin content of the blood meal and the state of the fat reserves. Some flies were caught while probing but before they were allowed to feed, and weighed by the same procedure. By subtraction of the mean weight of the unfed, probing flies from the mean weight of the fed flies, average blood meal sizes were calculated. All disabled flies were transferred to small, labelled, polythene tubes and desiccated over silica gel for 72 h before sealing for later analysis.

For both the laboratory and field experiments, the wings and legs of each fly were removed and the whole body was dried in a vacuum oven at 70°C for a minimum of 8 h and

weighed to ±0.005 mg. Stored lipid was extracted with three changes of chloroform over 72 h, and the flies were then dried again and reweighed to calculate the weight of the lipid removed. Finally, the abdomens were removed and crushed, and their contents spectrophotometrically analysed using the haemochromagen technique (Ford *et al.*, 1972; Rogers & Randolph, 1978a) with measurements at 417 nm, to estimate the haematin remaining in the fly. This measurement was used to correct the residual dry weights of the flies for the weight of the dry residual blood meal (see below). The field flies attracted to the bait ox were also analysed for wing fray and these data were used to aid the identification of immature flies (see below).

The fat contents of all the flies were expressed as the proportion of the dry body weight at death accounted for by the fat reserves held in the fly's body. The residual dry weights of all the flies were corrected for any residual blood meal. This was done by regressing the measurements of residual dry weight against their respective optical density measurements of haematin (Rogers & Randolph, 1978b) along with flies of lower haematin content which were analysed at the same time. The slopes of these regressions were then used to calculate the corrected residual dry weights of the flies, i.e. the real weight of the flies without the remaining blood meal or any stored fat. By adding the weight of the extracted fat back in, the corrected dry weight of the flies could be calculated, and thus their fat as a proportion of corrected dry weight.

Immature flies (those which are still building up their flight musculature) have lower corrected residual dry weights than do mature flies and would introduce confusing data if included in the analyses. In the laboratory, the flies were not used until they were known to be mature (because they had taken three blood meals), but in the field the immature flies had to be identified. Mature male flies remain within specific ranges of corrected residual dry weight once their flight musculature is fully developed and the technique developed by Rogers & Randolph (1978b) was used to differentiate between the two stages of development. The corrected residual dry weights of the flies were examined in conjunction with the fat content and state of wing fray, and this allowed separation of the immature flies, with low fat, low corrected residual dry weight and low wing fray, from mature flies with fully developed flight musculature and, therefore, higher corrected residual dry weight, variable levels of fat content and some wing fray. The lowest corrected residual dry weight of the mature *G. pallidipes* males caught in this study was *c.* 7 mg.

The mean blood meal size (±standard error) for *G. morsitans* males in the laboratory was 26.1 ± 0.9 mg (range 7.3–55.0 mg; $n = 127$). This is similar to the value published in Gaston & Randolph (1993). For *G. pallidipes* males in the field, the blood meal size was 47.3 ± 1.8 mg (range 19.5–79.4 mg; $n = 49$). Taylor (1976) estimated the mean blood meal size of male *G. pallidipes* in the same area as 53.9 mg, which is similar to that measured here. The differences between the two species are to be expected because of their respective sizes, and also because laboratory flies are known to take smaller meals than field flies, usually around 78% of the field values (Gaston & Randolph, 1993).

Figure 1 (circles) shows that in *G. morsitans* males blood meal size is correlated with the state of the fly's fat reserves. This trend of smaller blood meals with higher fat reserves is apparent if the absolute fat levels of flies are used, but the relationship becomes statistically significant only if the body size of the fly itself is controlled for. In the field data for *G. morsitans* shown in Randolph & Rogers (1978) (original unpublished data from Ford *et al.*, 1972), very few flies are present with fat reserves above 3.0 mg, and this is also the case with the laboratory flies. Laboratory flies with more than 0.38 fat as a proportion of corrected dry weight appeared reluctant to feed (unpublished observation), and the indications are that there may be some sort of feedback mechanism linked to a fly's fat reserves operating during the feeding process itself, and not just affecting the host-seeking aspects of tsetse feeding.

Loke & Randolph (1995) suggested from their laboratory work on *G. palpalis* males that 'flies do not regulate their blood intake according to their fat reserves', although they were uncertain as to whether this was a real effect or a laboratory artefact since 'fly feeding behaviour in the laboratory is notoriously variable'. This variability is evident in the blood meal sizes of the laboratory flies in the present study. Loke and Randolph were, however, measuring the absolute fat content of flies two and four days after their fourth blood meal after emergence and comparing this to the mean blood intake per day over the four meals. This could have masked effects of proportional fat content on blood meal sizes at individual feeds. They did, however, find that 'the more frequently flies were offered a meal, the smaller the mean blood meal size (14.1 ± 0.87 mg for 2-day flies, 15.7 ± 0.76 mg for 3-day flies and 22.3 ± 0.77 mg for 4-day flies)' and since the main result of increased rates of feeding is to increase the overall amount of fat laid down by flies, this would indicate that the shorter the inter-feed period, the greater the build-up of fat in a fly's body, and the smaller the blood meals ingested. Around 48 h is required for the maximum level of fat deposition from a blood meal to be reached under the conditions of their experiments (25°C in 7.5 × 2.5 cm tubes) before fat use exceeds fat deposition and levels decline (Brady, 1975). As the inter-feed periods of flies increase above 48 h, therefore, the more of their stored fat they will have used since their last blood meal, the lower

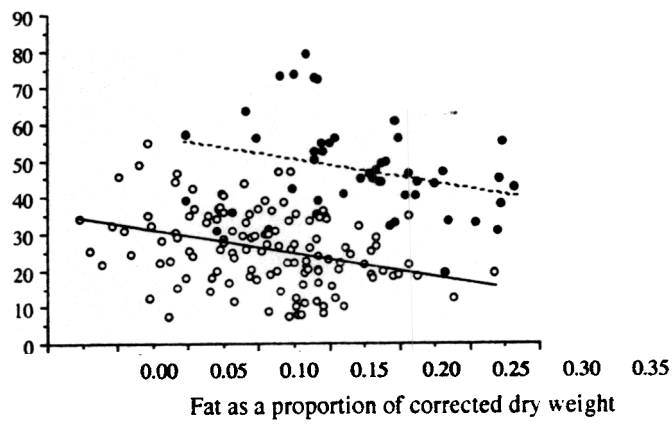


Fig. 1. The absolute blood meal size of mature male *Glossina morsitans* fed in the laboratory (circles and unbroken line) and mature male *G. pallidipes* captured after feeding on a bait ox in the field (dots and broken line) in relation to their fat content as a proportion of corrected dry weight. *G. morsitans*: $y = 35.70 - 62.54x$, $n = 127$, $r = 0.343$, $P < 0.001$; *G. pallidipes*: $y = 61.76 - 65.21x$, $n = 49$, $r = 0.296$, $P < 0.05$.

their overall fat levels, and the larger the subsequent blood meals. This can be seen in the mean blood meal sizes of the flies with increasing inter-feed periods in Loke and Randolph's experiments above, and would result in the negative correlation of stored fat reserves and blood meal sizes seen in the present study.

The *G. pallidipes* males killed immediately after feeding from the bait ox in the field showed the same relationship between blood meal size and proportional fat reserves as the flies in the laboratory (fig. 1, dots). Although it could be said that the range of proportional fat levels seen in flies feeding in the laboratory is an artefact of laboratory-feeding regimes, the data from the field indicate that flies with a great range of proportional fat levels do approach and feed on hosts in the field. It has also been shown that the presence of humans at a bait ox affects the nutritional composition of the flies approaching the ox, biasing the composition towards hungrier flies for which feeding is more important (Hargrove, 1976). This should not, however, affect the blood meal sizes of the flies which do land and feed in this situation, although fig. 1 suggests that flies under the prevailing field conditions were not dropping below a value of 0.10 fat as a proportion of corrected dry weight before successfully feeding at a host.

A possible reason for the relationship between proportional fat and blood meal size is that a larger fat body, contained predominantly in the abdomen of the fly, is physically occupying a greater proportion of the space available, and, as a result, there is less capacity available to hold ingested blood. If this was the case, however, there would probably be a closer relationship between the proportional fat content and the blood meal size. As Loke & Randolph (1995) noted, the previous blood meal should not physically affect the capacity of a fly for further feeding since after 48 h only 2.5%, and after 72 h only 0.4% remains in the gut (Langley, 1966).

In conclusion, therefore, it appears that in the wild, tsetse flies with a wide range of proportional fat levels approach and feed on hosts, and some sort of feedback mechanism linked to a fly's fat reserves affects the feeding process itself, and not just tsetse host-seeking behaviour.

## Acknowledgements

I thank Drs D.J. Rogers and S.E. Randolph for supervision of this work, and I thank them, Dr G.N. Stone, Mrs G. Petrokofsky and anonymous referees for their comments on the manuscript. I thank Drs J.W. Hargrove, F. Phelps, S.J. Torr and G.A. Vale for advice during the field work, Mr B. Mutauro and Mr M. Chinokoro for field assistance and Mr M. Chirinda for overall organization at Rekomitjie Research Station. I also thank the staff of the Tsetse Research Laboratory, University of Bristol (funded by the Overseas Development Administration) for the supply of pupae for the laboratory experiments. This work was supported by a postgraduate grant from the Science and Engineering Research Council.

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(Accepted 6 March 1997)