# Learning Affects Host Preference in Tsetse Flies

J. Bouyer [1,2] D. Cuisance [3] S. Messad [1] P.M. Guerin [4]

***Keywords***

*Glossina palpalis gambiensis* – Trypanosomosis – Feeding preference – Behavior – Animal learning – Burkina Faso.

***Summary***

Tsetse flies are very efficient cyclic vectors of African trypanosomosis. Since tsetse are generally infected by the first blood meal, as in the case of sleeping sickness for example, any propensity to feed on the same host a second time will improve transmission within this host species, whereas transmission between host species will decrease. To test this hypothesis we presented a monitor lizard and a cow in a stable to marked tsetse flies that had first fed on one of these two hosts. 80% of the teneral flies that fed did so on the cow when provided the choice. Among the flies having feeding experience, a disproportionately high number of flies that had fed on one host returned to this host for the second meal. We discuss the energetic advantages of such a learning behavior and its importance in sleeping sickness epidemiology. The findings are of relevance to the role played by such learning behavior in disease transmission by other insect vectors of zoonoses.



## ■ INTRODUCTION

Even though the tsetse fly vectors of African trypanosomosis are sometimes present at modest densities, any relaxation of sanitary measures results in recrudescence of the disease (14). There are therefore some underlying mechanisms serving to optimize transmission cycles. In West Africa, the main vectors of sleeping sickness are tsetse flies of the *palpalis* group (subgenus *Nemorhina*) that thrive in vegetation along rivers (3). These riparian subspecies have a well known host range of reptiles, ruminants and man (1, 4, 16). Local host availability contributes to significant differences in the origins of blood meals between populations of the same tsetse species (6). In hymenoptera, it is well known that foraging experience can affect host selection (8, 13). The existence of such learning mechanisms in insect vectors is being elucidated and has tremendous epidemiological implications (9), but has not been shown, as yet, for tsetse flies. Since tsetse flies are generally infected by *Trypanosoma brucei* in the first blood meal, any propensity to feed on the same host will improve transmission within this host species. Correspondingly, transmission between host species will decrease. Here we show that the first host chosen by teneral tsetse influences them to feed on the same one for the next blood meal.

1. Cirad, département d'Elevage et de médecine vétérinaire, TA30/A, campus de Baillarguet, 34398 Montpellier Cedex 5, France

2. Cirdes, BP 454, Bobo-Dioulasso, Burkina Faso

3. Conseil général vétérinaire, 25 rue de Vaugirard, 75732 Paris Cedex 15, France

4. University de Neuchâtel, Institut de zoologie, rue Emile-Argand 11, 2007 Neuchâtel, Suisse

## ■ MATERIALS AND METHODS

All the experiments took place at CIRDES, Burkina Faso. To test the effect of the first blood meal on subsequent host choice, separate groups of laboratory-reared male *Glossina palpalis gambiensis* (*palpalis* group) were at first exposed to either a caged (mesh size 2.5 x 5 cm) monitor lizard or a tethered dwarf cow in a stable (10.4 x 4.0 x 2.0 m high) where mosquito netting formed the upper half of the four walls. These groups constituted random samples from a population of more than 100,000 flies bred for more than twenty years at CIRDES using random mating. The experiment was first carried out during the dry season (mean daily temperature of 28.5°C and relative humidity of 10–25%) in February 2002 and repeated during the wet season (mean daily temperature of 25.9°C and relative humidity of 63–94%) in July 2003, in the fly-proof stable where these ambient climatic conditions prevailed. Marked one-day post emergence flies (acrylic paint on the pronotum) were released into the stable with either the cow or the monitor lizard (no-choice situation; 120 flies on the cow and 100 on the monitor lizard in 2002, and 155 each on the cow and the monitor lizard in a repeat experiment in 2003). Engorged flies were captured and released two days later (minimal inter-blood meal period in nature) with a similar number of teneral flies (n = 56 in 2002 and 105 in 2003) into the same stable but now holding both hosts (choice situation). Replete flies were caught and dissected to determine the origin of the blood in the crop where it stays for about 30 min. Blood meals were colored (in 10% Giemsa) and twice examined blind by microscope – monitor lizard erythrocytes are oval and nucleated whereas those of bovids are round and anucleated. The monitor lizard (*Varanus niloticus*) was 68 cm long and the dwarf cow (*Bos taurus*, Baoule breed) was 3 years old and weighed 165 kg.

Statistical analysis of fly preferences was made using contingency cross tables obtained from categorical variables characterizing the origin of the blood meals. Pearson's independency chi-square tests, relative risks and confidence intervals were calculated using R statistical software (7).

## ■ RESULTS

In the no-choice situations, 73 and 89% of the recaptured flies were found engorged during the dry and rainy season, respectively, on the cow. These percentages were 59 and 37%, respectively, for the flies exposed to the monitor lizard.

In the choice situations, over 80% of the teneral flies that fed did so on the cow when provided the choice between the cow and the monitor lizard (Table I). Despite this preference for the cow, the feeding preferences of previously fed flies turned out to be largely dependent on the source of their first blood meal (Table I). Disproportionately high numbers (in brackets) of the flies that had first fed on the monitor lizard returned to feed on this less favored host (48% in 2002 and 65% in 2003; Pearson $\chi^2$ test, $p < 0.001$). The probability for a fly that had first fed on the monitor lizard to choose this host for its second blood meal was 4.1 times greater (1.5–10.7, 95% confidence interval) in 2002 and 4.8 times (2.3–10.0) in 2003 in comparison to teneral flies' propensity to feed on this animal. Because of the high preference for the cow, no significant difference was found between flies that had first fed on the cow and teneral flies. However, the probability for a fly that had first fed on the cow to choose this host for its second blood meal was 1.6 time greater (1.2–2.2, 95% confidence interval) in 2002 and 2.8 times (1.7–4.8) in 2003 in comparison to flies that had first fed on the monitor lizard.

## ■ DISCUSSION

A significantly higher percentage of flies engorged on the cow in the no-choice experiments. This cannot be attributed to a genetic preference for one host or the other on the part of some flies, for if such a preference existed in the fly groups taken here at random from the same population, then the proportions feeding on the monitor lizard and the cow in these no-choice situations should add up to 100%. The data differ significantly from such complementarity. Moreover, *G. palpalis gambiensis* is considered to be opportunistic but not host specific (2, 16).

In the choice situations, teneral *G. palpalis gambiensis* showed a clear preference for the bovid over the monitor lizard, a fact that could be accounted for by the host size alone in the confines of the stable. Despite this, a disproportionately high number of flies that had fed on the monitor lizard for the first meal returned to this host for the second meal, even with the simultaneous presence of the cow in the stable. This cannot be ascribed to a density dependent factor since the total fly density bearing on any given fly on each host is the same, leaving no environmental factor but previous experience to influence the feeding preference. It has already been observed that tsetse flies (*G. palpalis gambiensis*) that had fed on goats in captivity persisted to exploit this host upon release into a forest where goats were very rare (2). The present findings are consistent with this observation and show that blood meal sourcing in tsetse flies is influenced by the first meal they take.

Within a given habitat host availability can change between successive generations of a tsetse species as well as between the habitats occupied by the same fly species. Learning permits a widening of the host range: the first choice is very opportunistic but, once a host has been found, tsetse flies become specialists, focusing on this available host species in a given habitat. Tsetse can thus learn to exploit an available, even less preferred, host rather than undertake energetically costly and potentially dangerous long-range flights in search of an unpredictable, though possibly preferred, alternative. The same phenomenon has been identified in hymenoptera (18) and in other insect vectors of disease (9). It is therefore not surprising that a species such as *G. palpalis gambiensis* can exploit a large range of habitats (from natural to man-made; 3) and show variable host preferences (6, 12).

The present findings have a bearing on host-parasite coevolution and pathogenicity of trypanosomes by reducing the probability of inter-host transmission of these parasites (5). In fact, when a trypanosome is transmitted only in cattle, highly pathogenic strains disappear and the disease takes on an endemic character. Where spatial encroachment between cattle and game favors vector confusion this can result in the transmission of highly pathogenic strains of trypanosomes that lead to epidemic situations (14).

Moreover, learning behavior in tsetse flies may play a crucial role in the epidemiology of human sleeping sickness in that animal breeding practices and human habits may inadvertently affect successful exploitation of an alternative host by tsetse simply by its proximity to a targeted species. This may afford a route for trypanosomosis transmission to man when people living in the same place as an infected domesticated or wild host use a common resource such as a water course (17). In current two-host trypanosomosis models, the probability of a tsetse fly to choose one or the other host species is assumed to be constant during its entire life (10, 11). This study shows that it is not the case. The transmission rate of parasites from one host species to another may thus be dependent on spatial encroachment between the host species and not only on innate preferences of the vector. Recently, Sané et al. wrote that "Everything leads one to conclude that the endemic and epidemic phases of sleeping sickness are more linked to vector opportunism than to its eclecticism. The number of hosts of the tsetse fly is less important than its tendency to alternate its blood-meals between animals and man" (12).

It is necessary to integrate such learning behaviors in tsetse into epidemiological models for forecasting risk. The main vectors of sleeping sickness belong to the *morsitans* and *palpalis* groups in East and West Africa, respectively. The former group is a less

**Table I**

Feeding preferences of three groups of tsetse flies *(G. palpalis gambiensis)* given a choice between a monitor lizard and a cow in the dry and wet seasons

| | Origin of the blood meal in the choice situation | Teneral flies[1] (unfed) | Origin of the first blood meal[2] (already fed flies) | |
|---|---|---|---|---|
| | | | Monitor lizard | Cow |
| Dry season | Monitor lizard | 12% (4) | 48% (21) | 17% (10) |
| | Cow | 88% (30) | 52% (23) | 83% (48) |
| Wet season | Monitor lizard | 14% (7) | 65% (17) | 2% (1) |
| | Cow | 86% (44) | 35% (9) | 98% (42) |

[1] Flies with no previous feeding experience
[2] Flies that had taken their first blood meal in a no-choice situation on either a monitor lizard or a dwarf cow; the numbers between brackets are those that fed on each host.

opportunistic feeder and shows rather innate preferences (16). As in certain hymenoptera (15), we believe that learning may be less important in the *morsitans* group, explaining why current tsetse fly feeding and disease transmission models fit well to East African epidemiological patterns. It is worth noting that whereas the two parasites, *Trypanosoma brucei rhodesiense* in East Africa and *T. brucei gambiense* in West Africa, are so closely related as to be hardly distinguishable genetically, the role of animal reservoirs is secondary in West Africa while preponderant in East Africa. This epidemiological difference between the regions cannot be explained by current models of disease transmission nor by trypanosome specificities since *T. b. gambiense* also occurs in a variety of wild and domesticated hosts. We believe that learning in *G. palpalis gambiensis*, which serves to confine the disease cycle to a given host reservoir, could provide an explanation for this epidemiological enigma.

Finally, the existence of this phenomenon in other vectors of zoonoses such as Rift Valley fever and West Nile virus (transmitted by mosquitoes) may have implications for human outbreaks and should be explored.

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## *Résumé*

**Bouyer J., Cuisance D., Messad S., Guerin P.M.** L'apprentissage modifie les préférences trophiques des glossines

Les glossines sont des vecteurs cycliques très efficaces des trypanosomoses animales. Etant généralement infectées dès le premier repas de sang, en particulier dans le cas de la maladie du sommeil, toute tendance à retourner sur la première espèce hôte rencontrée se traduirait par une augmentation de la transmission intra-spécifique du parasite au détriment de sa transmission interspécifique. Pour tester cette hypothèse, un choix entre deux hôtes (vache et varan) a été proposé en étable sous moustiquaire à des glossines marquées (*Glossina palpalis gambiensis*, Diptera : Glossinidae) préalablement nourries sur un de ces deux hôtes. Quatre-vingts pourcent des glossines ténérales choisirent d'exploiter la vache lorsque le choix leur fut proposé. Parmi les glossines nourries une première fois sur un des deux hôtes, une proportion significativement supérieure de glossines sont retournées sur le premier hôte rencontré. Les avantages énergétiques et les implications épidémiologiques de ce comportement dans la transmission du parasite de la maladie du sommeil du réservoir animal à l'homme sont discutés. Enfin, la question de l'impact de l'apprentissage dans l'épidémiologie des zoonoses à transmission vectorielle est posée.

***Mots-clés :*** *Glossina palpalis gambiensis* – Trypanosomose – Préférence alimentaire – Comportement – Apprentissage animal – Burkina Faso.

## *Resumen*

**Bouyer J., Cuisance D., Messad S., Guerin P.M.** El aprendizaje afecta las preferencias de huésped en las moscas tse-tse

Las moscas tse-tse son vectores cíclicos muy eficientes de la tripanosomosis africana. En vista de que las tse-tse generalmente se infectan con su primera alimentación de sangre, como por ejemplo en el caso de la enfermedad del sueño, cualquier propensión a alimentarse una segunda vez en el mismo huésped mejora la transmisión al interior de las especies huéspedes mientras que la transmisión entre especies huéspedes disminuye. Para probar esta hipótesis, presentamos una lagartija monitor y una vaca en un establo, para marcar las moscas tse-tse que se alimentan por primera vez en uno de estos huéspedes. 80% de las moscas tenerales que se alimentaron, lo hicieron en la vaca cuando la escogencia les fue propuesta. Entre las moscas que experimentaron la alimentación, un número desproporcionado de moscas que se alimentaron en uno de los huéspedes volvieron a este huésped para su segunda comida. Discutimos las ventajas energéticas de este comportamiento aprendido y su importancia en la epidemiología de la enfermedad del sueño. Estos hallazgos son relevantes para el papel que juegan estos comportamientos aprendidos en la transmisión de la enfermedad por otros insectos vectores de zoonosis.

***Palabras clave:*** *Glossina palpalis gambiensis* – Tripanosomosis – Preferencía alimentaria alimentaire – Comportamiento – Aprendizare animal – Burkina Faso.