

# Intraspecific variability in natural populations of *Glossina palpalis gambiensis* from West Africa, revealed by genetic and morphometric analyses

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**Abstract.** *Glossina palpalis gambiensis* Vanderplank (Diptera: Glossinidae) from West Africa (Senegal and Burkina Faso) were analysed for microsatellite DNA polymorphisms and size of the wings. In the overall sample a strong heterozygote deficiency was found at two polymorphic microsatellite loci. It led to a highly significant value of *F<sub>is</sub>* (within-sample heterozygote deficit) in the western zone of Sideradougou area in Burkina Faso. Genetic differentiation was significant on a macrogeographic scale, i.e. between tsetse coming from Senegal and Burkina Faso. Wing measures also differed between these two countries; flies from Senegal appeared to be smaller. Microsatellite loci further allowed differentiation of populations of *G. palpalis gambiensis* trapped on the same hydrographic network a few kilometres apart. The results are interpreted as indicating that further investigations will allow the study of genetic variability of tsetse flies in relation to the dynamics of transmission of human and animal trypanosomoses.

**Key words.** *Glossina palpalis gambiensis*, epidemiology, genetic differentiation, microsatellite DNA, morphometry, wings, Burkina Faso, Senegal.

## Introduction

*Glossina palpalis gambiensis* Vanderplank (1949) is a riverine tsetse species of the subgenus *Nemorhina* (*palpalis* group) which inhabits galleries or relic forests along the West African river systems (Challier, 1973; Cuisance *et al.*, 1985; Laveissière, 1986). This subspecies is involved in the transmission of trypanosomes causing both human and animal African trypanosomoses. These diseases are responsible for severe losses in sub-Saharan Africa (Touré & Mortelmans, 1996). The geographical distribution of *G. p. gambiensis* extends, in West Africa, from Senegal, east to Mali, Burkina Faso, Ghana, Togo and Benin (Challier & De Jardin, 1987). It is limited in the south by the savannah–forest transition where it is replaced by the subspecies *G. p. palpalis* (Robineau-Desvoidy).

Little is known about the genetic differentiation of these vectors in relation to geography or host specificity. Such information may be critical in our understanding of the ability of tsetse to avoid traps or treated animals for example (RTTCP,

1996), and for improving our knowledge of the ability of *G. palpalis* to transmit veterinary and/or medically relevant trypanosomes (Janssen & Wijers, 1974; Solano *et al.*, 1996; Reifenberg *et al.*, 1997). Furthermore, future work, using genetically modified tsetse, would benefit from such an appreciation of population genetic structure (Dale *et al.*, 1995). Allozyme studies have been conducted for the study of interspecific relationships among *Glossina* species (Gooding *et al.*, 1991) but these markers display little intraspecific polymorphism in *Glossina*, especially within the *palpalis* group (Gooding, 1981). Within *G. pallidipes*, a member of the *morsitans* group, genetic studies have been conducted in East Africa using allozymes and revealed population differentiation on a regional scale (Kence *et al.*, 1995; Krafur *et al.*, 1997).

Recently, microsatellite DNA markers were described, and shown to be powerful tools to measure genetic variation within and between populations (Bruford & Wayne, 1993; Jarne & Lagoda, 1996). Microsatellite loci are highly polymorphic loci, widely dispersed throughout the genome (Tautz & Renz, 1984). Alleles at microsatellite loci consist of several short tandem DNA repeats (1–6 bp). Length polymorphism which reflects variation in the number of repeats, is likely to be due to slippage during DNA replication (Schlötterer & Tautz, 1992).

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Different alleles are characterized by the length, in base pairs, of DNA fragments amplified by polymerase chain reaction (PCR) using primers derived from unique sequences flanking the microsatellite. Because of their codominance, supposed neutrality, and mendelian inheritance, microsatellites can be very useful for population genetics studies.

To study intraspecific genetic variability in *G. p. gambiensis* populations, we first isolated three microsatellite polymorphic markers from this species (Solano *et al.*, 1997). In the present paper, as part of a more global study, we describe the use of two of them (GT repeats) on natural populations collected in different areas of the geographical distribution of this tsetse taxa. We have already shown that these two loci were located on the X-chromosome and that alleles were inherited in a Mendelian fashion at these loci (Solano, 1998). The objective of this work was to investigate the potential use of these molecular markers for studies of the intraspecific genetic variability of *G. p. gambiensis* on different spatial scales. To support the genetic evidence, we also used morphometric measures on the same flies using a semiautomatic measure software based on wing measurements.

## Materials and Methods

### *Geographic location of the tsetse*

Genetic variability of *G. p. gambiensis* was studied at the scale of its geographical distribution. For this purpose, the flies originated from Dakar, Senegal coll. J. Seignot, and from south-west Burkina Faso, near Bobo-Dioulasso, in Sideradougou area. These two areas are separated by  $\approx 2000$  km.

The population originating from Dakar (sample name: SENE) lies at the extreme western and northern range of this species. In this region, tsetse inhabit the 'niayes', habitats composed mainly of dense thickets with palm trees (*Elaeis guineensis*), where a regular hygrometry is brought by the Atlantic coastal winds (Touré, 1971). Tsetse flies were captured near the city of Dakar using Nzi traps (S. Mihok, unpublished).

In the area of Sideradougou, the vegetation is typically south sudanian. Biconical (Challier & Laveissière, 1973) and monoconical (Laveissière & Grébaud, 1990) traps were set up along the Koba river in the western part (sample name: SIDW) and in the eastern part (sample name: SIDE) where *Glossina tachinoides* Westwood and *G. p. gambiensis* are present. This work was part of a multidisciplinary study, the aim of which was to identify the key factors affecting the occurrence of tsetse flies, using a geographical information system (de La Rocque & Cuisance, 1997). The western and eastern parts of the area are separated by a linear distance of about 15 km.

### *DNA extraction and microsatellite genotype scoring*

All the reactions were done at CIRDES Bobo-Dioulasso, Burkina Faso. From each fly, three legs were removed and

put in a single microfuge tube, and 300  $\mu$ l of 5% aqueous Chelex chelating resin was added (Walsh *et al.*, 1991; Dumas *et al.*, 1998) to each tube. After incubation at 56°C for 1 h, DNA was denatured at 95°C for 30 min. The tubes were then centrifuged at 12000g for 2 min and were frozen before further handling.

The PCR reactions were carried out in a thermocycler (MJ Research) in 50  $\mu$ l final volume, using 10  $\mu$ l of the supernatant of the extraction step. Specific primers had been designed to anneal with the regions flanking the microsatellite core sequences, and loci *Gpg55.3* and *Gpg19.62* (as described in Solano *et al.*, 1997) were amplified using the same conditions (annealing temperature was 50°C). After PCR amplification, the genotypes were scored on 4% agarose gels under ultraviolet illumination (Goldstein & Clark, 1995). Two sequential series of samples could be charged in each gel for migration, for economic purposes (see Fig. 2).

A total of 105 flies from Senegal and Burkina Faso were analysed for microsatellite DNA polymorphism: 23 females for Dakar, 61 for Sideradougou, Burkina Faso, and a total of 21 males. Females were mostly used because of the location of the microsatellite loci on the X-chromosome.

### *Morphometrics*

To compare the information obtained from our microsatellite sequences to morphometric characters, we used analysis of the wings of the flies, as has been done for other vector species (see for example Dujardin *et al.*, 1997, 1998). The size of the wing is known to be proportional to the size of the individual in insects (Bursell, 1960). We used a semiautomatic measure software, 'Fly Picture Measurement' (F. Borne *et al.*, unpublished) which allows the measurement of different morphometric characters on the wings of each individual. Briefly, the wing was mounted on a microscope slide under a cover slip, and observed using a binocular microscope. From the digitized image, eight points were selected using the mouse of the computer, which allows measurement of the surface of the hatchet cell (points 1–5) and the lengths and ratios of three identifiable segments on the wings (Fig. 1). A description of this package is freely available at the following address: <http://www.multimania.com/bornef/FPM/FPM.htm>

To avoid asymmetry problems, the left wing of each fly was studied (only females were used). Morphometric measures were conducted on individuals which had been subjected to genetic analysis, i.e. on 23 females from Senegal and on 49 females from Sideradougou.

### *Statistical analyses*

*Microsatellite loci.* Wright's *Fis* (within sample heterozygote deficit) and *Fst* (measure of population differentiation) were estimated with Weir & Cockerham's (1984) unbiased estimators (*f* for *Fis*,  $\theta$  for *Fst*). These estimators were computed with FSTAT v. 1.2 software (Goudet, 1995).

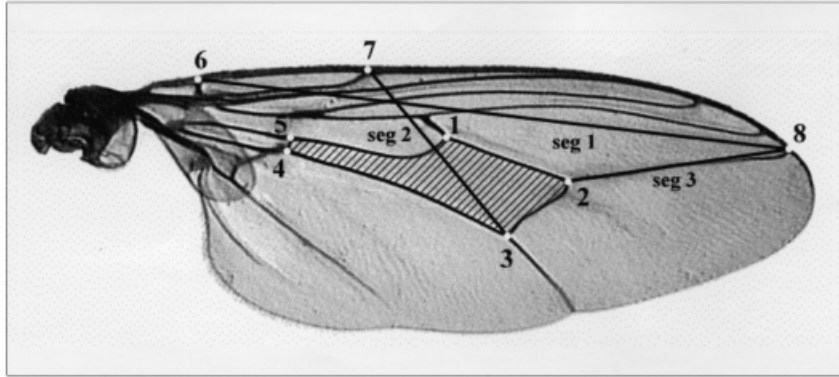


Fig. 1. Fly Picture Measurements: location of the points selected on the wing of each tsetse and presentation of the calculated parameters.

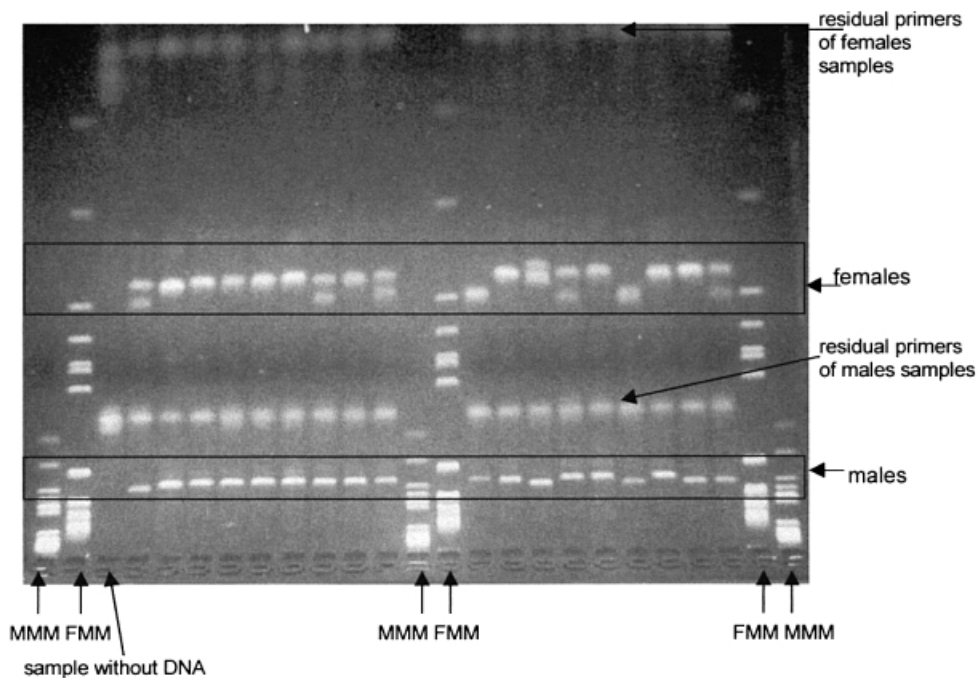


Fig. 2. Photograph of a 4% agarose gel showing migration of PCR products after amplification of microsatellite DNA at locus *Gpg19.62* on Chelex-extracted DNA from legs of *G. p. gambiensis*. Amplified DNA from females (top rectangle) was first charged on the gel with their molecular markers, then DNA from males (bottom rectangle) was charged 2 h later, in the same holes (also with their molecular marker), for economic purposes. For each migration, the molecular marker was added at the beginning, the middle, and at the end of the samples. Females showed either one or two bands, while males always showed one unique band, indicating that the locus was on the X chromosome. The total migration time for microsatellite genotype scoring was 9 h. MM, molecular weight marker ( $\phi$ X174/HaeIII); FMM, female molecular marker; MMM, male molecular marker.

For random mating (within samples) or random distribution of individuals (between samples),  $F$  statistics are expected to be null.

For each locus in each sample, and overall, the significance of  $F_{is}$  (panmixia) was tested using GENEPOP v. 3.1 (Raymond & Rousset, 1995). The probability test described by Guo & Thompson (1992) was used, using a complete enumeration method (Louis & Dempster, 1987) for loci with up to four alleles, and a Markov chain method (Guo & Thompson, 1992) for loci with more than four alleles. Measuring the deficit in heterozygotes simultaneously in several samples, the multi-

sample extension of the score test described by Rousset & Raymond (1995) was then applied to data. In this test, the alternative hypothesis is  $H1$  = heterozygote deficiency. The significance of  $F_{st}$  (population differentiation) was assessed using 10 000 permutations of genotypes among samples (FSTAT; Goudet, 1995). The alternative hypothesis here is  $F_{st} > 0$ . Linkage disequilibrium was tested by the exact test of GENEPOP for genotypic linkage disequilibrium (Raymond & Rousset, 1995).

*Wings.* Comparisons were conducted for three tsetse samples according to their geographical origin: Senegal (SENE) and

**Table 1.** Genetic results obtained from the eighty-four *G. p. gambiensis* females analysed at the two microsatellite loci. Mean *Fis* was 0.189 ( $P < 0.0001$ ). He, unbiased expected heterozygosity (Nei, 1978); Ho, observed heterozygosity; *P*, probability associated with each value of *Fis* obtained when testing for an absence of heterozygote deficit.

POP	He	Ho	Fis (P)	Number of females	Geographic location
SIDW98	0.5396	0.3625	0.331 <b><math>P &lt; 0.001</math></b>	40	Sideradougou (West), BF
SIDE98	0.3902	0.381	0.024 NS	21	Sideradougou (East), BF
SENE	0.5092	0.4931	0.033 NS	23	Dakar SENEGAL

**Table 2.** Pairwise *Fst* (population differentiation) measured between the three populations. The overall *Fst* value is 0.182 ( $P < 0.0001$ ). \**Fst* value is highly significant ( $P < 0.0001$ ).

	SIDW98	SIDE98	SENE
SIDW98	/	0.1163*	0.1848*
SIDE98		/	0.2791*
SENE			/

Sideradougou West (SIDW) and East (SIDE). First, homogeneity of the variances for each measured parameter was tested using Bartlett's test; if the test was significant (i.e. variances were heteroscedastic), a non-parametric test was used (Kruskal–Wallis one-way ANOVA). If not, an ANOVA using Student–Newman–Keuls procedure (SNK) was employed to compare the means (e.g. Sokal & Rohlf, 1981). When a parameter displayed an overall significant difference, multiple comparisons were carried out between pairs of localities.

## Results

### Genetic results at microsatellite loci

**Intrapopulation analyses.** Confirming the preliminary results observed in the insectary sample, males had only one band after migration of the PCR products, while females had either one (homozygotes) or two (heterozygotes) PCR signals (see Fig. 2). Statistically the loci did not appear to be in linkage disequilibrium, whatever the population analysed.

A total of nine and 10 alleles were observed in the whole sample analysed at loci *Gpg55.3* and *Gpg19.62*, respectively, with heterozygosities ranging from 0.3 to more than 0.5 (see Table 1). The first locus showed one predominant allele at a frequency of more than 0.7, whereas the second one showed several alleles at relatively low frequency (all  $< 0.6$ ).

Regarding the overall sample, a strong heterozygote deficiency appeared ( $Fis = 0.189$ ,  $P < 0.0001$ ). According to their geographical origins, the results were as follows (see Table 1). In the Senegal sample, no significant heterozygote deficit was found ( $P = 0.18$ ). With the flies collected in the area of Sideradougou, two situations were observed: in the eastern area, as in the Senegal sample, *Fis* measures did not deviate

**Table 3.** Morphometric values (mm) measured from the left wings of the females of *G. p. gambiensis* from Senegal and Burkina Faso (using the software 'Fly Picture Measurements') (see Fig. 1).

POP	Hatchet					
	cell	seg1	seg2	seg3	seg 1/2	seg 1/3
SENE	1.349	6.246	2.339	2.228	2.671	2.805
SIDE	1.396	6.325	2.396	2.267	2.643	2.791
SIDW	1.389	6.348	2.41	2.261	2.63	2.80
Test						
probability	$< 0.05$	NS	$< 0.001$	NS	$< 0.05$	NS

significantly from Hardy–Weinberg expectations ( $Fis = 0.024$ ;  $P = 0.38$ ). By contrast, in the western area, a strong and significant heterozygote deficiency was found ( $Fis = 0.331$ ;  $P < 0.001$ ).

**Interpopulation analyses.** Pairwise *Fst* values showed high and significant values whatever the level of structuring used. In the total sample, as well as at the between-country scale, the values remained significant (Table 2). Interestingly, the *Fst* value remained significant when measured at the scale of the hydrographic network, on a few kilometres in the area of Sideradougou.

These significant *Fst* values suggested that natural populations of *G. p. gambiensis* were genetically differentiated on a macrogeographic scale, between Senegal and Burkina Faso and also on a smaller scale, between the eastern and western part of Sideradougou.

### Morphometric results

Multiple comparisons showed that some of the measures differed significantly between Senegal and the two areas of Sideradougou. The differentiating parameters were: the surface of the hatchet cell, the length of segment 2, and the ratio segment 1/segment 2 (Table 3). The two first parameters had heteroscedastic variances, but the ratio segment 1/segment 2 displayed homoscedastic variances in the three samples. There was a general tendency for the flies from Senegal to have the lowest measures, indicating that their wings were smaller than those from Burkina Faso. In Sideradougou area, none of the parameters differed significantly between the western and the eastern part (Table 4).

**Table 4.** Morphometric results: multiple comparisons consecutive of the different ANOVAS between pairs of localities were carried out with the SNK procedure at the 0.05 level of significance. \*Significant. The sample from Senegal differs significantly from the two others for the three parameters: seg2, hatchet cell, seg 1/2.

	SIDW	SIDE	SENE
SIDW	/	NS	*
SIDE		/	*
SENE			/

## Discussion

In this work, we present preliminary data on the intraspecific genetic variability in *G. p. gambiensis*, a vector of both human and animal trypanosomes in West Africa. Two different techniques, molecular and morphometric, are used to characterize the tsetse originating from three sampling sites.

The advantage of microsatellites over other markers classically used, such as the allozymes, is their very strong polymorphism which makes it possible to have access to more information (Weber, 1990). As an example in our study the average heterozygosity is between 0.3 and 0.5, whereas Gooding (1981) obtained an average heterozygosity of 0.029 for five isoenzymatic loci out of four natural populations of *G. p. gambiensis*. This latter work did not find any population structuring within *G. p. gambiensis* in Burkina Faso. This discrepancy between allozymes and microsatellites is typical of that which has already been reported in other insects (Hughes & Queller, 1993; Lanzaro *et al.*, 1995a). Such differences can be related to mutation rates, which are hundreds or even thousands of times higher for microsatellite sequences (Weber & Wong, 1993; Dallas & *al.*, 1994), than for allozyme loci.

### Within-sample genetic analysis

The overall results of the genetic analyses showed a highly significant within-sample heterozygote deficiency (*Fis*) in populations of *G. p. gambiensis*. This overall value was mainly due to a strong *Fis* observed in the population coming from the western part of Sideradougou (SIDW). It should be pointed out that in this area of Sideradougou tsetse flies had been completely eradicated 15 years ago (Poltzar & Cuisance, 1984). Now they have reinvaded the area and are present at similar densities to those of before (de La Rocque, 1997). This western sample, in our study, was also the largest sample (40 females). This highly significant deviation from Hardy–Weinberg expectations might have arisen for a number of reasons. First, the occurrence of null alleles. Other studies have already shown the existence of null alleles, that is non-amplification of alleles at microsatellite loci due to mutations in the flanking sequences (Callen *et al.*, 1993; Paetkau & Strobeck, 1995; Dumas *et al.*, 1998). In our sample, both microsatellite loci were interpreted as located on the X-chromosome. If null alleles were the cause of the heterozygote deficits found, we would have expected the occurrence of null males. However, all of the analysed males showed one band.

Even in the females, the frequency of a null allele accounting for the observed deficiency would be so high that null homozygotes (females showing no PCR product) would have been expected (Brookfield, 1996), which was not the case. So it is unlikely that null alleles could explain the observed heterozygote deficits. Secondly, the result may have been due to endogamy (sib-mating). Systematic sib-mating which could explain these values has not been described so far in tsetse, to our knowledge. We would also have expected to find it as a general phenomenon, which was not the case in the present results. Finally, the Wahlund effect may be important. Artificially pooling separate gene pools may result in a Wahlund effect, in which significantly fewer heterozygotes are observed than expected, as in our work. This may be the consequence of the existence of sympatric cryptic species, of the arrival of new migrants from other locations, or of the trapping methods.

A more precise interpretation of this *Fis* seems critical, to better understand the biology of tsetse and the epidemiology of trypanosomosis in the Sideradougou area.

### Genetic differentiation

A significant genetic differentiation was detected between the western and eastern parts of Sideradougou at a 15-km scale. To our knowledge, this is the smallest scale at which such a differentiation level could be detected. Further investigations on the genetic structure of tsetse populations will take place in this area of Sideradougou, as these two sides of the hydrographic network have already been shown to display different epidemiological patterns (de La Rocque, 1997; Lefrançois *et al.*, 1998; Solano, 1998).

At the scale of the distribution range of *G. p. gambiensis*, a strong genetic differentiation is evident. The *Fst* measured between Senegal and Burkina Faso is of an order of magnitude that leads to a rough estimate of an effective number of migrants of one per generation. This suggests that these geographical areas may be considered strongly isolated.

Several factors may be involved in these genetic differences, such as geographical distance, ecological differences, or different trapping methods. One might expect these populations of *G. p. gambiensis* to display different vectorial capacities. Indeed, it might be pointed out that in forest relics of the Gambia (which is, with Senegal, the most western distribution of *G. p. gambiensis*), the riverine-forest-type of *Trypanosoma congolense* was the trypanosome most often detected in *G. p. gambiensis* (McNamara & Snow, 1991). On the other hand, in our Sideradougou sampling site in Burkina Faso, using PCR analysis, no mature infection involving this *T. congolense* forest-type was found in *G. p. gambiensis* (Lefrançois *et al.*, 1998). Experimental infections also showed variations of vectorial competence of *G. p. gambiensis* with respect to *T. brucei sensu lato* between two laboratory lines showing genetic differences (Elsen *et al.*, 1994).

At this spatial scale, the observed genetic differentiation is in accordance with other works on tsetse. For instance, Krafur *et al.* (1997) also described a limited gene flow between populations of *G. pallidipes* of East Africa using eight

isozymatic loci. In other vector insects, such as mosquitoes, Lehman *et al.* (1996) measured an average *F<sub>st</sub>* of 0.016 within the species *Anopheles gambiae* between Senegal and Kenya. This latter value is considerably lower than those found in *G. p. gambienseis*. Furthermore, the differences found between Senegal and Burkina Faso are of the same order of magnitude as those that can be found between different species in other insects (e.g. Estoup *et al.*, 1995).

### Wings

The genetic differences found with both microsatellite loci between Senegal and Burkina Faso were accompanied by a smaller size of the tsetse sampled in Senegal. However, within Sideradougou, the differentiation between the eastern and western parts of the area seems not to involve significantly different wing morphometry. This may suggest an environmental influence acting on wing size, as it has already been shown (Buxton, 1955; Glasgow, 1961). Among the three parameters that differed significantly between flies from Senegal and Burkina Faso, one was the ratio *seg1/seg2*. This might indicate that the difference concerned the shape of the wing, but could also mean that the hatchet cell was differentially positioned on the wing. Nevertheless, these points need further analyses with a greater number of samples to better assess factors influencing wing measurements and thus their taxonomical or ecological significance.

### Conclusion

This preliminary work shows that microsatellite DNA polymorphism can represent a powerful tool for studying population genetics of vector species in its area of distribution (Dumas *et al.*, 1998; Kamau *et al.*, 1998). It has already been shown that understanding the population genetics of the vector is an important element in our knowledge of the epidemiology of vector-borne diseases (Lanzaro & Warburg, 1995), even in trypanosomoses (Janssen & Wijers, 1974). However, few works have focused on the implications of tsetse genetic variability on the efficiency of trypanosome transmission because of the lack of reliable tools. Increasing the number of loci and natural sampling of tsetse in a given area will allow the integrative study of genetic variability of tsetse flies in relation to the dynamics of trypanosome transmission, to better understand the tsetse–trypanosome complex associations.

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