Insect Molecular Biology (2000) 9(4), 433–439

Microsatellite DNA markers reveal genetic differentiation among populations of *Glossina palpalis gambiensis* collected in the agro-pastoral zone of Sideradougou, Burkina Faso

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Abstract

Intraspecific genetic variability of *Glossina palpalis gambiensis* in the area of Sideradougou, Burkina Faso, was studied using polymorphic microsatellite DNA markers. This genetic study was combined with other epidemiological information on the same tsetse: bloodmeal identification, dissection of tsetse and molecular characterization of the trypanosomes detected. There was significant genetic differentiation among flies caught only a few kilometers apart, within the same riverine habitat. These distinct subpopulations were also differentially infected by trypanosomes. In part of the study area, a Factorial Correspondence Analysis

information is available to assess the spatial scale of this phenomenon. Recent developments in techniques for assessing variation at highly polymorphic microsatellite DNA markers (Tautz & Renz, 1984; Estoup *et al.*, 1993; Weber & Wong, 1993) have provided us with new tools to investigate the question. *Glossina palpalis gambiensis* VanderPlank 1949, is a riverine West African tsetse fly that transmits trypanosomes causing both human and animal African Trypanosomoses. Improving our understanding of the genetics of the vectorial capacity of tsetse is clearly a worthy endeavour (Janssen & Wijers, 1974; Maudlin, 1980; Reifenberg *et al.*, 1997; Kazadi *et al.*, 1998). Similarly, genetic studies may provide useful information on the potential for large-scale tsetse control particularly in terms of Sterile Insect Technique (Cheng & Aksoy, 1999; Dale *et al.*, 1995; Vreysen *et al.*, 1998).

Tsetse were eradicated from the agro-pastoral zone of Sideradougou, south-west Burkina Faso, 15 years ago through the combined use of insecticide-impregnated screens and Sterile Insect Technique (Politzar & Cuisance, 1984). In recent years, tsetse have reinvaded the area, and now once again inhabit the gallery along major rivers. As part of a larger

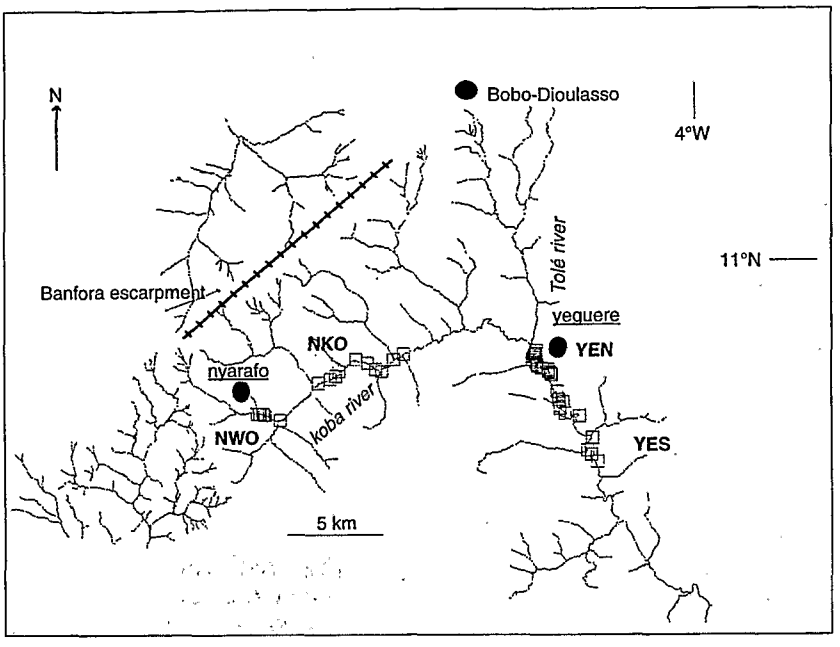

Figure 1. Map of the study area and geographical location of the traps (□) and subsamples (NWO, NKO, YEN, YES).

Epidemiological results

From 215 dissected flies, mean parasitological infection rates differed significantly between the areas: 20.3% (west) and 3.5% (east) (Fisher's exact test: $P = 0.0024$).

Using PCR to characterize the trypanosomes in the flies, some infections in the west could be characterized as *T. vivax* (40% of infected flies) or *T. congolense* «forest type» (6%). Surprisingly large numbers of infections (54%) did not react with any primers. These infections were localized only in the midgut of the flies. Because reptiles constituted the main food source (eight of twenty-one identified bloodmeals), this lead us to hypothesize that reptilian trypanosomes could account for these many infections. To date, specific primers for reptilian trypanosomes are not generally available (but see Gouteux & Gibson, 1996). Tsetse in both areas also fed on Suidae, followed by other species including cattle and man.

Infection rates were very low in the East, only two infected *G. palpalis gambiensis* were found; these harboured *T. vivax*.

Genetic analyses at the scale of the agropastoral zone

Among 201 analysed tsetse females, a total of twelve and seventeen alleles was recorded, respectively, at loci *Gpg55.3* and *Gpg19.62*. Seven subsamples (i.e. populations) were defined according to their geographical location and year of capture (Fig. 1).

No significant linkage disequilibrium was detected between loci in any of the subsamples.

The overall *Fis* value (0.163) across these two first microsatellite loci indicated a strong departure from panmixia,

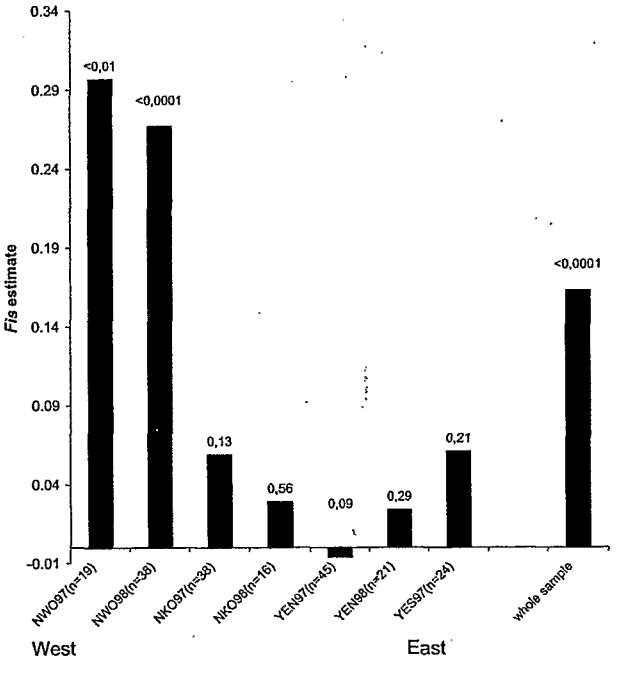

Figure 2. Graph showing within-sample heterozygote deficiency and its significance in each subsample. The subsamples are presented sequentially from the west to the east of the area and are plotted against the *Fis* estimate. Above each sample is represented the probability associated with the test of significance of the *Fis*. The number of females of each subsample is indicated in parentheses.

but this observation was dependent on location. Positive values of *Fis* (within sample heterozygote deficiency) were common only in the west. In the east, we could not reject the null hypothesis of panmixia (Fig. 2).

Table 1. Matrix of pair-wise *Fst* measured between the seven studied samples

	NWO97	NKO97	NKO98	NW098	YEN97	YES97	YEN98
NWO97	/	0.0211	−0.0086	−0.0085	**0.0590**	**0.0597**	**0.0831**
NKO97		/	0.0158	**0.0610**	**0.0810**	**0.0713**	**0.1399**
NKO98			/	0.0119	0.0213	0.0236	0.0627
NWO98				/	**0.0995**	**0.1077**	**0.1163**
YEN97					/	−0.0093	0.0354
YES97						/	0.0355
YEN98							/

Significant values after Bonferonni procedure are in bold (Bonferonni level is $\alpha' = \alpha/21$; $\alpha = 0.05$; so $\alpha' = 0.0024$).

The subsamples are presented from the west (four subsamples) to the east (three subsamples). Their names show the location and the year of capture. NOW = Nyarafo Wood; NKO = near Nyarafo on the Koba river; YEN = north of Yeguere; YES = south of Yeguere.

The overall *Fst* value was highly significant (*Fst* = 0.059, $P < 0.0001$) when measured at the scale of the entire

Taking into account these two groups of individuals (A and B) occurring in sympatry in the western side of the area in the sacred wood of Nyarafo, the population differentiation remained significant compared to the eastern zone.

Discussion

In this work, the study of *G. palpalis gambiensis* popula-

eastern and southern Africa (Nesbitt *et al.*, 1990; Kence *et al.*, 1995; Krafsur *et al.*, 1997), or through the use of the same microsatellite loci between geographically distant populations of *G. palpalis gambiensis* (Solano *et al.*, 1999), however these studies have worked on a macrogeographic scale, or have compared populations from ecologically distinct areas.

In the present study, as a second level of structuring, a highly significant heterozygote deficiency was observed

Table 3. Characteristics of the three microsatellite loci isolated from *Glossina palpalis gambiensis*

Microsatellite locus	Core microsatellite sequence	Annealing temperature	Number of alleles in the whole sample	Frequency of most common allele
Gpg55.3	$(GT)_{14}(GC)(GT)_4$	50 °C	12	0.79
Gpg19.62	$(GT)_{12}$	50 °C	18	0.49
Gpg69.22	$(TA)_{10}$	48 °C	8*	0.60

*Locus *Gpg69.22* was scored on ninety-seven females from the western area.

Because this tsetse species moves preferentially along riverine networks and needs high humidity to survive (Buxton, 1955), the geography of the area provides the most likely explanation for population substructuring.

It would be of interest to know if the genetic differences at the intraspecific level pointed out in the present work could lead to different vectorial competences (Maudlin, 1980; Reifenberg *et al.*, 1997; Moloo *et al.*, 1998).

The lack of evidence for any linkage disequilibrium is not definitive in our study due to the low number of loci analysed. The third group of the FCA (group C) might also represent individuals having migrated from other localities. However in the absence of clearer information that would come from the analysis of more loci, we cannot draw more precise conclusions. It should be noted that to the author's knowledge, these three microsatellite loci are the only ones that have been described in tsetse flies so far. Nevertheless the information expected from such approaches will contribute in designing future control strategies: genetic studies on vector populations combined with the study of other epidemiological factors will be of help in identifying 'epidemiologically dangerous points' to assess areas at risk for trypanosomosis (Cuisance *et al.*, 1980; de La Rocque, 1997; Hendrickx *et al.*, 2000; B. Bauer & B. Snow, personal communication), and will also help to assess the feasibility of the Sterile Insect Technique in tsetse eradication schemes (Vreysen *et al.*, 1998).

Experimental procedures

Study area

Sideradougou lies in the southern half of Burkina Faso in the Sudan savannah vegetation zone (4°20′W, 10°55′N). Two riverine tsetse species are present in the area: *Glossina palpalis gambiensis*

Field techniques

Each trap site was georeferenced using a GPS and tsetse species, sex and apparent density were recorded.

In the field camp, after removing of the tsetse from the trap, individual *G. palpalis gambiensis* were processed as follows:

- Three legs were removed and stored in dry eppendorf tubes for subsequent genetic analyses.
- The mouthparts, salivary glands and midgut were dissected to detect trypanosome infections by microscope examination (Lloyd & Johnson, 1924).
- The midgut content was collected for subsequent bloodmeal identification (Kaboré *et al.*, 1994).
- When a tsetse was found infected with trypanosomes, each of the three organs was collected separately in eppendorf tubes (dry for the mouthparts, and with 50 µl sterile distilled water for the midgut and salivary glands). This material was used for PCR identification of trypanosomes using DNA primers designed from genomic satellite sequences specific of the different taxonomic groups (Majiwa *et al.*, 1994; Solano *et al.*, 1995).

The techniques for identification of the trypanosomes by PCR and for the bloodmeal origin of the tsetse are described fully in Lefrançois *et al.*, 1998.

PCR reactions at microsatellite loci

At the CIRDES (Centre International de Recherche-Développement sur l'Elevage en zone Subhumide) laboratory in Bobo-Dioulasso, 300 µl of 5% Chelex® (BIORAD, Hercules, CA, USA) chelating resin were added to each tube containing the legs of the tsetse (Walsh *et al.*, 1991; Dumas *et al.*, 1998). After incubation at 56 °C for 1 h, DNA was denatured at 95 °C for 30 min. The tubes were then centrifuged at 12 000 ***g*** for 2 min and were frozen before further handling.

The PCR reactions were carried out in a thermocycler (MJ Research, Watertown, MA, USA) in 50 µl final volume, using 10 µl of the supernatant from the extraction step. Specific primers were designed in the regions flanking the microsatellite core sequences of loci *Gpg55.3* and *Gpg19.62* (as described in Solano *et al.*, 1997). Precise allele sizing on a limited number of samples had already

males in captive-bred flies (Solano, 1998). We therefore analysed only field-collected females. Captive breeding also demonstrated mendelian inheritance of the alleles at these microsatellite loci and an absence of null alleles (e.g. Callen *et al.*, 1993; Dumas *et al.*, 1998).

Data analysis on microsatellite loci

In each population, Wright's *Fis* (within sample heterozygote deficiency) and *Fst* (measure of population differentiation) were estimated using Weir & Cockerham's (1984) unbiased estimators (f for *Fis*, θ for *Fst*). These estimators were computed with FSTAT v.1.2 software (Goudet, 1995). For random mating (within samples) or random distribution of individuals (between samples), *F*-values were expected to be zero.

For each locus in each sample, and for all loci, the significance of *Fis* (panmixia) was tested using GENEPOP v.3.1 (Raymond & Rousset, 1995). The probability test described by Guo & Thompson (1992) was used, employing 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 multisample extension of the score test described by Rousset & Raymond (1995) was then applied. For this test, the alternative hypothesis was 'H1 = heterozygote deficiency'. The measure of *Fis* and its significance were conducted only on the females, because males were haploid at two loci (i.e. they have only one X-chromosome).

The significance of *Fst* (population differentiation) was assessed using 10 000 permutations of female genotypes among samples (FSTAT; Goudet, 1995). The alternative hypothesis here is 'Fst > 0'.

Linkage disequilibrium was tested by the exact test of GENEPOP for genotypic linkage disequilibrium (Raymond & Rousset, 1995).

To evaluate significance when multiple tests were performed, the sequential Bonferroni procedure was applied (see Rice, 1989).

A Factorial Correspondence Analysis (FCA) was undertaken to explore genetic structure within samples. The analysis was carried out on the multilocus genotypes obtained at the three loci: each individual was characterized for each existing allele by the values 2, 1 or 0 whether it had 2 (homozygotes), 1 (heterozygote) or 0 copies of the considered allele. Individuals were then analysed as active variables using FCA (e.g. Ba *et al.*, 1993). The analysis was carried out after eliminating individuals carrying rare alleles (< 5%), because rare alleles tend to hide the existing patterns.

Acknowledgements

This work was funded by CIRAD-EMVT (Action Thématique Programmée 'Santé-Environnement'), CNRS (Programme Interdisciplinaire 'Environnement, Vie et Sociétés') and AUPELF-UREF (LAF n°X/7.10.02/4581-306). We are indebted to Dr S. M. Touré, Director of CIRDES, for allowing us to work at CIRDES, and we thank all the technical staff for excellent work. Special thanks to S. Mihok for critically reviewing the manuscript.

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Volume 9 • Number 4 • August 2000

ISSN 0962-1075

Insect Molecular Biology

Editors: L. M. Field and A. A. James