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Acta Tropica 65 (1997) 175–180

ACTA TROPICA

Microsatellite markers for genetic population studies in *Glossina palpalis* (*Diptera*: *Glossinidae*)

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Received 5 November 1996; received in revised form 30 December 1996; accepted 10 February 1997

Abstract

Little is known about tsetse intraspecific variability and its consequences on vectorial capacity. Since isoenzyme analyses revealed little polymorphism, microsatellite markers have been developed for *Glossina palpalis gambiensis* species. Three loci have been identified and showed size polymorphisms for insectarium samples. Moreover, amplifications were observed in different species belonging to *palpalis* group. These molecular markers will be useful to estimate gene flow within *G. p. gambiensis* populations and analyses could be extended to related species. © 1997 Elsevier Science B.V.

Keywords: Microsatellite; *Glossina palpalis*; Population genetics; Trypanosomosis; *G. fuscipes*; *G. morsitans*

1. Introduction

Most species of the genus *Glossina* play a potential vector role in the transmission of African *Trypanosomosis*, which has considerable economic impact in subsaharan

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PII S0001-706X(97)00663-3

Africa (Janhke et al., 1988). In West Africa, species of the *palpalis* group (subgenus *Nemorhina*) are involved in transmission of Animal trypanosomosis (nagana) and Human trypanosomosis (sleeping sickness). Despite their importance little is known about tsetse population genetics and their implications for the transmission of trypanosomes: very little information is available on the possible structuration of tsetse populations which could lead them to express resistance to control measures, by avoiding traps or treated animals for example. Intraspecific variation and related differential vectorial capacity is suspected to occur in natural *G. palpalis gambiensis* populations (Bauer et al., 1995; Solano et al., 1996). However, previous studies using isozyme analyses were undertaken only for interspecies comparisons (Gooding et al., 1991) or for purpose of genetic assignment of loci (Gooding and Rolseth, 1995). Natural populations of tsetse flies of Burkina Faso showed little polymorphism using isozyme data on five loci (Gooding, 1981). Genetic studies were hence of limited value because of the lack of accurate technologies.

Among insects, microsatellite loci have mostly been developed for social species like ants (Gertsch et al., 1995), bees (Estoup et al., 1993, 1995), or wasps (Hughes and Queller, 1993). In the field of medical or veterinary entomology, studies are still rare, and microsatelllites have only been developped in *Anopheles gambiae* (Zheng et al., 1993, 1996; Lanzaro et al., 1995) and *Simulium damnosum* (Dumas, unpublished results).

This paper reports on the isolation of microsatellite sequences in *G. p. gambiensis*, a riverine species widespread in West Africa and their potential use for population genetics in this taxa and in related species.

2. Material and methods

DNA (20 μg) from 50 individual *G. p. gambiensis*, originating from the CIRAD/ORSTOM insectarium (Montpellier, France), was digested to completion overnight with *Hae*III. The 400- 800 bp fraction was recovered and ligated into the dephosphorylated EcoRV site of M13 BM 20 (Boehringer–Mannheim). Ligation products were used to transform *E.coli* XL1 Blue cells and 4500 recombinants clones were lifted on Hybond-N^+ membranes. Hybridizations were carried out with (CA)n and (GA)n probes, labelled with dCTP-$\alpha[^{32}P]$, using rapid hybridization buffer (Amersham) according to manufacturer instructions. Positive clones were dot-blotted and re-screened to ensure specificity. Eleven clones were kept after this secondary screening. Eight of them were sequenced by the dideoxy-chain termination method, using the Taq dye primer kit and an automatic sequencer (Applied Biosystems).

Template DNA for PCR was prepared by incubating two legs of a fly in 5% chelex for 1 h at 56°C, then 30 min at 95°C. Amplification reactions were performed in a Perkin Elmer thermal cycler, in a final volume of 50 μl containing as final concentrations 1 × Appligene incubation buffer with 1.5 mM $MgCl_2$, 200 μM of each dNTP, 15 pmol of each primer and 0.5 U Appligene Taq Polymerase. Samples were first denatured during 90 s at 92°C and then processed through 35 cycles consisting of 30 s at 92°C, 30 s at 50°C for loci 55.3 and 19.62 and 48°C for

locus 69.22 and 1 min at 72°C. The last elongation step was lengthened to 10 min. An amount of 15 μl of each amplified sample was resolved on 12% non-denaturing polyacrylamide gel.

3. Results

Of these eight clones sequenced, four were false positives and microsatellite sequences were successfully obtained for four clones. The presence of false positives can be explained by an imperfect homology of sequences between the clones and the microsatellite probes due to the low stringency of the hybridization washes. Two clones had microsatellite sequences located too close to the cloning site to allow primer selection; fortunately, as one of them (~~19.62~~ 69.22) owned a (TA) repeat also, three pairs of primers could be designed.

A sequence was considered as a microsatellite if the number of repetitions of the dinucleotide motif was six at a minimum (Stallings et al., 1991). According to Weber (1990), the three microsatellite loci were classified as 'perfect' for 69.22 and 19.62 ($(TA)_{10}$ and $(GT)_{12}$, respectively) and 'imperfect' for 55.3 $(GT)_{14}$ $GC(GT)_4$.

Eight individual *G. p. gambiensis* from the insectarium were individually tested by PCR with the three primer pairs and PCR products were size-fractioned on 12% acrylamide gels with appropriate markers. Results were as follows: locus 55.3 showed four alleles, locus 19.62 showed three and locus 69.22 showed two alleles (Table 1). Allele size was highly variable; for example, 20 bp separated the largest and smallest alleles at locus 19.62 (Table 1).

The three primer pairs gave also a strong signal with wild *G. p. gambiensis* from Mali; the three individuals tested had some alleles in common with some of the insectarium tsetse, for example allele 176 bp at locus 19.62, which appears as the most common. Wild *G. palpalis palpalis* from Cameroun and a laboratory colony of *G. fuscipes fuscipes* gave also scorable signals for the three primer pairs with intra-colony variability at the three loci for *G. p. palpalis*, and at loci 55.3 and 19.62 for *G. f. fuscipes* (Table 2). Only primer pairs 19.62 amplified an appropriate sized product from DNA of *G. tachinoides*. No amplification signal could be obtained at

Table 1
Characteristics of the three microsatellite loci among a laboratory sample of eight *G. p. gambiensis*

Locus	Repeat sequence	Allele sizes (bp)	Primer sequence
55.3	$(GT)_{14}(GC)(GT)_4$	181, 183, 187, 197	5'GTACTCAACGTGGTGCTTAAAGTTG3' 5'GTCTGAGATAGGACCATTTATCG3'
19.62	$(GT)_{12}$	176, 178, 196	5'CAGATATGCTACACTTGGTCAGC3' 5'GCATTTAATGTTATACACTGAAGG3'
69.22	$(TA)_{10}$	198, 200	5'CAAAACTCGACCAAATTGACCG3' 5'CGATAATGATACGATTAAATCAAACC3'

Table 2
Size of the bands (when observed) in other tsetse taxa

Locus	Bands observed (bp)				
	G. p. palpalis	*G. f. fuscipes*	*G. tachinoides*	*G. m. submorsitans*	*G. m. morsitans*
55.3	175, 171	181, 185	None	None	None
19.62	170, 174	174, 182	~~None~~ 160	None	None
69.22	194, 200	192	None	None	None

any locus with either *G. morsitans morsitans* nor than with *G. morsitans submorsitans*.

4. Discussion

The (CA)n and (GA)n probes used in this work allowed three primer pairs which showed size polymorphisms in a laboratory sample of *G. p. gambiensis*, to be designed. The fact that one locus (69.22) consisted of a (TA) repeat whereas the probes used (CA)n and (GA)n could be explained by the presence of a second GT repeat too close to the cloning site to allow primer selection.

The tsetse individuals from different origins used in this study were just tested for scorable amplifications. After this first step, heritability of the presumed alleles should be demonstrated, then the microsatellite markers will be used to estimate gene flows within species. *G. p. gambiensis* is of particular interest since previous work has shown great plasticity of behaviour in Burkina Faso (Challier, 1973; Bauer et al., 1995; Solano et al., 1996) and great variability in transmitting *Trypanosoma brucei gambiense* (Elsen et al., 1994). At the moment, there is no strong evidence that tsetse populations are structured, as is the case in *Anopheles gambiae*, for example (Lanzaro et al., 1995), but this could be due to the lack of studies of this type of tsetse.

All tsetse belong to one genus *Glossina*, that is divided into three subgenera, *Austenina* (*fusca* group), *Nemorhina* (*palpalis* group) and *Glossina* (*morsitans* group). *G. m. submorsitans* and *G. m. morsitans* are in the *morsitans* group and could not be amplified with any primer sets used in this study. In contrast, all three loci were correctly amplified in both *G. p. palpalis* and *G. f. fuscipes* from the *palpalis* group. Indeed these species were first classified as a single one, *G. palpalis* (Van der Planck, 1949), and they showed great similarity at isoenzyme loci (Fig. 1). Finally, *G. tachinoides* belongs also to the *palpalis* group, but is known to be quite different from *G. palpalis*, regarding ecological behaviour as well as genetic data (Gooding et al., 1991). In this work, the primer specificity reflected well the generally accepted phylogenetic relationships between the tsetse taxa (Fig. 1). In the future, these genetic studies could be extended to related species because interspecific conservation of flanking sequences will support use of these loci.

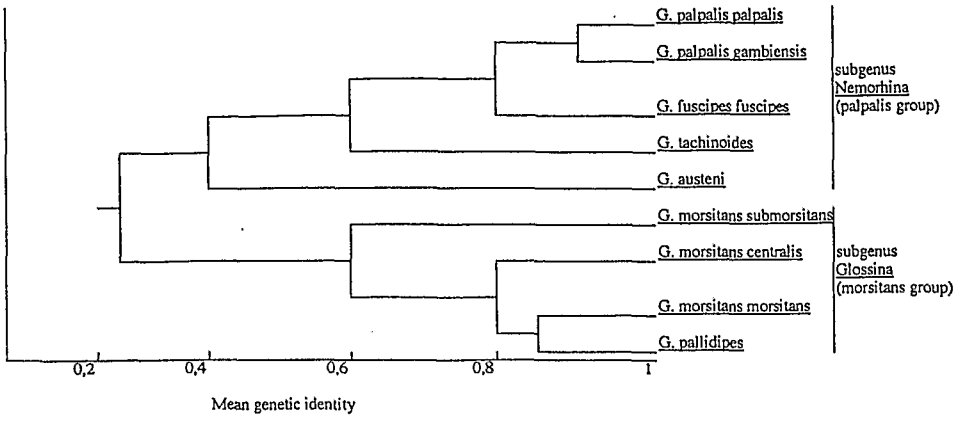

Fig. 1. Phenogram for nine taxa of tsetse flies, based upon loci for 12 enzymes (Gooding, 1981, modified).

Acknowledgements

This work was supported by the CIRAD-EMVT and the centre ORSTOM Montpellier, France, and will continue at the CrRDES Bobo Dioulasso, Burkina Faso. The authors acknowledge Dr O. Diall (Laboratoire Central Vétérinaire, Bamako, Mali) for the gift of the specimens from Mali.

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