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M. D. Baker
Iowa State University

E. S. Krafsur
Iowa State University, eskrafsur@gmail.com

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Identification and properties of microsatellite markers in tsetse flies *Glossina morsitans sensu lato* (Diptera: Glossinidae)

M. D. BAKER and **E. S. KRAFSUR**
Department of Entomology, 402 Science 2, Iowa State University, Ames, IA 50011–3222, USA

Abstract

Genomic libraries enriched for simple sequence repeats were constructed for *Glossina morsitans morsitans*, *G. m. submorsitans*, and *G. m. centralis*. Sixteen microsatellite markers were isolated from the libraries and evaluated on flies from natural *G. m. morsitans* populations and other *Glossina* species in the *Morsitans* and *Palpalis* species groups. The primers amplified appropriate sized DNA fragments in the *Morsitans* and *Palpalis* groups. In *G. morsitans s.l.*, eight of 12 dinucleotide repeats and four of 12 trinucleotide repeats were polymorphic. The polymorphic loci showed a mean 7.5 ± 4.8 alleles per locus and their mean heterozygosity was 55.8 ± 7.7%.

Keywords

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Tsetse flies (Diptera: Glossinidae) are exclusively haematophagous and confined to sub-Saharan Africa. Their medical and veterinary importance is great because they are the only vectors of African trypanosomiasis, ‘sleeping sickness’ in humans and nagana in cattle. In east and southern Africa, *Glossina morsitans sensu lato* and *G. pallidipes* are the most important vectors of trypanosomiasis. These species are assigned to the subgenus *Glossina*, the morsitans group (Leak 1998). Three allopatric subspecies of *G. morsitans* are recognized, *G. m. morsitans* (*Gmm*), *G. m. centralis* (*Gmc*), and *G. m. submorsitans* (*Gms*).

Some genetic markers have been developed in *Glossina*. Allozyme polymorphism were detected and mapped in *G. morsitans* and *G. palpalis* by Gooding (1992). Krafsur & Griffiths (1997) examined isozyme variation at 31–45 loci in *G. pallidipes*, *G. morsitans* sensu lato, and *G. swynnertoni*. They found 23% of the loci were polymorphic, and heterozygosity was a statistically homogeneous 6.2% averaged over taxa. Isozyme loci in tsetse are inconvenient because it is difficult to obtain liquid nitrogen in Africa. DNA techniques, in contrast, allow use of alcohol preserved or dried material and provides more genetic variation.

Solano *et al*. (1997) isolated an autosomal and two X-chromosome linked microsatellite loci in *G. palpalis gambiense*. Luna *et al*. (2001) recovered 13 polymorphic microsatellite loci from *G. palpalis palpalis. G. palpalis* primers did not amplify *G. morsitans* DNA. We therefore developed *G. morsitans s.l.* genomic libraries enriched for microsatellites and proceeded to identify and characterize them.

DNA was extracted as outlined (Wohlford *et al*. 1999). *G. m. morsitans*, *G. morsitans*, and *G. m. centralis* DNA was digested with *Rsa*I and electrophoresed on an agarose gel. Fragments between 300 and 1500 bp were excised and purified. Gel purified fragments were ligated to *Bgl*II adapter (21-mer: 5′-CTCTTGCTTAGATCTGGACTA-3′; 24-mer: 5′-

Correspondence: E. S. Krafsur. Fax: 515 294 5957; E-mail:ekrafsur@iastate.edu.

pTAGTCCAGATCTAAGCAAGAGCACA-3′), using T4 DNA ligase (Promega). Ligated DNA was PCR amplified in 50 μL reactions containing 3 μM 21-mer adapter primer for 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min.

Amplification products were enriched for simple sequence repeat (SSR) loci by either hybridizing to probes bound to nylon membranes (MBP) after Edwards *et al*. (1996) or to biotinylated probes bound to magnetic beads (BP) following Kijas *et al*. (1994). Enriched fragments were amplified to and cloned in pGEM7 plasmids (Promega) and plated. Colonies were picked and their DNA amplified by using polymerase chain reaction (PCR). Amplified products of appropriate size were sequenced and primers designed for those loci containing repeats.

G. m. morsitans DNA was used for the initial evaluations of presumptive loci for polymorphisms. Primers were also tested against DNA from *G. m. centralis*, *G. m. submorsitans*, *G. pallidipes*, *G. swynnertoni*, *G. austeni*, *G. fuscipes*, *G. palpalis*, *G. tachinoides*, *G. brevipalpis*, *G. longipennis*, and *G. fuscipleuris*. Genomic DNA was amplified in 10 μL reactions for 35 cycles of 94 °C for 40 s, 50 °C for 40 s, and 72 °C for 30 s. Samples were electrophoresed on 5% acrylamide gels and genotypes scored after silver staining.

The MBP method afforded 14 positive clones that were sequenced. Seven of the 14 possessed SSRs. Six were polymorphic (Table 1). No AT repeats were found.

Sixty-eight clones constructed by the BP method were sequenced. Primers were made for 16 of the cloned loci, 10 pairs of which produced appropriately sized amplification products, only four of which were polymorphic. Heterozygotes were found only in females at *Gmm127*, *Gmm14*, and *GmcCA16C*.

Allele numbers varied from three to 19. The mean was 7.5 alleles. Heterozygosities varied from eight to 92%. The mean diversity was 55.8 ± 7.7%. The frequency of polymorphic loci was greater among dinucleotide (eight of 12) than trinucleotide (four of 12) loci (Fisher's exact test $P = 0.11$). Dinucleotide repeats were more diverse (60.7%) than trinucleotide repeats (13.8%).

All primers derived from *G. morsitans s.l.* amplified template DNA from other members of the species complex (Table 2). Five *G. m. morsitans* loci amplified DNA in *G. swynnertoni* and *G. austeni* and the *Palpalis* group. Only *GmcCAG6* amplified DNA in *Fusca* group flies. The dinucleotide repeats in *G. morsitans* were more polymorphic than the trinucleotide repeats and variances were greater. Diversities at five dinucleotide ($H_E = 0.80$) and five trinucleotide loci ($H_E = 0.81$) in *Drosophila* did not differ but variances were much greater among the dinucleotide loci (Goldstein & Clark 1995).

Both the MBP and the BP methods were successful in isolating microsatellites. The BP library was 100% enriched for SSRs, whereas the MBP library was about 50% enriched. Moreover, the MBP method took 2–3 times longer to complete.

Our microsatellites are used to analyse the breeding structure of *G. morsitans s.l.* populations. We have successfully automated scoring genotypes on an ABI 377 Prism™ automated DNA sequencer by using GENESCAN™ software and fluorescent labelled primers.

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Table 1

Characteristics of microsatellite loci in *Glossina morsitans morsitans*

Locus	Repeat motif*	Allele size ranges	GenBank Accession no.	Primer pair sequences (5′–3′)	No. flies	No. Alleles	H_O	H_E	F_{IT}*
Gmm8	$(GA)_{11}$	125–131	AF372506	F: CGCGCTTCAATGTTTGCTTTC R: TGCAGATGCAATGCGGAGAG	44	3	0.59	0.51	−0.153
Gmm14	$(CA)_{32}$	153–211	AF372507	F: CACACCCTGGATTACAAA R: TGAAATGCAACCCTTCTT	26	19	0.77	0.90	0.223
Gmm15	$(GA)_{17}$	185–195	AF372508	F: CTAGCTTCACTACCCATC R: CATAGACGGATAACTGGA	44	6	0.55	0.63	0.156
Gmm22	$(GA)_{19}$	135–145	AF372509	F: CGTAAACGCGGGCTTGT R: CAATTTGGCTGGCTGTCC	43	10	0.35	0.54	0.351
Gmm5B	$(CA)_{14}$	155–175	AF372510	F: GAATTGTTATGAGTGCATGT R: ATGCGACACGACACAATAAG	22	9	0.50	0.86	0.422
Gmm9B	$(CA)_9TA(CA)_{11}$	140–180	AF372511	F: TTTCCTATATTGCGATTA R: CGTTTACGTTACCCAGAA	21	14	0.89	0.92	0.048
GmsCAG6	$(CAG)_{11}$	120–140	AF372512	F: CGGTGTTGGTGGCAATG R: GCTGGGGCTTGTTGATGA	72	4	0.08	0.08	−0.027
GmcCA16C	$(CA)_{11}$	200–210	AF372513	F: CGCAAAACGGAATCAC R: CATGTTAATCGCATTGAA	62	6	0.52	0.66	0.342
GmsCAG2	$(CAG)_8$	130–145	AF372514	F: GCTTTTCTCGTCCCATAA R: GCGTTGTTGATGACTGTG	72	4	0.15	0.19	0.260
GmsCAG29B	$(CAG)_6(CAA)_7$	175–190	AF372515	F: AACTATTGCTGGGCTCAC R: ATGTGGCGACGATGA	30	5	0.56	0.61	0.309
GpCAG133	$(CAG)_{12}$	185–205	AY033512	F: ATTTTTGCGTCAACGTGA R: ATGAGGATGTTGTCCAGTTT	73	7	0.38	0.36	−0.049
Gmm127	$(CA)_5$	295–301	AY029598	F: CTTCGCTTTTTCACAGACAGATTA R: GAAGCTTTAGCTATTCTTGTTCGC	72	3	0.52	0.44	−0.156
Means ± SD	—	—	—	—	—	7.5 ± 4.8	0.49 ± 0.07	0.56 ± 0.08	0.17 ± 0.08

*F_{IT} estimates departures from Hardy–Weinberg expectations.

†$P < 0.01$ by Chi-square. $\chi^2 = F^2_{IT}N(k-1)$ for k alleles and $k(k-1)$ degrees of freedom.

Table 2

Application of *G. morsitans morsitans* s.*l.* microsatellite primers against other *Glossina* species

Locus	Taxon									
	G. m morsitans	***G. allidipes***	***G. wynnertoni***	***G. usteni***	***G. uscipes***	***G palpalis***	***G. achinoides***	***G. revipalpis***	***G. ongipennis***	***G fuscipleuris***
Gmm8	+	+	+	+	+	+	+	0	0	0
Gmm14	+	+	0	0	0	0	0	0	0	0
Gmm15	+	+	0	0	0	0	0	0	0	0
Gmm22	+	+	0	0	0	0	0	0	0	0
Gmm5B	+	0	0	0	0	0	0	0	0	0
Gmm9B	+	0	0	0	0	0	0	0	0	0
Gmm127	+	0	0	0	0	0	0	0	0	0
GmcCAG6	+	+	+	+	+	+	+	+	+	+
GmcCA16C	+	+	+	+	+	+	0	0	0	0
GmsCAG2	+	+	+	+	+	+	+	0	0	0
GmsCAG17B	+	+	0	0	0	0	0	0	0	0
GmsCAG29B	+	+	+	+	+	+	+	0	0	0
GpCAG133	+	+	+	0	+	+	+	0	0	0

+ signifies amplification; 0 signifies no amplification.