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Characterization of microsatellite markers in the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae)

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Abstract

Glossina pallidipes is a vector of African trypanosomiasis. Here we characterize eight new polymorphic microsatellite loci in 288 *G. pallidipes* sampled from 12 Kenya populations. The number of alleles per locus ranged from four to 36 with a mean of 20.5 ± 10.1. Expected single locus heterozygosities varied from 0.044 to 0.829. Heterozygosity averaged 0.616 ± 0.246. No linkage disequilibrium was found. We also report results in eight other tsetse species estimated by using the primers developed in *G. pallidipes*. The primers worked best in *G. swynnertoni* and *G. austeni* and worst in *G. m. morsitans* and *G. m. submorsitans*.

Keywords

gene diversity; *Glossina pallidipes*; microsatellites; simple sequence repeats; tsetse flies

Tsetse flies (Diptera: Glossinidae) are obligate and exclusive blood feeders found in much of tropical Africa where they transmit *Trypanosoma* spp. that cause sleeping sickness in humans and nagana in livestock. Of the 32 known taxa, *Glossina pallidipes* is among the most important vectors of trypanosomes. It has a wide but patchy distribution in East and southern Africa (Ford 1971).

Three classes of genetic markers have been developed in tsetse flies and used to evaluate natural populations. Gooding (1992) detected allozyme polymorphism in *G. morsitans sensu lato* and in *G. palpalis*. Krafsur & Griffiths (1997) examined isozyme variation at 31–45 loci in *G. pallidipes, G. morsitans s.l.* (consisting of three reproductively isolated taxa), and *G. swynnertoni*. They observed that 23% of the loci were polymorphic, and heterozygosity averaged over loci and taxa was a statistically homogenous 6.2%. Studies on the breeding structure of *G. pallidipes* showed surprisingly high levels of genetic differentiation at allozyme loci (Krafsur *et al.* 1997) and at mitochondrial loci (Krafsur & Wohlford 1999). Microsatellite loci have also been developed in tsetse flies. Solano *et al.* (1997) isolated an autosomal and two X-linked microsatellite loci from *G. palpalis gambiensis*. Luna *et al.* (2001) identified 13 poly-morphic microsatellite markers in *G. palpalis palpalis.* Baker & Krafsur (2001) isolated and characterized 16 micro-satellite markers in *G. morsitans s.l.* The primers amplified DNA from other *morsitans* and *palpalis* groups. Three of these markers were found to be useful for population genetic studies of *G. pallidipes* (Krafsur 2002).

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There is need to develop more genetic markers in *Glossina* sp., particularly in *G. pallidipes*. Here we characterize eight new polymorphic microsatellite loci isolated from *G. pallidipes* and report their usefulness in related tsetse taxa.

G. pallidipes were obtained from a colony established at the International Atomic Energy Agency (IAEA), Seibersdorf, Austria. Enriched genomic libraries were constructed by Genetic Identification Services (GIS, http://www.genetic-id-services.com; Chatsworth, CA, USA), using 100 μg of DNA. Insert DNA from individual clones was amplified by polymerase chain reaction (PCR) following GIS guidelines. The PCR products were purified by using Qiaquick™ columns (Qiagen® Inc.) and sequenced using forward and reverse universal M13 primers and ABI Prism® BigDye™ Terminator chemistry. Clones with inserts less than 350 bp were not sequenced. Sequencing products were resolved on the ABI Prism® 377 Sequencer (PE Applied Biosystems).

Oligonucleotide primers were designed by using the software DESIGNERPCR version 1.03, 1994 (Research Genetics, Inc.). *G. pallidipes* DNA was used for the initial evaluations of presumptive loci for polymorphisms. Primers were also tested against DNA from other *Glossina* taxa (Table 2). PCR amplifications were performed in a PTC-100™. thermocycler (MJ Research Inc.) as 10 μL reactions containing 1 × Biolase™ PCR buffer,1.5 or 2.5 mM $MgCl_2$; 0.5 μm each of forward primer (labelled with FAM or HEX), and reverse primer; 0.4 mM dNTPs; 0.4 units Biolase™ polymerase (Bioline USA, Inc., Springfield NJ); and about 100 ng template DNA. The amplification profile consisted of an initial denaturation at 94 °C for 3 min followed by 34 cycles for 1 s at 94 °C, 15 s at the primer-specific annealing temperature (Table 1), 72 °C for 15 s, ending with an extension cycle of 72 °C for 10 min. Analysis of fragment size was performed on the ABI Prism 377 DNA sequencer, using GENESCAN™ 3.1.2 and the TAMRA-350 size standard.

Analyses of genetic diversity were carried out by using FSTAT version 2.9.3.2 (Goudet 1995). Tests for Hardy–Weinberg equilibrium of the genotypic frequencies were carried out with ARLEQUIN version 2.0 (Schneider *et al.* 2000). Genotypic disequilibrium was tested by using the log-likelihood ratio G-statistic. CERVUS (Marshall *et al.* 1998) was used to estimate genetic diversity parameters and to generate *P*-values for the Hardy-Weinberg tests in Table 2.

Polymorphisms at eight loci from 288 flies collected from 12 geographical populations are summarized in Table 1. Two loci contained imperfect repeats (*GpA19a* and *GpC26b*), while six had perfect repeats. A total of 164 alleles was detected. Numbers of alleles ranged from four to 36 per locus, with a mean of 20.5 ± 10.1. The difference between the longest and the shortest allele varied from nine (*GpD18b*) to 61 base pairs (*GpB20b*). Averaged across loci, the difference between the maximum and minimum allele size was 39 bp. Observed (H_O) and expected (H_E) heterozygosities ranged from 0.014 and 0.044–0.881 and 0.829, respectively. Mean $H_O = 0.590 \pm 0.260$ and $H_E = 0.616 \pm 0.246$; these lead to an estimate of departures from random mating $F_{IT} = 0.042$. No significant linkage disequilibrium was detected.

G. pallidipes primers amplified DNA from other *Glossina* morsitans group taxa (Table 2). *G. m. morsitans* DNA was not amplified at *GpA19a*, *GpB6b* and *GpD18b*. Mean expected heterozygosities among taxa ranged from 0.404 in *G. m. morsitans* to 0.700 in *G. m. submorsitans*. A significant paucity of heterozygotes was detected at 18 of 61 loci-taxa (*c.* 30%). The paucity was probably caused by null alleles. *G. brevipalpis* gave the best results and *G. m. submorsitans* and *G. m. morsitans* the worst. Sample sizes and loci numbers were too small to allow phylogenetic inferences.

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Referencmes

Baker MD, Krafsur ES. Identification and properties of micro-satellite markers in tsetse flies *Glossina morsitans sensu lato* (Diptera: Glossinidae). Molecular Ecology Notes 2001;1:234–236. [PubMed: 16479272]

Ford, J. The role of trypanosome in African Ecology. A Study of the Tsetse Fly Problem. London: George Allen & Unwin; 1971.

Gooding RH. Genetic variation in tsetse flies and implications for trypanosomiasis. Parasitology Today 1992;8:92–95. [PubMed: 15463582]

Goudet J. fstat: a computer program to calculate *F*-Statistics. Journal of Heredity 1995;86:485–486.

Krafsur ES. Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite and mitochondrial gene diversities. Insect Molecular Biology 2002;11:37–45. [PubMed: 11841501]

Krafsur ES, Griffiths NT. Genetic variation at structural loci in the *Glossina morsitans* species group. Biochemical Genetics 1997;35:1–11. [PubMed: 9238514]

Krafsur ES, Griffiths N, Brockhouse CL, Brady J. Breeding structure of *Glossina pallidipes* (Diptera: Glossinidae) populations in East and southern Africa. Bulletins of Entomological Society 1997;87:67–73.

Krafsur ES, Wohlford DL. Breeding structure of *Glossina pallidipes* populations evaluated by mitochondrial variation. Journal of Heredity 1999;90:635–642. [PubMed: 10589514]

Luna C, Bonizzoni M, Cheng Q, Robinson S, Aksoy S, Zheng L. Microsatellite polymorphism in tsetse flies (Diptera: Glossinidae). Journal of Medical Entomology 2001;38:376–381. [PubMed: 11372961]

Marshall TC, Slate J, Kruuk L, Pemberton JM. Statistical confidence for likelihood-based patemity inference in natural populations. Molecular Ecology 1998;7:639–655. [PubMed: 9633105]

Schneider, S.; Roessli, D.; Excoffier, L. ARLEQUIN, Version 2.000: a Software for Population Genetics Data Analysis. Switzerland: Genetics and Biometry Laboratory. University of Geneva; 2000.

Solano P, Duvallet G, Dumas V, Cuisance D, Cuny G. Micro-satellite markers for genetic population studies in *Glossina palpalis* (Diptera: Glossinidae). Acta Tropica 1997;65:175–180. [PubMed: 9177579]

Table 1

Polymorphic microsatellite loci in 288 *Glossina pallidipes* representing 12 geographical populations.

Locus	Allele size range (bp)	T_a (°C)	Repeat motif	No. alleles	H_O	H_E	F_{IT}	Accession number	Primer sequence (5′-3′)
GpA19a	142–189	48, 52	$(CA)_7GA(CA)_7$	10	0.556	0.590	0.058	AY220498	F: CATATCCACACCCACATACAT
									R: GCGATTATGGCTAGAGGTTT
GpA23b	172–215	48, 52	$(GT)_{21}$	27	0.581	0.609	0.046	AY220499	F: CTCCTGCTTGGGCTCTAT
									R: GCGATGAGTTGGTTTCTTT
GpB6b	187–224	48, 52	$(CT)_{15}$	21	0.542	0.626	0.134	AY220500	F: GTAAACCGCCTGTCACATC
									R: AGGGAGAGAGCCGTAAGAG
GpB20b	139–200	48, 52	$(GA)_{29}$	36	0.718	0.829	0.134	AY220501	F: AGTTTGCTTCTCAACGCAGTAG
									R: TTCGGCAGTAGATGGCAA
GpC5b	187–239	52	$(GAT)_{10}$	21	0.653	0.753	0.133	AY220502	F: GTTGTTTTCTGCTCCTCAATA
									R: CAAGGGTGTGTCGTCTTC
GpC10b	283–314	52	$(CAT)_9$	27	0.881	0.748	−0.178	AY220303	F: GTTGATGTTGTGATGGTAATGA
									R: GCTGGCAAAGAAACTAATGA
GpC26b	168–201	52	$(CAT)_3CGT(CAT)_{12}$	18	0.776	0.731	−0.062	AY220504	F: GGATCACCCTTCTTGAATG
									R: GGACGTTATTTGTTCGTGTAA
GpD18b	220–229	52	$(CAG)_7$	4	0.014	0.044	0.682*	AY220505	F: CCTGCGATGTTTACCGAG
									R: CGAATCCCTACCTACAAGTCA
Mean ±SD				20.5 ± 10.1	0.59 ± 0.26	0.616 ± 0.246	0.118 ± 0.253		

T_a is the annealing temperature. H_O, the observed heterozygosity, and H_E, the expected heterozygosity

*$P < 0.01$.

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Table 2

Genetic variability in *Glossina* taxa estimated by using primers derived from *G. pallidipes*

Locus	Taxa	*N*	No. of alleles	Size (bp)	H_O	H_E	F_{IS}	H-W (*P*-value)
GpA19a	*G. longipennis*	8	3	194–224	0.750	0.708	−0.059	0.464
	G. austeni	8	5	151–183	0.750	0.857	0.125	1.000
	G. f. fuscipes	8	1	177–178	0.000	0.500	1.000	NA
	G. m. submorsitans	8	7	142–168‡	0.400	0.733	0.454	< 0.001
	G. m. centralis	8	3	142–160	0.500	0.833	0.400	0.064
	G. m. morsitans	8	—	—	—	—	—	—
	G. swynnertoni	8	2	203–209‡	0.708	0.590	−0.200	0.491
	G. brevipalpis	8	2	127–140‡	0.375	0.325	−0.154	0.182
GpA23b	*G. longipennis*	7	6	131–187‡	0.875	0.692	−0.264	< 0.001
	G. austeni	7	8	140–184‡	0.625	0.508	−0.230	< 0.001
	G. f. fuscipes	8	6	149–162‡	0.750	0.500	−0.500	< 0.001
	G. m. submorsitans	8	6	171–188	0.500	0.433	−0.154	0.018
	G. m. centralis	8	10	195–215	0.875	0.867	−0.009	1.000
	G. m. morsitans	8	7	171–196	0.500	0.642	0.221	1.000
	G. swynnertoni	8	6	177–208	0.833	0.748	−0.114	1.000
	G. brevipalpis	7	5	168–198‡	0.875	0.817	−0.071	0.718
GpB6b	*G. longipennis*	8	3	191–200‡	0.750	0.575	−0.304	0.209
	G. austeni	4	5	211–238‡	0.875	0.675	−0.296	0.636
	G. f. fuscipes	8	2	217	0.000	0.000	NA	0.009
	G. m. submorsitans	5	4	153–220	1.000	0.867	−0.153	0.136
	G. m. centralis	2	3	174–188	1.000	0.592	−0.689	0.236
	G. m. morsitans	8	—	—	—	—	—	—
	G. swynnertoni	8	3	158–213‡	0.750	0.500	−0.500	0.118
	G. brevipalpis	8	2	173–206‡	0.875	0.525	−0.666	1.000
GpB20b	*G. longipennis*	8	3	126–185‡	0.286	0.681	0.580	0.127
	G. austeni	8	3	137–168‡	0.286	0.923	0.690	1.000

Locus	Taxa	*N*	No. of alleles	Size (bp)	H_O	H_E	F_{IS}	H-W (*P*-value)
	G. f. fuscipes	8	2	150–181‡	1.000	0.750	−0.333	1.000
	G. m. submorsitans	8	3	155–175	0.625	0.683	0.085	0.364
	G. m. centralis	8	7	157–186‡	1.000	0.900	−0.111	1.000
	G. m. morsitans	8	4	156–185	0.875	0.792	−0.105	< 0.001
	G. swynnertoni	8	6	155–172‡	0.875	0.767	−0.141	1.000
	G. brevipalpis	8	7	160–179‡	0.714	0.791	0.097	< 0.001
GpC5b	*G. longipennis*	7	7	197–212‡	0.125	0.575	0.783	< 0.001
	G. austeni	8	3	237–240	0.500	0.500	0.000	0.218
	G. f. fuscipes	8	6	224	0.000	0.000	NA	1.000
	G. m. submorsitans	8	5	215–221	0.250	0.783	0.681	< 0.001
	G. m. centralis	8	2	210–225	0.500	0.667	0.250	1.000
	G. m. morsitans	8	4	224–239	0.875	0.792	−0.105	< 0.001
	G. swynnertoni	8	2	225–230‡	0.125	0.125	0.000	0.018
	G. brevipalpis	8	4	191–206‡	0.375	0.325	−0.154	1.000
GpC10b	*G. longipennis*	7	5	306–337‡	0.429	0.593	0.277	0.591
	G. austeni	8	5	267–281	0.375	0.692	0.458	0.100
	G. f. fuscipes	8	2	300–306	0.000	0.233	1.000	0.046
	G. m. submorsitans	7	6	275–314	0.714	0.868	0.177	< 0.001
	G. m. centralis	8	5	275–285	0.750	0.600	−0.250	1.000
	G. m. morsitans	3	2	282–288	0.333	0.333	0.000	1.000
	G. swynnertoni	8	2	293–294	0.125	0.125	0.000	1.000
	G. brevipalpis	8	6	293–350	0.750	0.817	0.082	< 0.001
GpC26b	*G. longipennis*	8	3	147–204‡	0.286	0.890	0.687	< 0.001
	G. austeni	8	2	183–198	0.375	0.708	0.470	1.000
	G. f. fuscipes	8	1	151–215‡	0.625	0.683	0.085	NA
	G. m. submorsitans	8	5	180–198	0.375	0.600	0.375	< 0.001
	G. m. centralis	8	5	190–202	0.250	0.233	−0.073	< 0.001
	G. m. morsitans	8	5	190–194	0.875	0.675	−0.296	0.600
	G. swynnertoni	8	2	187–188	0.000	0.400	1.000	1.000
	G. brevipalpis	8	2	177–190‡	0.500	0.442	−0.131	1.000

Locus	Taxa	*N*	No. of alleles	Size (bp)	H_O	H_E	F_{IS}	H-W (*P*-value)
GpD18b	*G. longipennis*	8	2	139–140	0.000	0.400	1.000	0.064
	G. austeni	8	2	143–226‡	0.125	0.125	0.000	1.000
	G. f. fuscipes	8	3	142–264‡	0.375	0.608	0.383	0.091
	G. m. submorsitans	8	3	223–226	0.000	0.633	1.000	< 0.00
	G. m. centralis	8	2	222–223	0.000	0.533	1.000	< 0.001
	G. m. morsitans	8	—	—	—	—	—	—
	G. swynnertoni	8	2	216–222	0.000	0.233	1.000	0.082
	G. brevipalpis	8	2	226–227	0.000	0.500	1.000	< 0.001

N, sample size; H_O, observed heterozygosity, H_E, expected heterozygosity; —, no or inadequate amplification; H-W (*P*-value), Hardy–Weinberg probability

‡PCR yielded multiple bands, NA, not applicable.