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Polymorphic microsatellite markers for the tsetse fly *Glossina fuscipes fuscipes* (Diptera: Glossinidae), a vector of human African trypanosomiasis

J. E. BROWN,* K. J. KOMATSU,* P. P. ABILA,† A. S. ROBINSON,‡ L. M. A. OKEDI,† N. DYER,§ M. J. DONNELLY,§ M. A. SLOTMAN* and A. CACCONE*

**Department of Ecology and Evolutionary Biology, Yale University, 21 Sachem Street, New Haven, CT 06511, USA,*
†National Livestock Resources Research Institute, PO Box 96, Tororo, Uganda, ‡Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Insect Pest Control Sub-Programme, International Atomic Energy Agency, A-1400 Vienna, Austria,
§Vector Research Group, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK

Abstract

Our understanding of *Glossina fuscipes fuscipes*, a major vector of sleeping sickness, has been severely constrained by a lack of genetic markers for mapping and population genetic studies. Here we present 10 newly developed microsatellite loci for this tsetse species. Heterozygosity levels in Moyo, an Ugandan population, averaged 0.57, with only two loci showing very low heterozygosity. Five loci carried more than six alleles. Together with five recently published microsatellite loci, this brings the number of available microsatellite loci for this species to 15. Their availability will greatly facilitate future studies on the genetics of this important human disease vector.

Keywords: genetic marker, *Glossina fuscipes*, microsatellite loci, sleeping sickness, tsetse

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The tsetse fly *Glossina fuscipes* is the primary vector of human African trypanosomiasis (sleeping sickness) in Uganda, where it is represented by the subspecies *Glossina fuscipes fuscipes*. Although *G. fuscipes* is a major human disease vector, little information is available on the genetics of this species. One of the reasons for this dearth of knowledge is the lack of suitable genetic markers for population genetic and mapping studies.

A total of 16 microsatellite loci are available for *Glossina palpalis palpalis* (Luna *et al*. 2001) and the related *Glossina palpalis gambiensis* (Solano *et al*. 1997). Unfortunately, amplification of these microsatellite loci in *G. f. fuscipes* is inconsistent, with most loci failing to amplify in 20–80% of *G. f. fuscipes* individuals (Luna *et al*. 2001), limiting their usefulness for this species.

Previously we developed five microsatellite loci for *G. f. fuscipes* for a preliminary population genetic study of this subspecies in Uganda, where it transmits two geographically isolated *Trypanosoma* parasites (Abila *et al*. 2008). However, for more detailed studies, additional genetic markers are needed. Therefore, we developed 10 new microsatellite loci and examined their variability in two Ugandan populations.

A microsatellite-enriched library was constructed, as described in Abila *et al*. (2008), from a *G. f. fuscipes* strain established from the Central African Republic in 1986. This library consisted of 42 clones containing di- or trinucleotide repeats. From these we identified loci containing at least eight uninterrupted dinucleotide repeats. Primers were developed for 10 loci that matched these criteria using Primer 3 (Rozen & Skaletsky 2000). Sequences of these loci, as well as for the five loci recently published (Abila *et al*. 2008), have been submitted to GenBank under accession nos EU834297–EU834311.

Variability of the loci was examined in 25 *G. f. fuscipes* specimens from Uganda. Seventeen specimens were collected in 2004 in Moyo in northwestern Uganda and eight specimens were collected from Buvuma Island in Lake Victoria in 2007. DNA was extracted using the Easy-DNA kit (Invitrogen). Polymerase chain reaction (PCR) amplifications for four of the loci were performed using a HEX-labelled forward primer and unlabelled reverse primer (Table 1). Because of cost considerations, PCR amplifications

Correspondence: Michel A. Slotman, Fax: 203 4327394; E-mail: maslotman@ag.tamu.edu

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Table 1 Characterization of 11 microsatellite loci in the tsetse fly *Glossina fuscipes fuscipes*

Locus		Primer sequence	T_A	No. of alleles Moyo/Buv. Total	Size range	2 N	H_O	H_E	*P* value Moyo Buvuma
Gff_B8*	FOR	TGACCACTAAATCGTCCTCAC	48 °C	9/4	183–217	32/16	0.81	0.70	0.726
EU834303	REV	TCCCGAGCTTAGAGAGAGTG		10		48	0.25	0.53	0.370
Gff_C107*	FOR	CGCGGCACCTGTTAGTTAGT	50 °C	7/3	189–245	26/16	0.77	0.85	0.528
EU834304	REV	GGCACGTGAACTAATGCAAA		9		42	0.13	0.24	0.067
Gff_D6†	FOR	TAACGGGGAGCTAAAAGAAG	52 °C	3/3	259–279	34/16	0.47	0.40	1
EU834305	REV	AAATCATCAGCAGCATCATC		4		50	0.13	0.34	0.067
Gff_D109*	FOR	ATCTGCCAATAGCATGAATATC	58 °C	3/3	153–177	34/16	0.41	0.40	0.656
EU834306	REV	CAGTTGGTGTCCGTGTGT		4		50	0.25	0.34	0.200
Gff_A3†	FOR	CGAAGCAGGTAGATAGAATCC	48 °C	10/5	227–258	30/14	0.87	0.80	0.542
EU834297	REV	CTGCATTGGAAGTTACTTATCC		13		44	0.14	0.82	0.000
Gff_A6*	FOR	TACAATCAAAGGTGGGTCTATC	50 °C	6/2	257–267	32/16	0.69	0.75	0.380
EU834298	REV	CCGAACGAATACAGGTAAAG		6		48	0.13	0.33	0.200
Gff_A9*	FOR	AACTGTTTCTTCAACAAAAATCCA	46 °C	3/3	170–174	34/16	0.59	0.56	0.679
EU834299	REV	GAAAATCGCGCATATTGCTA		3		50	0.25	0.49	0.077
Gff_A112*	FOR	CGTTTCCTCTTCACCTCCAC	56 °C	3/2	121–133	34/16	0.12	0.12	1
EU834301	REV	CGGGCTGTCTTCTTTTGG		4		50	0.13	0.13	1
Gff_B101†	FOR	CGACTTTTTAGAGCCAGTGC	48 °C	11/6	268–308	30/16	0.93	0.89	0.423
EU834302	REV	TTTGGTTGTTCATTCCTCAAC		14		46	0.88	0.77	0.654
Gff_A10†	FOR	GCAACGCCAAGTGAAATAAAG	48 °C	2/2	184–213	34/16	0.06	0.06	1
EU834300	REV	TACTGGGCTCGCGTACATAAT		3		50	0.13	0.13	1

*PCRs of these loci were performed using a HEX or FAM labelled M13-tailed forward primer; †PCRs of these loci were performed using a HEX-labelled forward primer. N = sample size.

for the remaining six loci were performed using an M13-tailed forward primer, a fluorescently labelled M13 primer, and a reverse primer (Boutin-Ganache *et al*. 2001). The following programme was used to amplify all 11 loci: 96 °C for 10 min, followed by 40 cycles at 96 °C for 1 min, 48–58 °C (see Table 1) for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 15 min. PCRs were run on a Thermo Hybaid MBS 0.2 G (Thermo Hybaid). PCRs (25 μL) using a fluorescently labelled forward primer contained 1× PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 500 nmol of forward primer, and 500 nmol of reverse primer. PCRs using the M13-tailed forward primer contained 1× PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 200 μM of each dNTP, 40 nmol of forward primer, 500 nmol of reverse primer, 600 nmol of fluorescently labelled M13 primer, and 1.5 U of Ampli*Taq* Gold polymerase (Applied Biosystems). PCR products were run with a 500-bp size standard on an ABI 3100 capillary sequencer (PerkinElmer). Gels were analysed using GeneMapper version 3.7 (Applied Biosystems). One additional attempt was made to amplify samples that did not yield a PCR product on the first try.

Observed and expected heterozygosities were calculated using Arlequin, version 3 (Excoffier *et al*. 2005). This software was also used to test for deviations from Hardy–Weinberg (10 000 Markov chains) and linkage equilibrium. For the linkage disequilibrium analysis, the five loci we recently developed (Abila *et al*. 2008) were included. The program Micro-Checker (van Oosterhout *et al*. 2004), which distinguishes between various causes of deviations from Hardy–Weinberg equilibrium, was used to estimate the frequency of null alleles.

For five of the 10 loci, six or more alleles were observed in the sample from Moyo (Table 1). The average observed heterozygosity was 0.57 for the Moyo population, ranging from 0.06 to 0.93. Two loci, Gff_A10 and Gff_A112, showed relatively low variability in Moyo. However, it is possible that these loci are more polymorphic in other populations. With the exception of locus Gff_B101, all loci had very low heterozygosity in the Buvuma population. The average observed heterozygosity was 0.24, ranging from 0.13 to 0.88. Buvuma is a small island located in Lake Victoria and this low variability is likely due to a small effective population size or a founder effect or both.

No significant deviations from Hardy–Weinberg equilibrium were detected in the Moyo sample after sequential Bonferroni correction. Neither did Micro-Checker detect the presence of null alleles in the Moyo samples. In the Buvuma sample, locus Gff_A3 deviated significantly from Hardy–Weinberg equilibrium after sequential Bonferroni correction, while Gff-B8 deviated significantly only before correction. Micro-Checker also indicated significant deficiencies of heterozygotes in this population, indicating the possible presence of null alleles for locus Gff_A3.

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In the Moyo sample, none out of the 105 pairwise comparisons between 15 loci showed significant linkage disequilibrium after Bonferroni correction, indicating that these loci can be considered independent markers. Similarly, none of the loci showed significant linkage disequilibrium after Bonferonni correction in the Buvuma samples, but the power of this test was low because sample size was very small for this population.

Together with the five previously published polymorphic loci (Abila *et al*. 2008), 15 polymorphic microsatellite loci are now available for *G. f. fuscipes*. These loci will greatly facilitate a variety of studies of this important vector of human African trypanosomiasis. Such studies will increase our understanding of the transmission of sleeping sickness, which will no doubt contribute to our ability to combat this devastating disease.

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