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New Polymorphic Microsatellites in *Glossina pallidipes* (Diptera: Glossinidae) and Their Cross-Amplification in Other Tsetse Fly Taxa

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Abstract

We report the development and characterization of three new microsatellite markers in the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). Fifty-eight alleles were scored in 192 individuals representing six natural populations. Allelic diversity ranged from 9 to 28 alleles per locus (mean 19.3 ± 5.5). Averaged across loci, observed heterozygosity was 0.581 ± 0.209, and expected heterozygosity was 0.619 ± 0.181. Cross-species amplifications of the *G. pallidipes* loci in other tsetse fly taxa are reported.

Keywords

tsetse flies; *Glossina*; microsatellites

INTRODUCTION

Tsetse flies (Diptera: Glossinidae) are discontinuously distributed throughout sub-Saharan Africa (Rogers and Robinson, 2004). They feed exclusively on blood and transmit hemoflagellate protozoan parasites, trypanosomes that cause sleeping sickness in humans and nagana in livestock. Tsetse taxa are subdivided into three subgenera, *Glossina* (*morsitans* group), *Nemorhina* (*palpalis* group), and *Austenina* (*fusca* group). *Glossina pallidipes* belongs to the *morsitans* group and is among the most important vectors of trypanosomes.

Earlier genetic studies on tsetse flies were based largely on cytological and allozyme methods (Krafsur and Griffiths, 1997; Gooding and Krafsur, 2005). Adequately sampling geographically diverse tsetse fly populations is difficult and expensive. Recent progress in tsetse fly population genetics includes the Solano *et al.* (1997) examination of microsatellite variation in *Glossina palpalis gambiensis* and *G. p. palpalis* natural populations. Baker and Krafsur (2001) and Ouma *et al.* (2003) uncovered microsatellite loci in the *morsitans* group of tsetse flies, and the population genetics of diverse natural *morsitans* group populations have now been investigated (reviews in Krafsur, 2003; Gooding and Krafsur, 2005). Additional genomic DNA markers would be most useful, and here we report three new microsatellite loci and their homologs in other tsetse fly taxa.

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METHODS AND MATERIALS

Genomic DNA was extracted from *Glossina pallidipes* obtained from a colony established at the International Atomic Energy Agency, Seibersdorf, Austria. Approximately 100 µg of DNA was used to construct four genomic libraries enriched for CA, GA, ATG, and CAG microsatellites. Libraries were constructed by Genetic Identification Services (http://www.genetic-id-services.com; Chatsworth, CA). Enriched *G. pallidipes* DNA fragments were ligated into the *Hin*dIII cut site of pUC19 plasmid and electroporated into *Escherichia coli* strain DH5α (Electro-Max, Gibco). After transformation, *E. coli* cells were screened for inserts between 350 and 700 bp by polymerase chain reaction (PCR) amplification. PCR and sequencing of individual clones was performed using forward and reverse universal M13 primers and ABI Prism BigDye Terminator Chemistry as previously described (Ouma *et al.*, 2003).

Fifty microsatellite clones were sequenced. PCR primers were designed for 28 of these clones by using the software DesignerPCR, version 1.03 (Research Genetics, Inc.). Three to eight primer pairs were designed for each locus, and the pair yielding a specific PCR as determined by a single band on 1% agarose gel was selected for use. Oligonucleotides were synthesized by Integrated DNA Technologies, Coralville, Iowa.

To evaluate presumptive loci for polymorphisms, *G. pallidipes* were sampled from six natural populations in Kenya and Tanzania. In all, 24–48 individuals were genotyped per population for a total of 192 flies (136 females and 56 males). PCR was carried out on a PTC-100 thermocycler (MJ Research). Reaction volumes were 10 µL and contained 25 ng template DNA, 1 × Biolase PCR buffer, 2.5 $MgCl_2$, 0.4 mM dNTPs, 0.4 units Biolase polymerase (Bioline USA, Springfield, NJ), 0.5 µM each of forward and reverse primers. The forward primers were fluorescently labeled with FAM or HEX. Primers were also tested for cross-amplification of DNA from nine species representing the three *Glossina* subgenera.

FStat version 2.9.3.2 was used to estimate genetic diversity (Goudet, 1995). Arlequin version 2.0 (Schneider *et al.*, 2000) was used to test for Hardy–Weinberg equilibrium. Micro-Checker (van Oosterhout *et al.*, 2004) was used to test for causes of departures from Hardy–Weinberg equilibrium. Genotypic disequilibrium was tested using the log-likelihood ratio G-statistic. Here F_{IT} estimates departures from random mating from all causes, and F_{IS} the departure from random mating within populations.

RESULTS AND DISCUSSION

All loci were autosomal. Two were trinucleotide repeats, one of which, *GpC107*, was compound (Table I). The loci were highly polymorphic in *Glossina pallidipes.* The number of alleles per locus ranged from 9 to 28, with a mean of 19.3 (Table I). Mean observed and expected heterozygosity was 0.581 and 0.619, respectively, leading to a modest F_{IT} estimate of 0.06. The least diverse locus was *GpC107*; it did not occur in Hardy–Weinberg proportions in any population. Rules postulated in Micro-Checker software indicated that large allele dropouts and allelic stutterings were unlikely causes of the deficit, thereby pointing to a null allele frequency of 0.125. Allele frequency distributions showed 43 rare (<5%) of a total of 58 alleles summed over loci, for an overall mean of 74% (Fig. 1). No significant genotypic linkage was detected in 18 locus–population combination tests.

Cross-amplification of species within a genus is commonly observed, and a pronounced ascertainment bias was recorded. The mean number of alleles among the *fusca* and *palpalis* group was 2.78, and in the *morsitans* group (apart from *G. pallidipes*) it was 4.8 alleles per locus.

Our primers (Table I) did not amplify *GpB115* in *Glossina brevipalpis*, *G. longipennis*, or *G. palpalis gambiensis* (Table II). *GpC101* and *GpC107* were monomorphic in *G. fuscipes fuscipes*; *GpC101* was monomorphic in *G. brevipalpis*. No homozygotes were recorded at *GpB115* in *G. m. morsitans* and *G. austeni* (Table II), the reason for which is obscure. Significant departures from random mating (F_{IS}) were observed at *GpC101* in *G. longipennis* and *G. swynnertoni*, *GpB115* in *G. austeni*, and *GpC107* in *G. m. submorsitans*. The best amplifications were in *morsitans* group flies. The three loci can be used in *G. m. morsitans* and *G. m. centralis*. There now is at least one new and well-behaved locus in *G. m. submorsitans*, *G. austeni*, and *G. swynnertoni*.

This report brings to 12 the number of microsatellite loci isolated from *Glossina pallidipes*.

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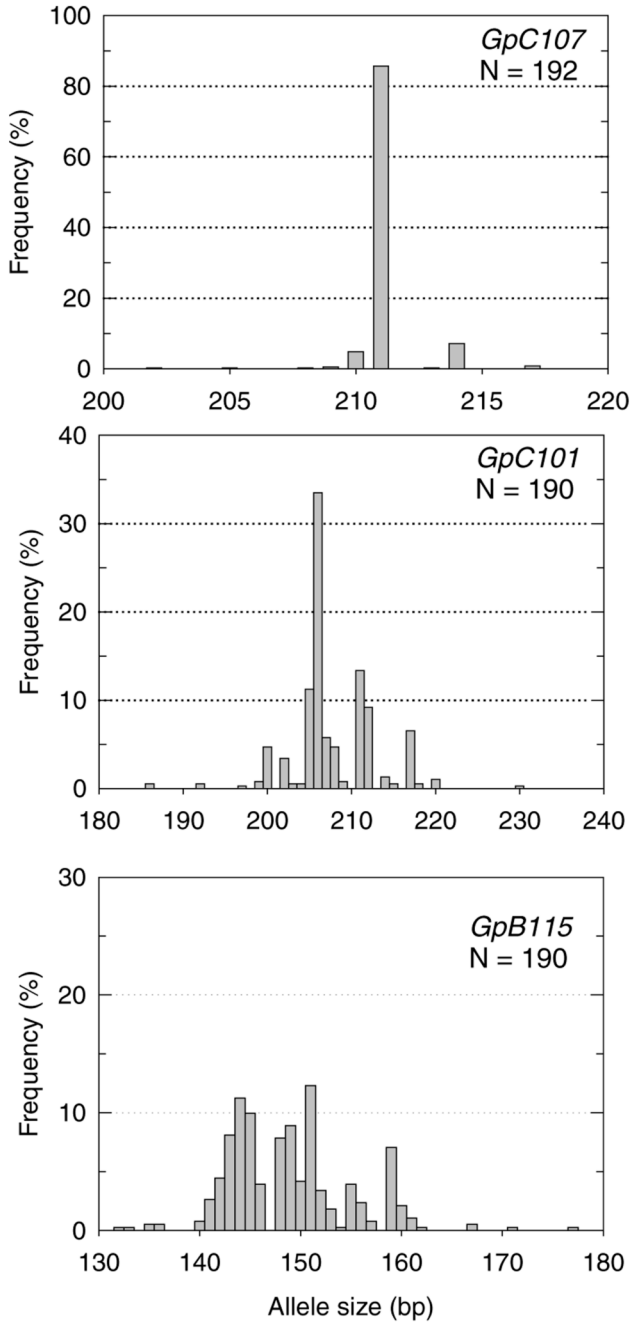

Fig. 1.
Allele frequency distribution in *G. pallidipes* microsatellite loci.

Table I

Characterization of New Microsatellite Loci in 192 *Glossina pallidipes* Representing Six Populations

Locus	Repeat motif	No. alleles	Allele size (bp)	H_O	H_E	F_{IT}	Primer sequence (5′–3′)	GenBank accession no.
GpB115	$(CT)_{10}$	28	132–177	0.916	0.825	−0.110	**F**:AGCGATAGAAAGGGTCAATC **R**:CGTAGAGATAGCGAGAGTGTG	DQ168823
GpC101	$(TGA)_{11}$	21	186–230	0.630	0.773	0.185	**F**:CCTCAATACAGCAGCAGATG **R**:CAAGGTGTGTTGTCGTCTTC	DQ168824
GpC107	$(CAG)_5(CAA)_5$	9	202–217	0.197	0.259	0.239***	**F**:CAATCGCAACAACATCAAAC **R**:GGCAATAACAACTGTCTGTGG	DQ168825
Mean		19.3		0.581	0.619	0.105		
S.E		5.5		0.209	0.181	0.072		

*** γ^2 (36) = 86.8, $P < 0.001$.

Table II

Cross-Amplification and Variability in Microsatellite Loci in Tsetse Fly Taxa

Group (subgenus)		Locus	*N*	No. alleles	Allele size range	h_o	h_e	F_{IS}
Fusca (*Austenina*)	*G. brevipalpis*	*GpB115*	—	—	—	—	—	—
		GpC101	15	1	187	0.000	0.000	NA
		GpC107	8	2	205–208	0.438	0.514	0.148
	G. longipennis	*GpB115*	15	6	213–224	0.467	0.818	0.429
		GpC101	16	3	173–193	0.063	0.280	0.775***
		GpC107	16	2	208–211	0.375	0.315	−0.190
Palpalis (*Nemorhina*)	*G. f. fuscipes*	*GpB115*	—	—	—	—	—	—
		GpC101	16	1	208	0.000	0.000	NA
		GpC107	16	1	211	0.000	0.000	NA
	G. p. gambiensis	*GpB115*	—	—	—	—	—	—
		GpC101	15	5	199–211	0.800	0.733	−0.091
		GpC107	16	4	205–214	0.563	0.569	0.011
Morsitans (*Glossina*)	*G. austeni*	*GpB115*	14	3	194–209	1.000	0.553	−0.808***
		GpC101	14	6	210–234	0.714	0.812	0.121
		GpC107	16	1	211	0.000	0.000	NA
	G. m. centralis	*GpB115*	14	4	129–163	0.500	0.476	−0.050
		GpC101	12	6	200–212	0.750	0.685	−0.095
		GpC107	12	4	214–220	0.667	0.772	0.136
	G. m. morsitans	*GpB115*	15	6	137–162	1.000	0.697	−0.435
		GpC101	14	10	202–228	0.643	0.860	0.252
		GpC107	15	5	205–217	0.267	0.308	0.133
	G. m. submorsitans	*GpB115*	16	9	144–168	0.813	0.784	−0.037
		GpC101	15	5	200–206	0.267	0.595	0.551*
		GpC107	16	4	211–217	0.063	0.413	0.847***
	G. swynnertoni	*GpB115*	16	4	131–140	0.688	0.534	−0.085
		GpC101	16	3	208–210	0.000	0.476	1.000***
		GpC107	16	2	205–211	0.063	0.063	0

*$P \sim 0.05$.

***$P < 0.001$

$\gamma^2 = F^2 N\,(k-1)$, df = $k\,(k-1)/2$, where k = number of alleles and N = sample size.