

Shared Microsatellite Loci in *Glossina morsitans sensu lato* (Diptera: Glossinidae)

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ABSTRACT Estimation of allelic frequencies at three microsatellite loci among 20 populations of *Glossina morsitans morsitans* Westwood, *Glossina morsitans submorsitans* Newstead, and *Glossina morsitans centralis* Machado indicated only two of 99 alleles were shared between three subspecies and 18 between any two subspecies; 81 alleles were unshared. The conserved flanking regions of each locus were completely shared. Genetic differentiation among subspecies, based on allele size, was $R_{ST} = 0.87$, close to the theoretic maximum value. All evidence suggests longstanding and complete reproductive isolation in nature among the sibling species. They should be elevated to specific rank.

KEY WORDS microsatellite loci, tsetse flies, sibling species, species complex

Glossina morsitans sensu lato (s.l.) is a complex of three allopatric subspecies: *Glossina morsitans morsitans* Westwood, *Glossina morsitans centralis* Machado, and *Glossina morsitans submorsitans* Newstead. Their approximate geographical distributions have been mapped (Ford 1971, Robinson et al. 1997, Rogers and Robinson 2004). Although the subspecies freely intermate in cages, hybrid male progeny are sterile and the females nearly so (Curtis 1972, Gooding 1990, Gooding and Krafur 2005). Their morphological differentiation was deemed insufficient to confer specific status; indeed, Machado (1970) referred to them as only "major geographic races." But speciation without morphological differentiation is not uncommon, particularly among the Diptera. There are numerous examples of sibling species in well studied families such as the Culicidae (Coluzzi et al. 2002), Psychodidae (Lanzaro et al. 1993), and Simuliidae (Vajime and Gregory 1990).

Materials and Methods

Microsatellite loci, composed of simple sequence repeats, were isolated from the nuclear genomes of *Glossina morsitans* s.l. and *Glossina pallidipes* Austen (Baker and Krafur 2001). These loci were used to confirm or deny hypotheses about gene flow among 20 populations of *G. morsitans* s.l. that included seven populations of *G. m. submorsitans*, seven populations of *G. m. centralis*, and six populations of *G. m. morsitans* (Krafur et al. 2001, Krafur 2002, Krafur and Endsley 2002). Three of 11 loci were found to amplify via the polymerase chain reaction (PCR) consistently well in each subspecies. Annealing sites are often found to be shared among congeners. Here, we examine the re-

sults to see what they show about the genetic relationships among taxa.

Details of sampling tsetse flies and amplification and demonstration of microsatellite loci were described previously (Wohlford et al. 1999, Krafur et al. 2000, Krafur and Endsley 2002). Primers and GenBank accession numbers for loci *GpCAG33*, *Gmm5B*, and *Gmm22* were cited in Baker and Krafur (2001). PCR products were electrophoresed on 5% denaturing polyacrylamide gels (32 by 50 cm by 0.4 mm) by using a Sequi-Gen GT nucleic acid electrophoresis system (Bio-Rad, Hercules, CA) run at 100 W and 50°C. Gels were silver stained according to methods outlined in Black and DuTeau (1996). Alleles were distinguished by their mobilities relative to DNA standards. Because sample numbers were not equal, allelic richness was estimated by rarefaction, which provides the expected number of alleles and private alleles unbiased by sample size and number of genes (Kalinowski 2004). Genetic statistics were computed by using FSTAT 2.9.3 (Goudet 1995), Genepop (Raymond and Rousset 1995), and HP-RARE (Kalinowski 2005).

Results

Shared and Unshared Variation among Subspecies. Three loci common to each *G. morsitans* subspecies permitted a straightforward comparison among taxa. Six annealing sites were shared, allowing PCR amplification of the microsatellite repeats. In total, 99 alleles were detected among the three microsatellite loci (Table 1), 81 of which were confined to only one or another subspecies. None of 27 *GpCAG133* alleles was shared among subspecies. The three loci occur on the same chromosome (*G. morsitans* s.l. have two pairs of autosomes), but *Gmm5B* segregated independently in

Table 1. Shared and unshared microsatellite alleles among *G. morsitans* subspecies

Locus	<i>G. m. centralis</i> (n = 155)		<i>G. m. submorsitans</i> (n = 162)		<i>G. m. morsitans</i> (n = 138)		Shared alleles, 2 subspecies
	Alleles	Private	Alleles	Private	Alleles	Private	
<i>GpCAG133</i>	12	12	9	9	6	6	0
<i>Gmm5B</i>	6	6	20	11	20	12	9
<i>Gmm22</i>	10	6	22	13	12	6	9
Total	28	24 (86%)	51	33 (65%)	38	24 (63%)	18

Unshared alleles among taxa: $\chi^2_{(2)} = 4.72, P \approx 0.09$; unshared alleles among loci: $\chi^2_{(2)} = 8.34, P \approx 0.015$.

G. m. submorsitans and *G. m. morsitans*. *Gmm5B* genotypes were not independent in *G. m. centralis*. *Gmm22* and *CAG133* genotypes were not independent in *G. m. submorsitans* and *G. m. morsitans*.

The proportions of private (i.e., unshared) alleles were homogeneous among taxa, but significant differences were detected among loci (Table 1). Only two alleles, at *Gmm22*, were shared among three subspecies. Thirteen of 89 alleles (15%) were shared between *Glossina m. morsitans* and *G. m. submorsitans*. *G. m. centralis* shared four of 28 alleles with *G. m. submorsitans* (14%) but only two alleles with *G. m. morsitans* (7%). Rarefaction of the genotypic data estimated the expected numbers of alleles to be the same as recorded in each taxon.

Genetic Differentiation among Taxa. R_{ST} estimates the variance in allele size (i.e., base pairs) among taxa. The weighted mean over loci was 0.87, not greatly different from the maximum possible value of 1.0. Pairwise estimates of R_{ST} are in Table 2 and suggest that *G. m. submorsitans* and *G. m. morsitans* are more closely related to each other than either is to *G. m. centralis*.

Discussion

Some shared alleles probably arose by homoplasy, similar in state but not by common ancestry. Homoplasy in microsatellites is common because the number of repeats is limited, and mutation rates tend to be high (Xu et al. 2000).

The small proportion of variation shared between subspecies testifies to their long reproductive isolation. It is particularly interesting that only two alleles were shared between *Glossina m. centralis* and *G. m. morsitans* ($R_{ST} = 0.82$) because these taxa occupy contiguous but not overlapping areas in southern Africa. In Zambia, the Muchinga mountains separate the two taxa, which are in proximity in central Zambia (Robinson et al. 1997, Rogers and Robinson 2004). The microsatellite data are consistent with the notion that *G. m. submorsitans* and *G. m. morsitans* are more closely related to each other than either is to *G. m.*

centralis, but three loci are inadequate to accept such a conclusion. It may be noted, moreover, that *G. m. centralis* showed the least mitochondrial and microsatellite diversity (Krafsur et al. 2001). The relative paucity of variation in *G. m. centralis* probably is related to earlier bottlenecks in population size. Bottlenecked populations rapidly lose by genetic drift alleles in the lowest frequencies and the return to mutation-drift equilibrium is slow (Nei 1987). The historical record suggests the rinderpest epizootic of 1889–1896 was severe in southern Africa and was said to have killed $\approx 90\%$ of the mammalian fauna (reviewed by Ford 1971). Mitochondrial and microsatellite diversities indicate greater loss in *G. m. centralis* populations than in *G. m. morsitans* (and greater losses in southern than in northern *G. pallidipes* populations; Krafsur 2002), but further sampling throughout the entire range of *G. m. centralis* is required to confirm this hypothesis.

Abundant evidence exists to elevate *G. morsitans* subspecies to specific rank. The evidence is 1) allopatry, 2) sterile male hybrids and sterile or semisterile female hybrids when subspecies are interbred in the laboratory, 3) gene flow within but not between subspecies, and 4) ancient separation of taxa. All data indicate strong measures of intrinsic and extrinsic reproductive isolation, the ultimate test of speciation (Wheeler and Meier 2000).

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References Cited

Baker, M. D., and E. S. Krafsur. 2001. Identification and properties of microsatellite markers in tsetse flies *Glossina morsitans* sensu lato (Diptera: Glossinidae). *Mol. Ecol. Notes* 11: 234–236.

Black, W. C., and N. M. DuTeau. 1996. RAPD-PCR and SSCP analysis for insect population genetic studies, pp 361–383. *In* J. M. Crampton, C. B. Beard, and C. Louis [eds.], *Molecular biology of insect disease vectors: a methods manual*. Chapman & Hall, London, United Kingdom.

Coluzzi, M., A. Sabatini, A. della Torre, M. A. Di Deco, and V. Petrarca. 2002. A polytene chromosome analysis of

Table 2. Pairwise estimates of R_{ST} among *G. morsitans* s.l.

	<i>G. m. submorsitans</i>	<i>G. m. centralis</i>
<i>G. m. centralis</i>	0.871	
<i>G. m. morsitans</i>	0.087	0.817

- the *Anopheles gambiae* species complex. *Science* (Wash. DC.) 298: 1415–1418.
- Curtis, C. F. 1972. Sterility from crosses between sub-species of the tsetse fly *Glossina morsitans*. *Acta Trop.* 29: 250–68.
- Ford, J. 1971. The role of the trypanosomiasis in African ecology. A study of the tsetse fly problem. Clarendon Press, Oxford, United Kingdom.
- Gooding, R. H. 1990. Postmating barriers to gene flow among species and subspecies of tsetse flies (Diptera: Glossinidae). *Can. J. Zool.* 68: 1727–1734.
- Gooding, R. H., and E. S. Krafur. 2005. Tsetse genetics: contributions to biology, systematics, and control of tsetse flies. *Annu. Rev. Entomol.* 50: 101–123.
- Goudet, J. 1995. FSTAT: a computer program to calculate F-statistics. *J. Hered.* 86: 485–486.
- Kalinowski, S. T. 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conserv. Genet.* 5: 539–543.
- Kalinowski, S. T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* 5: 187–189.
- Krafur, E. S. 2002. Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite, and mitochondrial gene diversities. *Insect Mol. Biol.* 11: 37–45.
- Krafur, E. S., and M. A. Endsley. 2002. Microsatellite diversities and gene flow in the tsetse fly, *Glossina morsitans* s.l. *Med. Vet. Entomol.* 16: 292–300.
- Krafur, E. S., M. Madsen, D. L. Wohlford, S. Mihok, and N. T. Griffiths. 2000. Population genetics of *Glossina morsitans submorsitans* (Diptera: Glossinidae). *Bull. Entomol. Res.* 90: 329–335.
- Krafur, E. S., M. A. Endsley, D. L. Wohlford, N. T. Griffiths, and R. Allsopp. 2001. Genetic differentiation of *Glossina morsitans centralis* populations. *Insect Mol. Biol.* 10: 387–395.
- Lanzaro, G., K. Ostrovska, M. Herrero, P. Lawyer, and A. Warburg. 1993. *Lutzomyia longipalpis* is a species complex: genetic divergence and inter-specific hybrid sterility among three populations. *Am. J. Trop. Med. Hyg.* 48: 839–847.
- Machado, A. de Barros. 1970. Les races géographiques de *Glossina morsitans*, pp. 471–486. In J. F. de Azevedo [ed.], *Tsetse fly breeding under laboratory conditions and its practical application*. First International Symposium, 11–23 April 1969, Lisbon. Junta Invest. Ultramar.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- Raymond, M., and F. Rousset. 1995. GENEPOP: population genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248–249.
- Robinson, T. P., D. J. Rogers, and B. Williams. 1997. Univariate analysis of tsetse habitat in the common fly belt of southern Africa using climate and remotely sensed vegetation data. *Med. Vet. Entomol.* 11: 223–234.
- Rogers, D. J., and T. P. Robinson. 2004. Tsetse distribution, pp 139–179. In I. Maudlin, P. Holmes, and M. Miles [eds.], *The trypanosomiasis*. CAB International, Oxford, United Kingdom.
- Vajime, C. G., and W. G. Gregory. 1990. Onchocerciasis: species complex of vectors and epidemiology. *Acta Leidensia* 59: 235–252.
- Wheeler, Q. D., and R. Meier. 2000. *Species concepts and phylogenetic theory. A debate*. Columbia University Press, New York.
- Wohlford, D. L., E. S. Krafur, N. T. Griffiths, J. G. Marquez, and M. D. Baker. 1999. Genetic differentiation of some *Glossina morsitans morsitans* populations. *Med. Vet. Entomol.* 13: 377–385.
- Xu, X., M. Peng, and Z. Fang. 2000. The direction of microsatellite mutations is dependent upon allele length. *Nat. Genet.* 24: 396–399.

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