Molecular Ecology Notes (2002) **2**, 531–533 doi: 10.1046/j.1471-8278 .2002.00307.x

PRIMER NOTE

Characterization of 16 microsatellite marker loci in the Maasai giraffe (*Giraffa camelopardalis tippelskirchi*)

RYAN M. HUEBINGER,* DONNA J. PIERSON,† THOMAS W. DE MAAR,‡ DAVID M. BROWN,§ RICK A. BRENNEMAN† and EDWARD E. LOUIS, JR.†

**Department of Wildlife and Fisheries, Texas A & M University, College Station, TX 77843, USA, †Center for Conservation and Research, Henry Doorly Zoo, 3701 South 10th Street, Omaha, NE 68107, USA, ‡Ol Jogi, Ltd, Nanyuki, Kenya, §Department of Organismic Biology, Ecology, and Evolution, University of California, 621 Charles E. Young Drive, Los Angeles CA 90095–1606*

Abstract

Sixteen polymorphic microsatellite markers with an average allele size of k = 4.3 are identified from a genomic plasmid library constructed for giraffe (*Giraffa camelopardalis* ssp.). Primer sequences and marker data are reported in tabular form. The markers were screened in a population of 25 Maasai giraffe (*G. c. tippelskirchi*) collected near the Athi River, Kenya. The average observed heterozygosity for each marker was 0.36 with an average expected heterozygosity of 0.535. Hardy–Weinberg deviations are reported from this population, which is suspected to be inbred. The markers will be used to screen the captive giraffe population for subspecific or hybrid classification.

Keywords: genetic markers, Giraffa, giraffe, inbreeding, microsatellite

Received 27 May 2002; revision received 22 July 2002; accepted 26 July 2002

Giraffe (*Giraffa camelopardalis*) are classified into nine subspecies (*G. c. angolensis*, *antiquorum*, *camelopardalis*, *giraffa*, *peralta*, *reticulata*, *rothschildi*, *tippelskirchi*, and *thornicrofti*) based on trunk spotting patterns and historic geographical location (Ansell 1968). The Maasai giraffe (*G. c. tippelskirchi*) are generally distinguished by a leaf-shaped pelage pattern, liver-coloured over cream, and range throughout Tanzania into southeastern Kenya. Samples were obtained via remote system biopsy darts on 25, free-ranging Maasai giraffe from the Athi River Ranch in southeastern Kenya.

Genomic DNA was isolated from tissue from a captive giraffe housed at Omaha's Henry Doorly Zoo (HDZ), in Omaha, NE, using a standard protocol (Sambrook *et al*. 1989). Procedures for construction of the genomic library, identification of plasmids containing $(GT)_n$ inserts as well as plasmid preparation and sequencing were carried out as described by Hillis *et al*. (1996). Of 4826 clones screened, 72 were positive for a microsatellite insert. Primers for polymerase chain reaction (PCR) amplification were designed from the flanking regions, that were selected for analysis based on a high repeat number using MACVECTOR 6.5.3 (Oxford Molecular Group, Campbell, CA). Sixteen were determined to be polymorphic based on PCR fragment size when screened across 10 giraffe individuals from the HDZ tissue bank.

Genomic DNA was isolated from tissue samples and PCR amplification was performed in a 25-μL reaction volume using an ABI480 thermocycler (Perkin-Elmer; Foster City, CA) with approximately 50 ng of genomic DNA as template. Final amplification conditions consisted of 12.5 pmol unlabelled reverse primer, 12.5 pmol fluorescently labelled forward primer, 1.5 mM $MgCl_2$, 200 μM each dNTP, and 0.5 units of *Taq* DNA polymerase (Promega; Madison, WI). The PCR amplification profile was 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, a primer-specific annealing temperature for 30 s (Table 1), 72 °C for 30 s, ending with a single extension of 72 °C for 10 min.

Allele sizes were determined by separation on a 7% polyacrylamide gel run on an ABI377 DNA Analyser (Applied Biosystems, Inc; Foster City, CA). Fragment lengths were assigned by the GENESCAN software program (Applied Biosystems, Inc.) using GENESCAN-500 (Tamra) size standard. Heterozygosity values for each locus were calculated and tests for Hardy–Weinberg equilibrium (HWE) of the genotypic frequencies were carried out using

Correspondence: Rick A. Brenneman. Fax: (402)733–0490; E-mail: rabr@omahazoo.com.

Table 1 Primer sequences, allele characteristics, sizes, and number (k) of 16 *Giraffa camelopardalis* microsatellites, observed and expected heterozygosities with Hardy–Weinberg probabilities, as screened across 25 Maasai giraffe

Locus	Primer sequence (5′ to 3′)	Repeat motif	Annealing temp. (°C)	GenBank accession No.	Fragment Size (bp)	k	H_O	H_E	Prob HWE
11HDZ073	F: AGA CCT AAT GCC ACC AGA ATG R: GAG GGT AGT GGA ACT GGG A	$(GT)_{12}$	54	AY102406	221–229	4	0.48	0.533	0.428
11HDZ102	F: TGG AAT AGG AAA TGG CAA CC R: GAT TGA AGG AAA CCA GAC ACG	$(CA)_{10}$	56	AY102407	193–195	2	0.36	0.565	0.020*
11HDZ334	F: TTC ACT CAT TGT CCA TTT AGG G R: TAG GCT GGC TTC TGC TGC	$(TG)_{13}$	54	AY102408	311–321	5	0.52	0.605	0.013*
11HDZ443	F: CAT AAA ATT AAA AGG CAC TTG TTC C R: ATG GGG GTC ACA AAG AGT CTG	$(GT)_{17}$	52	AY102409	139–147	5	0.32	0.682	0.000*
11HDZ447	F: CTC AAC AGA CAG CTC AAT ACT AGA AC R: AGT TCC TTC AAT AAG CCC ATA TC	$(CA)_7(AT)_2CA(AT)_5$	54	AY102410	158–188	3	0.00	0.340	0.000*
11HDZ480	F: TGC TTT AGT AAA GTG TGT GAA ATG C R: CAC AGA ATC TAC ACA CAT CAC ACA TC	$(TG)_{15}$	54	AY102411	122–126	3	0.20	0.621	0.000*
11HDZ550	F: GGA CAG TGG ACT AGG AGA AAA GG R: GCC TGG GAT TCC TGG TAA AC	$GTCT(GT)_{14}CT(GT)_4$	54	AY102412	168–180	7	0.48	0.728	0.011*
11HDZ561	F: CAA CAA AGA CAA ACT GGA TAG C R: TCT AAC ATC TGA GCC ACC G	$(CA)_3G(CA)_{18}$	58	AY102413	174–180	2	0.32	0.411	0.334
11HDZ562	F: AAA GAG TTA GAT GCA ACT GAG TGA C R: TCA GCA TCC TAT ATT TTC ACA CC	$(CA)_{19}$	54	AY102414	138–144	4	0.40	0.647	0.002*
11HDZ567	F: GGT TTC AGA AGG TTT GTT GGC R: TGC ATT ATC CCA AGT TCT TTA GC	$(CA)_{13}$	52	AY102415	182–212	5	0.12	0.155	0.112
11HDZ582	F: TTC CTA AGT TAC CCT CTC TGC C R: TTA GCA CCA CCC CTC TCA AC	$(CA)_3(GT)_6A(GT)_6$	50	AY102416	122–126	3	0.32	0.425	0.000*
11HDZ626	F: CAT TGG CAG GTG GAT TCT TTA C R: AGC CCA ATT ATT CTT TTA CTT CCC	$(GT)_{16}$	50	AY102417	182–194	6	0.88	0.822	0.035*
11HDZ665	F: GCC CCT TGC CTA GCT TAA C R: CCG ACT GTA GAA ATG AAG CG	$(CA)_{16}$	54	AY102418	194–228	10	0.40	0.782	0.000*
11HDZ748	F: TTT TGG AGA GGA TTG AAA TCT G R: GAA TCA TCT GTG GCT AAG CAT C	$(GT)_{14}$	56	AY102419	241–245	3	0.28	0.589	0.003*
11HDZ835	F: CCC ACA CTG CAA CTA AAC CTG R: AAG AAA CTC AAA AGC CTG CAA G	$(CA)_{12}$	54	AY102420	216–220	3	0.16	0.153	1.000
11HDZ1004	F: CTC ATG TCT CTT GCA CTG GC R: GTA ATG GCA TAT TTC ACT CTT TTT C	$(CA)_6(TA)_2(CA)_5TA(CA)_6$	54	AY102421	148–164	4	0.44	0.503	0.454

* = significant deviation from Hardy–Weinberg equilibrium at alpha = 0.05.

the GENEPOP v3.1 software package (Raymond & Rousset 1995). Primer sequences, annealing temperature, repeat motif, and GenBank accession number for each locus, number and size of alleles, and the observed and expected heterozygosity values for each of the markers are presented in Table 1. The excess of homozygotes detected as deviations from Hardy–Weinberg equilibrium support the contention that inbreeding may influence the confined population.

The marker suite will be used to screen representatives of the other 8 from free-ranging African populations (Dagg 1971). Data generated from wild populations will be the baseline for comparing captive giraffe held in North America to validate or dispute the expected subspecies of origin or identify hybrids (Lackey & LaRue 1997).

Acknowledgements

The authors wish to acknowledge the generosity of Bill and Berniece Grewcock for their support of student interns. This research was also supported by grants from the Ahmanson Foundation, which have provided the laboratory with four ABI automated DNA sequencers. The authors would also like to thank the staffs of the Kenya Wildlife Services, Athi River Ranching, Ltd, and Ol Jogi, Ltd, for their assistance in collecting the giraffe samples.

References

Ansell WFH (1968) Artiodactyla (excluding the genus. *Gazella*.) *Preliminary identification for African mammals* (ed. Meester JAJ) Smithsonian Institution, Washington, D.C.

Dagg AI (1971) Giraffa camelopardalis. *Mammalian Species*, **5**, 1–8.

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic Acids IV: Sequencing and Cloning. In: *Molecular Systematics*, 2nd edn (eds Hillis DM, Moritz C, Zimmer EA), pp. 321–381. Sinauer Associates, Inc., Sunderland, MA.

Lackey LB, LaRue FV (1997) 1997 North American regional studbook, Giraffe Species Survival Program, Lackey LB (registrar). 1–193.

Raymond M, Rousset F (1995) GENEPOP v1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Sambrook J, Fritch EF, Maniatus T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Press, New York.