Molecular Ecology Notes (2005) **5**, 223–225 doi: 10.1111/j.1471-8286.2005.00885.x

PRIMER NOTE

Six new polymorphic microsatellite loci isolated and characterized from the African savannah elephant genome

SILVESTER NYAKAANA,* JOHN B. A. OKELLO,* VINCENT MUWANIKA* and HANS R. SIEGISMUND†

**Institute of Environment and Natural Resources, Makerere University, PO Box 7298 Kampala, Uganda, †Department of Evolutionary Biology, Biological Institute, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark*

Abstract

The African savannah elephant (*Loxodonta africana*) is a 'keystone' species that plays a vital role in regulating the dynamics of both plant and animal communities and yet it is endangered and its numbers have been reduced to approximately 500 000 across their entire continental range. Molecular genetic markers are important tools for providing genetic information useful in formulating effective management and conservation strategies for the surviving elephant populations. We describe the isolation and characterization of six new polymorphic microsatellite markers in the African savannah elephant and demonstrate that these loci can be PCR (polymerase chain reaction)-multiplexed, a desirable attribute that saves costs in large-scale microsatellite screening.

Keywords: African elephant, *Loxodonta africana*, microsatellite markers

Received 03 September 2004; revision accepted 18 November 2004

Ever since the first elephant microsatellite loci were isolated, characterized and shown to be highly variable (Nyakaana & Arctander 1998), they have increasingly become the marker of choice for elephant population biologists in addressing behavioural and phylogenetic questions at different hierarchical levels (e.g. Nyakaana *et al*. 2001, 2002). This is mainly attributed to their high variability, the speed and ease with which they can be analysed in large sample sets and their ability to be amplified from degraded DNA collected noninvasively from dung.

Although a number of elephant microsatellite loci have been isolated and characterized to date (see Nyakaana & Arctander 1998; Comstock *et al*. 2000; Eggert *et al*. 2000; Fernando *et al*. 2001; Comstock *et al*. 2002; Archie *et al*. 2003), there is a continual demand for new polymorphic genetic markers for studying the behaviour, ecology and biology of this endangered species' effective management and conservation. In this study, we report the isolation and characterization of six new polymorphic loci from the elephant genome.

Correspondence: Dr. Silvester Nyakaana, Fax: + 256 41 530134; E-mail: snyakaana@muienr.mak.ac.ug

An enriched genomic library was constructed from total genomic DNA digested to completion using *Sua3A1* as described in Nyakaana & Arctander (1998), directly cloned using the PCR-Script Amp SK(+) cloning kit (Stratagene) and transformed into *Escherichia coli* XL1-Blue MRF' Kan supercompetent cells (Stratagene). Recombinant clones were selected, amplified directly using T3 and T7 primers and then cycle sequenced.

Six of the sequenced recombinant clones had good flanking regions to allow for the design of suitable primers using the program AMPLIFY 1.2 (Engels 1992). We BLAST- searched our new clones against the previously isolated and published microsatellite loci in the GenBank database [Nyakaana & Arctander 1998 (AF061840–AF061844); Comstock *et al*. 2000 (AF206275–AF206286); Comstock *et al*. 2002 (AF364123–AF364125); Eggert *et al*. 2000 (AF311670–AF311675); Fernando *et al*. 2001 (AF352833–AF352837); Archie *et al*. 2003 (AY172173–AY172184)] and found no significant sequence similarity between any of them and those previously published, thereby confirming their uniqueness.

Four of the loci (LafMS06, LafMS07, LafMS08 and LafMS09) were amplified in 15 μL reaction volumes containing 25–50 ng of DNA, 1.5 mM $MgCl_2$, 10 mM Tris, 50 mM KCl, 200 μM of each dNTP, 2 pmol of each primer and 0.5 units of Ampli*Taq* Gold DNA polymerase (Boehringer

Table 1 PCR cycling parameters for the six isolated elephant loci

Multiplexed loci	Step	*T* (°C)	Time	Remarks
LafMS06 & LafMS08	1	95	10 min	
	2	95	30 sec	10 cycles; 1 °C drop for each cycle up to 59 °C
	3	69	30 sec	
	4	72	30 sec	
	5	95	30 sec	28 cycles
	6	58	30 sec	
	7	72	30 sec	
	8	72	60 min	
	9	4	Infinity	
LafMS07 & LafMS09	1	95	10 min	
	2	95	30 sec	10 cycles; 1 °C drop for each cycle up to 58 °C
	3	68	30 sec	
	4	72	30 sec	
	5	95	30 sec	28 cycles
	6	57	30 sec	
	7	72	30 sec	
	8	72	60 min	
	9	4	Infinity	
LafMS10 & LafMS11	1	95	15 min	
	2	94	30 sec	5 cycles; 1 °C drop for each cycle up to 56 °C
	3	60	1 min 30 sec	
	4	72	1 min	
	5	94	30 sec	25 cycles
	6	55	1 min 30 sec	
	7	72	1 min	
	8	60	30 min	
	9	4	Infinity	

Mannheim GmbH). The forward primers for LafMS06 and LafMS08 were labelled with the fluorescent dyes, JOE and FAM. Similarly, the forward primers for loci LafMS07 and LafMS09 were also labelled with the dyes, JOE and FAM, respectively. LafMS06 and LafMS08 were multiplexed and LafMS07 and LafMS09 were also multiplexed and PCR-amplified using a touch down protocol (see Table 1). Amplification products for these four loci were size-fractionated on a 4% polyacrylamide gel using an ABI 377 DNA sequencer and sized using ROX-500 as an internal lane standard (Applied Biosystems), scored using the programs GENESCAN 3.1 and GENOTYPER 2.1 (Applied Biosystems).

Forward primers for the last two loci (LafMS10 and LafMS11) were labelled with infrared dyes 700 and 800, respectively, and amplified in 10 μL reaction volumes containing 1 pmol of each primer using the multiplex PCR kit (QIAGEN) following the manufacturer's instructions. A touchdown PCR procedure was used with the cycling parameters indicated in Table 1.

The PCR products were genotyped using the global IR 2LI-COR DNA system and the data scored using the SAGA^{GT} software. Polymorphism for all loci was analysed using the program POPGENE (Yeh *et al*. 1997).

All the loci were polymorphic and the number of alleles among up to 48 individuals randomly sampled from populations from east, west and southern Africa ranged from 4 to 12. Their characteristics, primer sequences, observed and expected heterozygosities are shown in Table 2.

Significant genotypic linkage disequilibrium was only observed between loci LafMS06 and LafMS08 while all the loci significantly deviated from Hardy–Weinberg proportions as calculated using POPGENE ($P < 0.05$) (Yeh *et al*. 1997). As the loci were tested on a set of individuals randomly sampled from various populations from eastern, southern and western Africa, we attribute these deviations to non-random mating and/or a Wahlund effect but not to null alleles as no heterozygote deficiency was observed at all loci.

Their cross-species amplification was not investigated in the African forest elephant or the Asian elephant, *Elephas maximus*, because of the lack of samples of this species.

Table 2 Characteristics of six African elephant microsatellite loci. The number of individuals analysed/number of alleles per locus (n/N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) are also provided

Locus	Repeat motif	Primer sequence (5′–3′)	Size range	n/N_A	H_O	H_E	Accession no.
LafMS06	$(AC)_{14}$	F: AGCTGTCCTAAGTCATAAATACACA R: ACAGCCACTGAAACCCCATGGA	138–156	48/12	0.96	0.82	AY817498
LafMS07	$(GATA)_4(GACA)_{13}(GATA)_3$	F: TACCCCCACTCCAATTCCATGTCT R: GGACAGGCAGAATCTAGTGGAGG	154–170	21/4	0.86	0.62	AY817499
LafMS08	$(TC)_8(AC)_{13}$	F: CTGTGGCCTCCAAATGAAGGG R: CAGACACTGGGAGGGAGATG	175–189	47/8	0.89	0.68	AY817500
LafMS09	$(GATA)_2(GACA)_7$	F: CTGGGGCAGTAAGCTGTATTTATC R: ACGAGGATGACAGACCAGGCAACA	144–160	41/5	0.27	0.48	AY817501
LafMS10	$(CA)_4CT(CA)_6CT(CA)_{11}$	F: GTCATGGGGGATACACAGTCA R: TCATGTGTGCATGGGGT	108–116	35/5	0.69	0.62	AY817502
LafMS11	$(CA)_5TA(CA)_{11}$	F: TAGGTTCATAGATCATAGGGT R: TACGTCTGTGATCAGTGGGCCT	130–136	35/4	0.49	0.47	AY817503

F, forward primer; R, reverse primer.

Acknowledgements

This work was carried out under the auspices of the Wildlife Genetics Project and funded by DANIDA through the ENRECA (ENhancement of REsearch CApacity) program based in Makerere University Institute of Environment and Natural Resources, Uganda. We thank Pernille S. Olsen for her help with laboratory work.

References

Archie EA, Moss CJ, Alberts SC (2003) Characterization of tetranucleotide microsatellite loci in the African savannah elephant (*Loxodonta africana africana*). *Molecular Ecology Notes*, **3**, 244–246.

Comstock KE, Georgiadis N, Pecon-Slattery J *et al.* (2002) Patterns of molecular genetic variation among African elephant populations. *Molecular Ecology*, **11**, 2489–2498.

Comstock KE, Wasser SK, Ostrander EA (2000) Polymorphic microsatellite DNA loci identified in the African elephant (*Loxodonta africana*). *Molecular Ecology*, **9**, 993–1011.

Eggert LS, Ramakrishnan U, Mundy NI, Woodruff DS (2000) Polymorphic microsatellite DNA markers in the African elephant (*Loxodonta africana*) and their use in the Asian elephant (*Elephas maximus*). *Molecular Ecology*, **9**, 2223–2225.

Engels B (1992) *AMPLIFY 1.2*. Department of Genetics, University of Wisconsin, Madison, W1, 53706.

Fernando P, Vidya TNC, Melnick DJ (2001) Isolation and characterization of tri- and tetranucleotide microsatellite loci in the Asian elephant, *Elephas maximus*. *Molecular Ecology Notes*, **1**, 232–233.

Nyakaana S, Arctander P (1998) Isolation and characterization of microsatellite loci in the African elephant, *Loxodonta africana*. *Molecular Ecology*, **7**, 1436–1437.

Nyakaana S, Arctander P, Siegismund HR (2002) Population structure of African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. *Heredity*, **89**, 90–98.

Nyakaana S, Abe EL, Arctander P, Siegismund HR (2001) DNA evidence for elephant social behavior breakdown in Queen Elizabeth National Park, Uganda. *Animal Conservation*, **4**, 231–237.

Yeh FC, Yang RC, Boyle T (1997) *POPGENE, version 1.21: A Microsoft Window-Based Freeware for Population Genetic Analysis*. Department of Renewable Resources, University of Alberta, Edmonton, Canada.