Molecular Ecology Notes (2001) 1, 232–233

PRIMER NOTE

Isolation and characterization of tri- and tetranucleotide microsatellite loci in the Asian elephant, *Elephas maximus*

PRITHIVIRAJ FERNANDO,* T. N. C. VIDYA† and DON J. MELNICK*‡

*Center for Environmental Research and Conservation, Columbia University, 1200 Amsterdam, Avenue, New York, NY 10027, USA, †Center for Ecological Sciences, Indian Institute of Science, Bangalore 560012, India, ‡Departments of Anthropology and Biological Sciences, Columbia University, 1200 Amsterdam Avenue, New York, NY 10027, USA

Abstract

Asian elephants (*Elephas maximus*) are an endangered species. Their future survival depends on intensive conservation and management, based on in-depth knowledge of particular populations. Molecular genetic methods, especially microsatellite analysis through noninvasive sampling, provides an effective means of obtaining such information. The use of tri- and tetranucleotide microsatellite markers is advantageous in noninvasive sampling through dung analysis. Here we describe the isolation and characterization of five tri- and tetranucleotide markers in the Asian elephant. All five loci were found to be polymorphic in a sample of 20 Asian elephants from Sri Lanka.

Keywords: Asian elephant, *Elephas maximus*, microsatellite markers

Received 8 March 2001; revision accepted 26 April 2001

Asian elephants (*Elephas maximus*) have become endangered due to the rapid decline of populations over the past few decades (Sukumar 1989). Future survival of fragmented and isolated Asian elephant populations depends on intensive conservation and management efforts, which require an in-depth knowledge of those particular populations (Fernando 1993). Asian elephants usually inhabit poor visibility habitat, and the ubiquity of human–elephant conflict over most of their range leads to their developing avoidance or aggressive responses toward humans. Consequently, they are difficult to study by observational methods alone, and molecular genetic techniques can be an effective means of obtaining data critical for their management and conservation (Fernando & Lande 2000).

In combination with noninvasive sampling, microsatellite analysis is a powerful tool in the study of free-ranging organisms. While dinucleotide repeats are more prevalent in mammalian genomes and possibly more variable than tri- or tetranucleotide repeats, we have found them to be more prone to false alleles generated by polymerase slippage during polymerase chain reaction (PCR). This is especially a concern when using suboptimal sources of DNA (Taberlet *et al.* 1996). Here we describe the isolation and characterization of five tri- and tetranucleotide microsatellite loci in the Asian elephant, using a protocol based on Kelly & Willis (1998).

Phenol-extracted genomic DNA from a male Asian elephant was digested to completion with *Eco*RV, *Hae*III, *Rsa*I and *Sau*3A restriction enzymes and *Eco*RI adapters (Promega) ligated to size selected (200–600 bp) fragments. A partial genomic library was constructed in XLI-Blue MRF′ transformed with the fragments ligated into *Eco*RI digested λZAPII (Stratagene) vector. Approximately 15 000 plaque forming units from the library were plated with XLI-Blue MRF′ on LB agar/agarose and replica-plated onto nylon membranes. Colonies were screened with [γ^{32}P]-dATP end-labelled oligonucleotides $(GTT)_8$ and $(AAGG)_8$. Positive colonies were cored, suspended in SM buffer and PCR amplified with M13 primers and products sequenced with T3 and T7 primers. From 20 sequenced positive clones, primers were designed for 10 loci, using OLIGO 5.0 (MBI). Amplification conditions were optimized for five primer sets that gave clear PCR products in the expected size range (Table 1).

DNA was extracted from elephant blood using a phenol/chloroform/isoamyl-alcohol and Qiagen column protocol (Fernando & Lande 2000). Forward primers were labelled with fluorescent dye markers TET, FAM or HEX (Biosynthesis).

Correspondence: Prithiviraj Fernando. Fax: (212) 854 8188; E-mail: pf133@columbia.edu

Table 1 Characterization of *Elephus maximus* microsatellite loci

Locus	Repeat motif	GenBank no.	Size (bp)	Primers (5′→3′)	T_a (°C)	No. of alleles	Size range	H_O	H_E
EMX-1	$(GTT)_{14}$	AF352833	152	F: AGGACTTATTTGCTTAGATGG R: AGGCAATGTTTCGTTCTGT	64	4	137–152	0.5000	0.4713
EMX-2	$(GTT)_5$	AF352834	223	F: CCCATGAGTCGGAATCCACTT R: CCATAGGGTTGCCAAGGAATG	70	2	217–223	0.3500	0.4888
EMX-3	$[GGAA]_3..(GAA)_5..$ $..(GGAA)_4$	AF352835	254	F: CATGGTTAACTCATTGCTTGC R: GTGTTCCCTCCCTCTCATCAT	64	2	238–254	0.6500	0.4888
EMX-4	$(GGAA)_3A(GA)_3A(GGAA)_3$	AF352836	379	F: AGTTCGTGTCTCGGTGCTGTA R: GTATGCTGATGGAAATGTCTA	61	3	351–387	0.2000	0.1838
EMX-5	$(GGAA)_3....$ $(GGAAGGGA)_4..$ $..(GGAA)_3....(AGGG)_3$	AF352837	263	F: AAATAGGAAAAGTCTGAGGTT R: CCCCTGGATTTTCTTCACCTG	59	3	248–263	0.2000	0.405

T_a, annealing temperature.

Reactions were amplified in a Perkin Elmer 9700 thermocycler in 12.5 μL reaction volumes at the appropriate temperature profile for each primer pair (Table 1), using 1 μL DNA extract, 1 μL 100 mg/mL BSA, 1.25 μL 10× PCR buffer (100 mM Tris-HCl pH 8.4, 500 mM KCl, 15 mM $MgCl_2$) 1.25 μL 8 mM dNTP mix, 0.25 μL 10 μM primers, 0.1 μL *Taq* DNA polymerase (Perkin Elmer), and 7.4 μL water. Reactions were amplified for 25 cycles of 1 min each at: the appropriate annealing temperature; 72 °C extension; and 92 °C denaturation. Cycles were preceded by a 3 min denaturation step at 93 °C, and followed by a 72 °C 15 min extension step. Amplification products were electrophoresed on 5.3% 0.2 mm urea-acrylamide gels in an ABI 377 DNA sequencer. Allele sizes were analysed using GENESCAN (ABI) software.

Microsatellite polymorphism was assessed in 20 Asian elephants from Sri Lanka. All five loci were polymorphic in Asian elephants (Table 1). We are currently using these markers to assess population subdivision and identification of populations with unique evolutionary trajectories in the Asian elephant.

Acknowledgements

We would like to thank Chandana Rajapakse, Ashoka Dangolla and LKA Jayasinghe for assistance in obtaining samples from Asian elephants, Senarath Gunasena and National Zoological Gardens Sri Lanka for permission to obtain samples from the Pinnawela Elephant Orphanage Sri Lanka, Alan Kelly for advice on laboratory techniques, Michael Pfrender and Justin Hicks for laboratory assistance, and Russ Lande for advice and encouragement. This work was funded in part by NSF grant DEB 9225127 to R Lande, WWF US, WWF International and USFWS Asian Elephant Conservation Fund grants to PF and DJM for the development of molecular tools for management and conservation of Asian elephants, and additional support from the Center for Environmental Research and Conservation (CERC).

References

Fernando P (1993) Implications of socioecology and genetics on the conservation and management of the Sri Lankan elephant. In: *A Week with Elephants. Proceedings of the International Seminar on Asian Elephants* (eds Daniel JC, Datye H), pp. 298–313. Bombay Natural History Society, Oxford University Press, Bombay.

Fernando P & Lande R (2000) Molecular genetic and behavioral analysis of social organization in the Asian elephant (*Elephas maximus*). *Behavioral Ecology and Sociobiology*, **48**, 84–91.

Kelly AJ & Willis JH (1998) Polymorphic microsatellite loci in *Mimulus guttatus* and related species. *Molecular Ecology*, **7**, 769–774.

Sukumar R (1989) *The Asian Elephant: Ecology and Management*. Cambridge University Press, Cambridge.

Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **24**, 3189–3194.