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TECHNICAL NOTE

Isolation and characterization of 10 microsatellite loci for White-headed Langur (*Trachypithecus leucocephalus*)

Sun Tao · Wang Bo-Shi · Liu Zhi-Jin · Huang Cheng-Ming · Zhou Qi-Hai · Li Ming

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Abstract The polymorphic microsatellite loci were isolated and characterized from a microsatellite DNA-enriched DNA library for the white-headed langur (*Trachypithecus leucocephalus*), which is listed as one of the top 25 most endangered primate species in the world during the year of 2004–2006. These loci showed polymorphism information content ranging from 0.590 to 0.874, allele numbers ranging from 4 to 14, and observed and expected heterozygosities from 0.660 to 0.906 and 0.526 to 0.920 respectively. Thus, we expect that these markers will be useful for conservation genetic of white-headed langur.

Keywords White-headed Langur · *Trachypithecus leucocephalus* · Microsatellites isolation · Conservation

The white-headed langur (*Trachypithecus leucocephalus*), is one of the numerous critically endangered species. It is distributed only in Karst hills in four counties in the southern Guangxi province of China. The total extant population was estimated to be less than 700 individuals (Groves 2001; Huang 2002; Huang et al. 2002). Therefore, it was listed as one of the top 25 most endangered primate species in the world during 2002–2006 (Konstant et al. 2002). Proper knowledge of the population genetics and conservation genetics of *Trachypithecus leucocephalus* will contribute to appropriate conservation management. However, little remains known about its genetic status and currently no molecular markers are available about this species. Microsatellite marker is an ideal tool for conservation genetic study (Ross 2001). Therefore, the development of highly polymorphic microsatellite markers for the white-headed langur would be useful in studies such population structure, migration and mating system. In this paper, we report the first isolation and characterization of 10 microsatellite loci in the white-headed langur.

Microsatellites were isolated as the enrichment protocols by Hamilton et al. (1999) with slight modifications as follows. Whole genome DNA was extracted from a blood sample of white-headed langur using Animal Genomic DNA Extraction Kit (PUEX). Approximately 9 μg of genomic DNA was digested with MboI restriction enzyme (TaKaRa) and ligated to an adaptor generated by annealing together oligo A and oligo B (Refseth et al. 1997). Then the 400–1,000 bp DNA fragments were isolated from the ligated products and enriched by polymerase chain reaction (PCR) using Oligo A as the PCR primer. Enrichment was carried out using $(CA)_{15}$ biotin-labelled probe and streptavidin coated magnetic beads (Promega). First, 4 μg of the adaptor-ligated size fractionated genomic DNA was hybridized to 2 μg of probe in 6× SSC at 50°C overnight, and the complex was added to 600 μl of streptavidin coated beads and the two were mixed for 1 h at 43°C. The enriched fragments that released from the probe were amplified to double-stranded

S. Tao · W. Bo-Shi · L. Zhi-Jin · H. Cheng-Ming · L. Ming (✉)
Key Lab of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, 1 BeiChen Xilu 100101 Chaoyang, Beijing, China
e-mail: lim@ioz.ac.cn

S. Tao · W. Bo-Shi
Graduate School of the Chinese Academy of Sciences, 100039 Beijing, China

S. Tao · H. Cheng-Ming · Z. Qi-Hai
Key Laboratory of Ecology of Rare and Endangered Species and Environmnetal Protection, Ministry of Education, Guangxi Normal University, 541004 Guilin, China

form, and ligated into pMD18-T vector (TaKaRa) and transformed into competent Top10 cells. Approximately 300 recombinant colonies were screened by PCR amplification directly from bacterial colonies using CA repeat and universal M13 primers (Lunt et al. 1999). About 71 positive clones were sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and ABI 3730 Genetic Analyzer. Twenty-four sequences having adequate flanking regions were selected for primer design. PCR primers were designed using PrimerSelect (DNASTAR) and Oligo Analyzer (Integrated DNA Technologies). Unlabelled primers were used to determine optimal amplification conditions. PCR amplifications were performed in 10 μl reaction volume containing approximately 20 ng of template DNA, 0.2 mM of each dNTP, 0.15 μM of each primer, 1.5 mM Mg^{2+}, 1× PCR buffer and 0.15 U of *Taq* DNA polymerase (PUEX). The amplification conditions were as follows: 95°C for 5 min, 35 cycles at 94°C for 30 s, T_a for 30 s, 72°C for 45 s and a final extension at 72°C 10 min. All reactions were amplified using an MBS Satellite thermal cycler (Thermo Electron Corp., USA).

Finally, a total of 24 primers were selected and one of each of the 24 primer pairs was labelled with one of the fluorescent dye (FAM, TAMRA or HEX) for polymorphism detection. Eight of them failed to amplify or showed complex amplification, six were monomorphic and ten were polymorphic. Ten polymorphic loci were tested in 64 individuals from the Guangxi population at South China. Genotyping of the microsatellite loci for each individual was done by electrophoresis on an ABI Prism 3700 Genetic Analyzer (Applied Biosystems), and fragment length was determined in comparison to an internal size standard (Genescan 500 LIZ Size Standard, Applied Biosystems) using GeneMapper 3.5 software (Applied Biosystems). We used The Excel Microsatellite Toolkit (Park 2001) to calculate number of alleles per locus (k), observed and expected heterozygosities (H_o and H_E), and polymorphic information content (PIC) and GENEPOP 4.0 (Rousset 2008) to test for significant deviations from expected Hardy–Weinberg proportions and genotypic disequilibrium (Table 1). The 10 loci were highly polymorphic with numbers of alleles per locus ranging from 4 to 14, observed heterozygosity from 0.660 to 0.906 and expected heterozygosity from 0.526 to 0.920 (Table 1). All loci except for WHL-04 and WHL-05 locus followed Hardy–Weinberg expectations. No significant linkage association was found among all these loci. The 10 novel polymorphic microsatellite loci will be useful for conservation genetic study in this species.

Table 1 Characterization of 10 microsatellite loci in *Trachypithecus leucocephalus*

Locus	Primer sequence(5′–3′)	Labelling dye	Size range (bp)	N_a	T_a (°C)	H_o	H_E	*P* value	GenBank accession No.
WHL-01	F: GAG GAC AAA GAT TGG CA R: CTG GGA ATG ACA CGG AGA	FAM	187–227	14	50	0.810	0.906	0.1239	GU253977
WHL-02	F: AGA GAG TTG GGG TGG GTA CT R: CTC CAT CAG GTC CTT GCC ATA G	FAM	207–213	4	50	0.600	0.660	0.2312	GU253978
WHL-03	F: GCT CAC TGC AGC CTA GA R: TTG GGC AAC ATA GCA AGA CC	HEX	140–160	10	50	0.920	0.832	0.9295	GU253979
WHL-04	F: TTA CAG TGG CTT GGA GTA GA R: AAC AAA CAA ACC CAA ACA	TAMRA	119–131	7	50	0.526	0.794	0.0020	GU253980
WHL-05	F: ACA GAA GGC AAA GCT AAG R: TGA CAG AGC AAG ACT CCA TC	HEX	118–130	7	48	0.529	0.811	0.0125	GU253981
WHL-06	F: CAG TCT TCC TCC AGA GGG TAT T R: GCT ACA CTG TGC CAT TGC	FAM	154–175	9	50	0.760	0.793	0.2139	GU253982
WHL-07	F: CCT GGG TGA CAG AGC GAG R: GTG CTT TCA GCT TGA CAT TC	TAMRA	143–151	7	50	0.700	0.745	0.4627	GU253983
WHL-08	F: GCA AGA GCG AAG CTC CGT CC R: GTT CAG CCC CTA CAG TGC CA	FAM	160–186	7	50	0.542	0.730	0.0743	GU253984
WHL-09	F: TCT TTA TTT CAG GCT TGT TC R: ACC CCA TCT CTA CTA AAC	FAM	144–156	6	48	0.714	0.701	0.6834	GU253985
WHL-10	F: TCA ATG ATT CTC TGT CTC TC R: GAG AAT CAC TTG AAC CTG	FAM	145–161	7	48	0.818	0.805	0.3483	GU253986

T_a annealing temperature, N_a number of alleles, H_o observed heterozygosity, H_E expected heterozygosity

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