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TECHNICAL NOTE

Characterization of 18 microsatellite marker loci in the white-collared lemur (*Eulemur cinereiceps*)

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Abstract The white-collared lemur (*Eulemur cinereiceps*) is found in southeastern Madagascar. This species is endangered due to the loss and fragmentation of its natural habitat and hunting. Eighteen nuclear microsatellite loci were isolated from genomic DNA derived from an animal from Mahabo Forest in Madagascar. Population genetic parameters were estimated from a population of white-collared lemur sampled at Vevembe Forest 7 years apart to evaluate the potential utility of this marker suite.

Keywords Genetic markers · White-collared lemur · *Eulemur cinereiceps* · Microsatellites

The white-collared lemur, *Eulemur cinereiceps* (=*albocollaris*; Johnson et al. 2008), is a medium sized (1.6–2.7 kg), cathemeral, frugivorous lemur that is sexually dichromatic (Tattersall 1982; Johnson et al. 2005). Males are grey–brown with distinctive white cheeks and beard, while females are not bearded, have grey faces, and are more rufous in color. *Eulemur cinereiceps* live in rain forests between the Andringitra Massif and the Mananara River in southeastern Madagascar (Petter and Petter-Rousseaux 1979; Johnson and Wyner 2000). It is estimated that there is approximately only 700 km^2 of habitat remaining within this species range (Irwin et al. 2005). As a result of habitat loss and hunting, *E. cinereiceps* is one of the 25 most endangered primates in the world (Mittermeier et al. 2007). We report here the isolation and characterization of 18 polymorphic markers for *E. cinereiceps*.

Genomic DNA was isolated from tissue from a free-ranging *E. cinereiceps* collected from Mahabo Forest. Procedures for construction of the genomic DNA library, identification of plasmids containing $(GT)_n$ inserts as well as plasmid preparation and sequencing were carried out as generally described by Hillis et al. (1996). Isolated genomic DNA was digested using Sau3A restriction enzyme. The digested DNA was sized using Clontec© chromaspin columns to remove fragments under 400 bp. Sized DNA was ligated to primers forming blunt-ended DNA pieces. Ligated DNA was enriched using a PCR-based method by Moraga-Amador et al. (2001), a modification of Kandpal et al. (1994). Enriched DNA was denatured and a biotinilated probe annealed to the DNA. The biotinilated DNA was captured using Vector Laboratory (Burlingame, CA) Vectrex Avidin D© and non-annealed DNA was washed away. After releasing captured DNA from the Vectrex Avidin D©, a second round of PCR enrichment was performed. An Invitrogen (Carlsbad, CA) TOPO A© plasmid ligation was performed following this PCR. Following transformation, cells were plated onto LB agar plates including ampicillin and X-gal. Plates were picked for positive white colonies that were placed on Pall (East Hill, NY) Biodyne B nylon membranes. A Southern blot of the colonies was done using DIG-labeled oligonucleotide. Plasmid preps of positive colonies from the Southern blot were sequenced and primers were designed from the two regions flanking the microsatellite repeat motif. Of 1,878

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clones screened, 268 were positive for a microsatellite insert and the first 18 were designed and polymorphic are being reported here.

Tissue samples were collected and stored in ambient temperature storage buffer (Longmire et al. 1992) from 21 white-collared brown lemurs in multiple locations in Vevembe Forest, Madagascar, in 2000 and 2007. Genomic DNA was isolated using standard protocols (Sambrook et al. 1989). PCR amplification was carried out in a 25 µl reaction volume using a MBS Satellite 0.2G thermal cycler (Thermo Electron Corporation; Gormley, ON) with approximately 50 ng of genomic DNA as template. Final amplification concentrations consisted of 12.5 pmol unlabelled reverse primer, 12.5 pmol fluorescently labeled forward primer, 1.5 mM $MgCl_2$, 200 µM each dNTP, and 0.5 units of *Taq* DNA polymerase (Promega; Madison, WI). The thermal profile for PCR amplification was 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, a

Table 1 Primer sequences with fluorescent dye label used, repeat motif, optimized annealing temperature, number of alleles (*k*), allele size range and GenBank accession numbers of 18 *Eulemur cinereiceps*—specific microsatellite loci

Locus	Prime sequence (5′–3′)	Repeat motif	Annealing temp (°C)	*k*	Size range	GenBank range accession no
104HDZ	^{HEX}GAT TTG ACC CCA GCC AGA C R: CAG ATG ATT ATT TCC CTC CCA C	$(GT)_{15}$	60	5	212–220	FJ424710
104HDZ11	F: ^{FAM}CCA AGA AGG GAT TTG TAG GAG T R: TGA AAC CAT CAC CAG CAC TTA T	$(CA)_4$ CG $(CA)_{13}$	60	3	114–118	FJ424711
104HDZ107	F: ^{FAM}TGT GTG GAA GGC AGT GTA GG R: GTT CTT TTG ATT TCT GAG GTG C	$(CA)_{12}$ GAG-GGA $(CA)_5$	54	4	206–212	FJ424712
104HDZ109	F: ^{FAM}CCC TAA CCC ACC CAC CAC R: ACT TTT TCT AAG GAG GCA GTG C	$(CA)_{14}$	60	5	189–197	FJ424713
104HDZ120	F: ^{FAM}CAG GTG CTT TCT TGG TGG TC R: TGA CAG GAT TTC TTT TTT ATG GC	$(CA)_6$ C $(CA)_{13}$	60	5	216–224	FJ424714
104HDZ127	F: ^{FAM}CCA AAG AGA AGT GAG AAG AAC ATC R: CAA ATG GTA AAC TGT GCG GAG	$(GT)_4$ CA $(TG)_2$-CCC $(GT)_{13}$	54	5	215–223	FJ424715
104HDZ132	F: ^{HEX}GAC CAA AAG ATA GGA AAG GGG R: GCA GGA TGT GTT GCT TCA TTT	$(CA)_{15}$ CG $(CA)_3$	56	5	176–184	FJ424716
104HDZ136	F: ^{HEX}CCT GAG TCA CAC ACC CAT TG R: TGA GCA CCC AGT AGG AGC C	$(CA)_{15}$	56	4	179–185	FJ424717
104HDZ137	F: ^{HEX}GGT TTC CTT ACC CCA AGC C R: TTC TTC ACA TTG CCC TCT CC	$(GT)_5$ CC $(GT)_{11}$-CC $(GT)_{14}$	58	5	111–137	FJ424718
104HDZ161	F: ^{FAM}GAA AAG TGA TTC CAA GGA TGA R: ATT TAG AAA GAT ACA TTT GAT AGT GTT AT	$(CA)_{13}$	52	2	240–242	FJ424719
104HDZ162	F: ^{FAM}AGG GAA GGA GGG AGG AAG T R: GAG TTT GGG CAT CTT TGT AGC	$(CA)_{12}$ TA $(CA)_3$	62	4	176–182	FJ424720
04HDZ163	F: ^{HEX}GCA GCA CAC GCC AAT GA R: CCC CAA ATA GCA AAA ATG TTA CT	$(GA)_2$ $(CA)_{11}$	58	4	170–178	FJ424721
104HDZ170	F: ^{FAM}GCA CAA TAA CCA GAA ATC ATA CAA R: GCC TCC CAG TCA GCA GTG	$(CA)_{11}$	52	4	254–260	FJ424722
104HDZ197	F: ^{HEX}ATT CAC TTG TAA AGC CAT CTA TTC R: GGG AGA ACA CTA AGA ACT AAA GAA A	$(GT)_3$ GA $(GT)_{10}$	56	3	112–116	FJ424723
104HDZ206	F: ^{HEX}AGG TTA CTG TAG GTC AAA TCA CG R: GGT TTC TGC TGG AGG GTG	$(CA)_{12}$ CG $(CA)_3$	52	5	120–128	FJ424724
104HDZ215	F: ^{FAM}GCC ACG GAA GTT AGC CTG R: TGG GAA AGG GAA GTG ATT ATG	$(CA)_{13}$ TG $(CA)_5$	56	3	175–181	FJ424725
104HDZ224	F: ^{HEX}ACC CAC AAA GCA CAT TAG AAT C R: GCA GGA AGT CTC ACA CAG GA	$(GT)_{12}$	62	6	129–143	FJ424726
104HDZ230	F: ^{FAM}CCA CAG ACA AAA AGA GGG AGG R: CCG AGT GAA GGA AAA TGA AAT AAT	$(CA)_2$ CGCACG $(CA)_{14}$	62	4	208–222	FJ424727

primer-specific annealing temperature for 30 s (Table 1), 72°C for 30 s, ending with a single 10 min extension of 72°C. Allele sizes were determined by separation of PCR products via POP 4 capillary buffer electrophoresed in an ABI 3100 DNA Analyzer (Applied Biosystems, Inc; Foster City, CA). Fragment length genotypes were assigned by GeneScan (Applied Biosystems, Inc.) using GeneScan-500 [Tamra] size standard. Loci characterizations are presented in Table 1.

The data set was analyzed for errors using MICRO-CHECKER (Van Oosterhaut et al. 2004) and Microsatellite Analyzer (MSA; Dieringer and Schlötterer 2003). Null alleles and polymorphic information content (PIC) were estimated using CERVUS v.2.0 (Marshall et al. 1998; Slate et al. 2000). Marker independence was verified following Bonferroni correction for multiple tests in FSTAT (Goudet 1995, 2001) before population genetic parameters were estimated in FSTAT and web-based Genepop (Raymond and Rousset 1995).

Global population genetic parameter estimates for the marker suite are presented in Table 2. Two loci deviated significantly from Hardy Weinberg Equilibrium and two others were estimated to have excessive null allele frequencies (>0.10). The average observed heterozygosity for the data was 0.577 with an expected heterozygosity of 0.631. Polymorphic information content for the loci averaged 0.568 ($0.214 < PIC < 0.719$). This novel marker suite will be useful to further the understanding of population dynamics in *Eulemur cinereiceps*.

Table 2 Hardy–Weinberg exact tests *P*-values with standard errors, within population fixation indices (F_{IS}), observed (H_O) and expected (H_E) heterozygosities, and polymorphic information content (PIC) among 18 *Eulemur cinereiceps* microsatellite loci screened across 21 white-collared lemurs from Vevembe Forest

Locus	*P*-val[b]	SE	F_{IS}	H_O	H_E	PIC
104HDZ9	0.801	0.0016	−0.097	0.810	0.740	0.677
104HDZ11[a]	0.174	0.0012	0.273	0.286	0.390	0.336
104HDZ107	0.970	0.0004	−0.064	0.714	0.672	0.590
104HDZ109	0.069	0.0011	−0.043	0.810	0.777	0.719
104HDZ120	0.220	0.0017	0.201	0.619	0.771	0.710
104HDZ127	0.061	0.0011	−0.056	0.810	0.768	0.706
104HDZ132	0.119	0.0015	0.135	0.667	0.768	0.711
104HDZ136	0.410	0.0018	−0.020	0.667	0.654	0.586
104HDZ137	0.569	0.0023	0.165	0.619	0.739	0.668
104HDZ161	1.000	0.0000	−0.143	0.286	0.251	0.215
104HDZ162	0.418	0.002	−0.152	0.667	0.581	0.510
104HDZ163	0.433	0.0014	0.124	0.667	0.758	0.692
104HDZ170	0.000	0.0000	0.603	0.286	0.710	0.644
104HDZ197	0.820	0.0008	0.147	0.476	0.556	0.439
104HDZ206[a]	0.007	0.0004	0.265	0.524	0.708	0.644
104HDZ215	0.845	0.0006	0.095	0.524	0.577	0.502
104HDZ224	0.922	0.0015	−0.014	0.714	0.705	0.655
104HDZ230	1.000	0.0000	−0.058	0.238	0.225	0.214
Average	0.005		0.095	0.577	0.631	0.568

[a] Null allele frequency >0.10

[b] Estimated with the Markov Chain Monte Carlo method 400 batches and 5,000 iterations per batch

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