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PRIMER NOTE

Microsatellite markers for woolly monkeys (*Lagothrix lagotricha*) and their amplification in other New World primates (Primates: Platyrrhini)

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Abstract

Seven polymorphic microsatellite loci were identified for woolly monkeys (*Lagothrix lagotricha*) from an 'enriched' genomic library. For a wild population of 66 animals, these markers averaged over 10 alleles per locus and provided a combined probability for excluding a random individual from parentage of over 98%. These loci were screened in up to 13 other genera of New World monkeys, and many were variable in multiple taxa. Few other platyrrhine-specific microsatellite markers have been identified; thus, these loci should prove valuable for studying the population genetic structure and mating system not just of *Lagothrix* but also of other neotropical primates.

Keywords: cross-taxon amplification, *Lagothrix*, microsatellites, PCR, platyrrhines, woolly monkeys

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Woolly monkeys (genus *Lagothrix*) are large-bodied neotropical primates. Traditionally, the genus has been divided into two species: the yellow-tailed woolly monkey (*Lagothrix flavicauda*), one of the world's most-endangered primates (Konstant *et al*. 2002), and the lowland woolly monkey (*Lagothrix lagotricha*), with four subspecies distributed throughout western Amazonia. As part of a long-term study of lowland woolly monkeys, we isolated and characterized seven novel polymorphic microsatellite markers for the genus. Because few other platyrrhine-specific microsatellites have been developed (Ellsworth & Hoelzer 1998; Witte & Rogers 1999; Escobar-Páramo 2000; Nievergelt *et al*. 2000; Grativol *et al*. 2001), and because many human-derived markers useful in genetic studies of Old World primates have limited utility in platyrrhines, we also tested these loci in a panel of other neotropical primates.

Markers were identified using a subtractive hybridization enrichment protocol. Roughly 6 μg of genomic DNA were digested with restriction enzymes *Hae*III, *Rsa*I, and *Nhe*I, and the resultant fragments were ligated to double-stranded 'SNX' linker (Hamilton *et al*. 1999) and amplified by polymerase chain reaction (PCR) using the linker as a primer. The set of PCR products was then 'enriched' for microsatellite-containing fragments by hybridization to 5′-biotinylated $[GT]_{15}$, $[CT]_{15}$, and $[CAG]_{10}$ oligonucleotides and recovery of the DNA-oligonucleotide hybrids on streptavidin-coated magnetic beads (Dynabeads®, Dynal). Recovered fragments were cloned in supercompetent *E. coli* (XL1-MRF′®, Stratagene) using pBluescriptII SK + (Stratagene) vector. Bacterial colonies bearing plasmids with putative microsatellite-containing inserts were identified by probing nylon transfer membranes (MagnaLift®, Micron Separations, Inc.) with the enrichment oligonucleotides using Phototope®-Star Chemiluminescent Detection kits (New England Biolabs) and were then picked directly into 'Tris-low-EDTA' buffer (10 mM Tris-HCl, 0.1 mM EDTA) and boiled.

Cloned inserts were PCR-amplified from colony boils using standard T3 and T7 primers, purified on QIAquick™ columns (Qiagen), and cycle-sequenced with ABI Prism™ DyeTerminator kits. Sequencing products were separated on ABI™ 373XL and Prism® 377 automated sequencers. A total of 73 'positive' clones were sequenced; 12 were redundant, and all but seven contained discrete microsatellites. Primers were designed from the microsatellite-flanking regions of 20 clones using PRIMER3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3_http://www.cgi) and tested

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Table 1 Characterization of seven variable woolly monkey-specific microsatellite loci and PCR conditions. F and R indicate forward and reverse primers, respectively, and the fluorescently labelled member of each primer pair is noted with an asterisk. Expected and observed heterozygosities were calculated with CERVUS 2.0 (Marshall *et al*. 1998) based on genotype data for up to 66 wild animals sampled in lowland Ecuador

Locus Name	Oligonucleotide Primer Sequences	Repeat Motif for Cloned Allele	$MgCl_2$	BSA	Annealing Temp.	Allele Size Range	# of Alleles	H_E	H_O	# of Ind.	Accession Number
1110	F* 5′-GGTGAATGAGAGAATCAAAG-3′ R 5′-TATGTTCCACAGTAGAAAGC-3′	$[GT]_{20}$	1.5 mM	0.5 µg	53 °C	202–222	11	0.857	0.848	66	AY450288
1115	F* 5′-GCTCATATTCATACATCCCTTGG-3′ R 5′-TTTGCTTGCTCATTCATTGC-3′	$[GT]_3[GA]_1[GT]_5$ $[CT]_1[GT]_{11}$	1.5 mM	0.5 µg	53 °C	196–226	11	0.836	0.864	66	AY405289
1118	F 5′-TTTCTCCCTCTCAGATTACCAG-3′ R* 5′-CCTTGAGGTTTTTGGGTTCC-3′	$[CA]_2[TA]_1[CA]_{17}$	2.0 mM	1.0 µg	50 °C	128–165	14	0.889	0.894	66	AY405290
157	F 5′-TGGCAAGTCTGGTTTCAAGC-3′ R* 5′-TTCCAGACTGAGCTAGGATGC-3′	$[GA]_4[GT]_4[CT]_1$ $[GT]_2[AT]_1[GT]_{13}$ $[GA]_9[GT]_5$	1.5 mM	0.5 µg	53 °C	215–223	6	0.734	0.758	66	AY405291
311	F 5′-CTTCCGAAAGCCATTTCTCC-3′ R* 5′-TTAATGCCAGATGATTTTGG-3′	$[GAA]_3[GAG]_9$ $[CAG]_6$	1.5 mM	0.5 µg	52 °C	191–203	5	0.297	0.318	66	AY405292
312	F* 5′-GAGACAACGACATTAACAATGC-3′ R 5′-GCTTCTGGTTTCTGATTGAGG-3′	$[GCT]_{10}$	1.5 mM	0.5 µg	50 °C	186–195	4	0.587	0.697	66	AY405293
113	F 5′-GCAAAACTCCCCTGTGACTG-3′ R* 5′-CCCACTCTCCTCCACAAAGG-3′	$[GT]_{15}$	1.5 mM	0.5 µg	50 °C	177–225	22	0.898	0.931	58	AY405294
						Average	10.4 ± 2.4 SE	0.728 ± 0.08 SE	0.759 ± 0.08 SE		

for self-complementarity with AMPLIFY version 1.2. Primer sets for seven variable loci appear in Table 1. The PCR profile for each locus, optimized on MJ Research and Bio-Rad thermal cyclers, was as follows: 94 °C for 2 min; 40 cycles of 94 °C for 30 s, the locus-specific annealing temperature for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 5 min. The PCR mix for all loci consisted of 2.5 µL Mg-free 10 × Promega™ PCR Buffer, 2.0 µL 10 mM dNTP mix (2.5 mM each dNTP), $MgCl_2$ and BSA to specifications noted in Table 1, 1.2 µL each of 10 µM F and R primers, 0.625 Units Promega™ *Taq* DNA polymerase, ~50 ng DNA template, plus ddH_2O up to a total volume of 25 µL.

The loci were used to genotype up to 69 woolly monkeys. Samples from three captive individuals were donated by the Woolly Monkey Sanctuary (Looe, England). The remaining samples came from local populations of *Lagothrix lagotricha* in Amazonian Ecuador; most were tissue punches collected from arboreal animals using biopsy darts (Karesh *et al*. 1987), but several tissue samples were salvaged from hunters' kills, and a few fecal samples were collected fresh. Tissue was stored in 'preservation buffer' (20% DMSO, 5 M NaCl, 0.25 M EDTA) or 90–100% ethanol; faeces were desiccated with silica gel or stored in 'lysis buffer' (1% SDS, 100 mM Tris-HCl, 100 mM NaCl, 10 mM EDTA). Samples were kept for several months in the field at room temperature and up to several years at –20 °C prior to analysis. For the wild population, the suite of markers averaged 10.4 alleles per locus with an average observed heterozygosity of 0.759 (Table 1) and provided an exclusionary power for parentage of 0.9837 when both parents are unknown and of 0.9986 when one parent is known (Marshall *et al*. 1998). Observed heterozygosities did not differ significantly from those expected under Hardy–Weinberg equilibrium, nor was there evidence of null alleles, for any locus.

We subsequently tested the ability of the primer sets to amplify homologous fragments in up to 13 other genera of platyrrhines. PCR mixes and cycling profiles for the cross-genus survey were identical to those used previously, except that annealing temperatures were dropped 1 °C. Results are summarized in Table 2. All seven *Lagothrix*-derived loci amplified and were variable in between two and eight other platyrrhine genera; moreover, all but one genus showed variation in at least one locus, and five genera, from three different families, showed variation in over half the loci. Thus, this suite of markers should prove useful for studying mating systems and population genetic structure in many neotropical primates. Given that only a few samples of each genus were available for screening, a more rigorous estimate of variability in these loci in other New World monkeys is not practical; nonetheless, this study reflects the broadest cross-taxon screening of platyrrhine-derived primers yet undertaken.

Table 2 Amplification success and allele size data for woolly monkey in other New World primates. Columns for each locus indicate the number of alleles and, in parentheses, the number of individuals genotyped. Data presented for woolly monkeys are based on up to 66 wild animals from lowland Ecuador and three captive animals of unknown provenience

Species			Locus 1110	Size	Locus 1115	Size	Locus 1118	Size	Locus 157	Size	Locus 311	Size	Locus 312	Size	Locus 113	Size	Proportion Variable
Family	Atelidae	*Lagothrix lagotricha*	13 (69)	194–222	11 (69)	196–226	17 (69)	128–168	6 (69)	215–223	5 (69)	191–203	4 (69)	185–195	23 (61)	173–225	7/7
		Alouatta seniculus[a]	5 + (31)	191–212	3 (11)	214–220	3 (11)	145–162	3 (29)	215–223	2 (32)	197–200	1 (31)	177	3 (2)	179–203	6/7
		Ateles belzebuth	1 (1)[b]	218	1 (1)[b]	206	4 (3)	134–163	3 (2)	261	1 (3)	194	3 (2)	183–192	1 (2)	181	3/7
		Brachyteles sp.	2 (1)[b]	218–222	4 (3)	196–208	4 (2)	142–166	4 (3)	229–237	1 (2)	194	2 (2)	176–179	3 (2)	181–186	6/7
	Pithecidae	*Callicebus* sp.	2 (1)[b]	188–214	3 (2)	214–220	1 (1)[b]	158	3 (2)	228–263	3 (2)	181–190	no amp (2)	—	1 (2)	181	4/7
		Chiropotes satanus	1 (1)	192	1 (1)	203	1 (1)	146	1 (1)	248	1 (1)	209	1 (1)	189	1 (1)	190	0/7
		Pithecia pithecia	2 (1)[b]	212–218	1 (1)	217	1 (1)	162	3 (2)	187–228	1 (1)	206	1 (1)	186	1 (1)	163	2/7
	Cebidae	*Callimico goeldii*	not tried	—	2 (1)	201–213	not tried	—	not tried	—	not tried	—	not tried	—	not tried	—	1/1
		Callithrix pygmaea	no amp (1)	—	no amp (1)	—	2 (1)	144–146	1 (1)	200	no amp (1)	—	no amp (1)	—	1 (1)	173	1/7
		Saguinus oedipus[c]	2 (1)	174–176	1 (1)	206	2 + (1)	189–191	2 (1)	208–226	2 (1)	215–218	1 (1)	177	no amp (1)	—	4/7
		Leontopithecus rosalia	no amp (1)	—	no amp (1)	—	2 (1)	179–181	1 (1)	241	2 (1)	230–233	no amp (1)	—	1 (1)	172	2/7
		Aotus vociferans	no amp (1)	—	2 (1)	188–192	2 (1)	166–168	2 (1)	170–172	1 (1)	200	1 (1)	177	1 (1)	172	3/7
		Cebus albifrons	no amp (1)	—	1 (1)	201	no amp (1)	—	1 (1)	203	2 (1)	191–194	1 (1)	177	1 (1)	167	1/7
		Saimiri sp.[d]	no amp (2)	—	3 (2)	216–225	2 (2)	149–152	3 (2)	231–235	3 (2)	225–234	1 (2)	182	2 (2)	150–172	5/7

[a]The *Alouatta* sample comprised up to two individuals from eastern Ecuador and up to 30 individuals from central Venezuela. For locus 113, both animals genotyped were from Ecuador. For locus 1115, all animals genotyped were from Venezuela. For loci 1110 and 1118, one animal was from Ecuador and the rest were from Venezuela. For loci 157, 311, and 312, 2 animals were from Ecuador and the remainder from Venezuela. For locus 311, all Venezuelan animals were homozygous for a single allele.

[b]In each of these cases, a second individual failed to amplify in the PCR reaction.

[c]The single *Saguinus* sample comes from blood, which is a chimeric tissue in this and other callitrichine primates. Locus 1118 for this individual appears to possess four alleles, consistent with blood chimerism between two individuals each heterozygous for different alleles.

[d]For loci 113 and 1118, each of the two animals sampled was homozygous for a different allele. These animals may, in fact, represent different species of *Saimiri*.

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References

Ellsworth J, Hoelzer GA (1998) Characterization of microsatellite loci in a New World primate, the mantled howler monkey (*Alouatta palliata*). *Molecular Ecology*, **7**, 657–666.

Escobar-Páramo P (2000) Microsatellite primers for the wild brown capuchin monkey *Cebus apella*. *Molecular Ecology*, **9**, 107–118.

Grativol AD, Ballou JD, Fleischer RC (2001) Microsatellite variation within and among recently fragmented populations of the golden lion tamarin (*Leontopithecus rosali*a). *Conservation Genetics*, **2**, 1–9.

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *Biotechniques*, **27**, 500–507.

Karesh WB, Smith F, Frazier-Taylor H (1987) A remote method for obtaining skin biopsy samples. *Conservation Biology*, **1**, 261–262.

Konstant WR, Mittermeier RA, Rylands AB *et al*. (2002) The world's 25 most endangered primates. *Neotropical Primates*, **10**, 128–131.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Nievergelt CM, Digby LJ, Ramakrishnan U, Woodruff DS (2000) Genetic analysis of group composition and breeding system in a wild common marmoset (*Callithrix jacchus*) population. *International Journal of Primatology*, **21**, 1–20.

Witte SM, Rogers J (1999) Microsatellite polymorphisms in Bolivian squirrel monkeys (*Saimiri boliviensis*). *American Journal of Primatology*, **47**, 75–84.