

## PERMANENT GENETIC RESOURCES

**Isolation and characterization of microsatellite markers in the white-faced capuchin monkey (*Cebus capucinus*) and cross-species amplification in other New World monkeys**

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Seventeen polymorphic microsatellite loci were identified for white-faced capuchin monkeys from an enriched genomic library in addition to one locus found through cross-species comparisons. In a sample of 187 wild individuals, these loci exhibited an average of five alleles and an observed heterozygosity of 0.62. The combined probability of exclusion of a random individual from parentage was 0.99. These loci were screened in 23 other New World monkeys and an average of seven loci was variable per species, suggesting that these loci could be of use in studies of other Neotropical primates.

*Keywords:* cross-species amplification, genotyping, noninvasive sampling, null alleles, paternity, platyrrhines

*Received 17 May 2007; revision accepted 27 July 2007*

Capuchin monkeys (*Cebus*) live in diverse forested habitats in Central and South America and are one of the most intensely studied New World primates. Although human-specific markers are routinely used in studies of Old World monkeys and apes (e.g. Bradley *et al.* 2000), the success of cross-species amplifications in New World primates tends to be low (Clisson *et al.* 2000).

Microsatellite detection is challenging and time-consuming (Scott *et al.* 2000). Thus, for this study, four enriched libraries were obtained commercially (Genetic Identification Services, USA) from 2 µg of white-faced capuchin genomic DNA digested with *EcoRI* restriction enzyme. DNA was extracted using a standard phenol–chloroform protocol from fresh blood samples obtained during veterinary procedures at the Centre de Primatologie, Strasbourg, France. Libraries consisted of recombinant pUC19 plasmids containing CA, GA, CAG, and TAGA nucleotide repeat fragments, respectively. We obtained 25 sequences for each of the di- and tetranucleotide repeat libraries, and three sequences for the trinucleotide repeat library. The CAG sequences showed broken or complex repeat patterns not useful for

genetic analyses. An additional 55 sequences were subsequently obtained by L.M. from the TAGA-enriched library following cloning using a TOPO TA Cloning Kit (Invitrogen).

The 108 resulting unique sequences were examined and oligonucleotide primers for four CA, one GA and 23 TAGA repeat sequences were developed using PRIMER 3 freeware (Rozen & Skaletsky 2000). Seventeen tetranucleotide repeat loci amplified well and were variable (Table 1). The polymerase chain reaction (PCR) mix for these loci consisted of 1× SuperTaq buffer (HT Biotechnology), MgCl<sub>2</sub> as in Table 1 and 200 nM of each primer, 200 µM of each dNTP, 0.3 U SuperTaq (HT Biotechnology) previously mixed 2 : 1 with Taqstart Antibody (BD Biosciences), 6 µg BSA, 25–200 pg template DNA, and water to 15 µL. Amplifications were run on a PTC-200 thermocycler (MJ Research) with denaturation at 95 °C for 3 min; up to 45 cycles of: 30 s at 95 °C, 30 s at  $T_a$  °C (Table 1) and 30 s at 72 °C; with a final extension for 30 min at 72 °C. Each forward primer was labelled with 6-FAM or HEX at its 5' end. PCR products were separated in an automated sequencer (ABI PRISM 3100, Applied Biosystems), and alleles were sized relative to an internal standard (HD400 with ROX fluorescent label) using GENESCAN 2.0 software (Applied Biosystems). A total of 99 human-derived microsatellite markers were

**Table 1** Characterization of 18 primer pairs for amplifying polymorphic microsatellite loci in white-faced capuchins (*Cebus capucinus*) and PCR conditions. F and R indicate forward and reverse primers, respectively. Observed and expected heterozygosities ( $H_O$  and  $H_E$ ) were calculated from the genotypes of 187 wild individuals from the Lomas Barbudal Biological Reserve area in Costa Rica

Locus	Primer sequences (5'-3')	Repeat motif	MgCl <sub>2</sub> (mM)	T <sub>a</sub> (°C)	Allele size range	No. of alleles	H <sub>O</sub>	H <sub>E</sub>	Accession nos
Ceb01	F: CCAGGCAAGCCAGCAATC R: GAGCCAATTCGCCCTAATAAATGTC	[TATC] <sub>12</sub>	1.5	58	197–205	3	0.487	0.509	EU019196
Ceb02	F: ACAGCGAGCAATATAACCT R: TCCTTCCTATGCAAATTC	[TCTA] <sub>9</sub>	1.5	55	225–233	3	0.207	0.209	EU019197
Ceb03	F: TGGAAGCTGTGGGTATCAGTGT R: TGTCAATGCTTTTAGGGGTTTC	[GATA] <sub>11</sub>	1.5	58	177–201	6	0.684	0.672	EU019198
Ceb04	F: CTTGAACTCGGGAAATGG R: TGTGAGGCTTGTCTTTTAAC	[TCTA] <sub>14</sub>	2.0	57	174–198	5	0.524	0.504	EU019199
Ceb07	F: ACCCAGGACAGGCAAAGG R: ATTATGGAGGGTCCGGTGTG	[AGAT] <sub>10</sub>	1.5	55*	119–135	4	0.567	0.567	EU019200
Ceb08	F: GCCTGGGTAACAAGAGCA R: TATTTGAAACGGTGGGTCCAG	[TAGA] <sub>12</sub>	1.5	58	161–189	6	0.642	0.598	EU019201
Ceb09	F: GGGCTTCTCAGCCTCCAC R: CAGGGTTCTCCAAAGAAAGAGA	[ATCT] <sub>10</sub>	1.5	60*	153–189	8	0.690	0.712	EU019202
Ceb10	F: TTGCTGATGCTTGCCTTC R: TGGCAGATTGTGGACTTCTC	[AGAT] <sub>13</sub>	1.5	61	238–250	4	0.594	0.644	EU019203
Ceb11	F: GCTTTCTGACTTGGGCTGAC R: TGGTTTGGATGCCTCTGAC	[TCTA] <sub>11</sub>	1.5	59	223–251	7	0.813	0.755	EU019204
Ceb105	F: GCACCTCCCCTGTCTGTTC R: TAGGACTTGGGCTGGCTTC	[TAGA] <sub>10</sub>	2.0	60	236–244	3	0.583	0.575	EU019208
Ceb115	F: CCTGGGCAACAGAGTGAG R: TACACACAGTATTGGGAGACCA	[ATCT] <sub>11</sub>	1.5	58	122–134	5	0.749	0.755	EU019209
Ceb119	F: TGGGCAACAGAGCAAGAC R: ACTTGAGAGGTTGAAGCATGAG	[GATA] <sub>12</sub>	2.0	62	229–257	6	0.674	0.664	EU019210
Ceb120	F: TTTGGGACTTGGACTGGTTC R: CCGGGTGTATTAGGGTCCCTC	[CTAT] <sub>13</sub>	1.5	60*	210–230	6	0.658	0.683	EU019211
Ceb121	F: CCATTTAGGGGAGGAGAAGG R: TTGGTTGGTAGGCAGGTAGG	[CTAT] <sub>10</sub>	1.5	59	140–184	5	0.695	0.667	EU019212
Ceb127	F: TGAGGCTTTGAGAGGGTATGTG R: AGGCAGGCAGGCAGACAG	[TATC] <sub>9</sub>	1.5	60	243–255	4	0.524	0.530	EU019213
Ceb128	F: CAGCGAGGTTTCATCTCAAG R: TATTGCCAGGTCCAAAAGTG	[CTAT] <sub>10</sub>	1.5	60	190–206	5	0.738	0.716	EU019214
Ceb130	F: CAAAGTCCACTCACTTAACCAC R: AGAAGACCCCTGCCTCAAG	[ATCT] <sub>9</sub>	1.5	59*	182–218	8	0.727	0.692	EU019215
D7S794†	F: GCCAATTCCTCAACAAATCC R: TATGCCCATGTGTTAGGGTT	[GATA]	1.5	52	133–145	3	0.658	0.648	G08607
					<i>Average</i>	<i>5.1</i>	<i>0.623</i>	<i>0.617</i>	

\*The first two cycles were run at T<sub>a</sub> +2 °C, followed by two cycles at T<sub>a</sub> +1 °C, and remaining cycles at T<sub>a</sub>.

†Primers designed for human typing.

also screened in *C. capucinus*; only one was found to exhibit appreciable variation and is also listed in Table 1.

Non-invasive faecal samples were collected since 1994 from habituated wild individuals belonging to three study groups in and around Lomas Barbudal Biological Reserve, Costa Rica (10°29–32'N, 85°21–24'W), and stored using silica desiccant or RNAlater solution as described in Nsubuga *et al.* (2004). Samples were extracted using the QIAamp DNA Stool Mini Kit (QIAGEN) with modifications of the manufacturer's protocol. DNA was extracted from approximately 100 mg of dried faeces following

Morin *et al.* (2001), while RNAlater samples were extracted starting from 2 mL of the solution-sample mixture, as described in Nsubuga *et al.* (2004). DNA was eluted in a final volume of 200 µL of AE buffer and the template concentration estimated using quantitative PCR as previously described (Morin *et al.* 2001).

The set of one human and 17 newly identified markers was used to genotype 187 wild white-faced capuchins. Loci had an average of five alleles with an average observed heterozygosity of 0.623 and exclusionary power for the first parent of 0.99 (Marshall *et al.* 1998) (Table 1).

**Table 2** Cross-species amplifications of white-faced capuchin primers in other New World primates. Number of alleles and allele size range (in parentheses) are given for each locus, and *N* indicates the number of individuals tested

Family*	Species	<i>N</i>	Ceb01	Ceb02	Ceb03	Ceb04	Ceb07	Ceb08	Ceb09	Ceb10	Ceb11	Ceb105	Ceb115	Ceb119	Ceb120	Ceb121	Ceb127	Ceb128	Ceb130	Proportion variable	
Cebidae	<i>Callithrix geoffroyi</i>	2	2 (295–299)	unsp.	2 (168–180)	1 (181)	1 (188)	1 (129)	2 (124–128)	4 (227–255)	2 (231–239)	unsp.	unsp.	1 (152)	1 (173)	1 (142)	2 (197–201)	2 (212–216)	1 (239)	7/14	
	<i>Callithrix jacchus</i>	2	1 (377)	1 (277)	2 (168–176)	1 (180)	1 (288)	1 (128)	3 (120–132)	2 (230–238)	3 (234–242)	unsp.	unsp.	1 (152)	1 (173)	1 (142)	3 (197–205)	3 (203–211)	1 (267)	6/15	
	<i>Callithrix pygmaea</i>	1	—	—	1 (169)	unsp.	1 (114)	1 (132)	2 (215–219)	1 (220)	—	—	1 (175)	1 (205)	2 (173–189)	unsp.	1 (200)	1 (200)	1 (259)	2/11	
	<i>Leontopithecus chrysomelas</i>	2	3 (301–313)	1 (230)	4 (192–212)	unsp.	unsp.	1 (128)	1 (142)	3 (199–207)	1 (194)	unsp.	unsp.	2 (179–198)	unsp.	2 (168–172)	1 (197)	2 (207–211)	2 (293–297)	7/12	
	<i>Leontopithecus</i> sp.	1	—	—	1 (212)	1 (233)	unsp.	1 (128)	1 (143)	1 (200)	—	—	unsp.	2 (179–198)	unsp.	1 (176)	1 (197)	2 (207–211)	2 (289–293)	3/10	
	<i>Saguinus nigricollis</i>	1	na	1 (229)	1 (191)	unsp.	2 (181–189)	1 (424)	1 (148)	1 (249)	2 (231–243)	1 (495)	unsp.	1 (223)	2 (216–220)	2 (205–209)	1 (249)	2 (193–213)	—	5/13	
	<i>Saguinus oedipus</i>	2	na	2 (269–273)	1 (196)	1 (179)	1 (189)	1 (162)	1 (148)	2 (218–222)	3 (225–237)	1 (495)	unsp.	1 (211)	1 (173)	1 (175)	1 (251)	2 (202–210)	2 (154–162)	5/15	
	<i>Callimico goeldii</i>	2	2 (291–299)	1 (224)	1 (138)	unsp.	1 (188)	1 (163)	1 (142)	2 (207–211)	2 (297–309)	2 (292–320)	unsp.	1 (191)	1 (224)	1 (167)	1 (197)	1 (191)	3 (240–252)	5/15	
	<i>Cebus apella</i>	10	3 (181–193)	1 (243)	2 (150–154)	2 (174–178)	4 (157–169)	3 (158–170)	5 (169–189)	4 (247–263)	1 (233)	5 (220–244)	3 (128–136)	2 (250–254)	7 (249–281)	1 (145)	5 (248–264)	2 (178–198)	6 (263–283)	14/17	
	<i>Cebus apella apella</i>	4	—	—	1 (190)	1 (174)	2 (165–169)	1 (162)	2 (169–181)	3 (247–255)	—	—	3 (128–136)	2 (250–254)	1 (281)	1 (145)	1 (256)	2 (178–190)	2 (192–204)	7/13	
	<i>Cebus olivaceus olivaceus</i>	3	3 (194–206)	4 (223–235)	3 (194–206)	5 (191–211)	2 (123–127)	3 (173–185)	3 (149–165)	2 (247–255)	3 (249–257)	2 (240–244)	2 (132–136)	1 (246)	2 (224–228)	2 (174–178)	3 (236–260)	2 (195–199)	3 (278–290)	16/17	
	<i>Cebus xanthosternos</i>	3	2 (181–197)	3 (231–247)	1 (146)	4 (170–186)	2 (131–135)	1 (165)	3 (169–193)	3 (246–258)	2 (229–233)	2 (228–244)	4 (120–136)	2 (262–270)	2 (220–224)	4 (137–173)	4 (240–264)	1 (178)	3 (280–288)	14/17	
	<i>Saimiri boliviensis peruviansis</i>	17	2 (179–203)	3 (261–269)	3 (151–159)	1 (433)	unsp.	1 (242)	2 (142–146)	4 (281–293)	4 (222–234)	unsp.	1 (180)	1 (215)	1 (192)	5 (187–203)	1 (207)	1 (158)	2 (145–149)	8/15	
	<i>Saimiri sciureus</i>	2	2 (204–208)	2 (272–280)	3 (147–155)	1 (432)	1 (262)	1 (242)	1 (142)	1 (252)	1 (231)	unsp.	1 (180)	unsp.	2 (191–199)	3 (191–199)	2 (207–211)	1 (158)	2 (145–149)	7/15	
	Nyctipithecidae	<i>Aotus azarae</i>	4	2 (242–246)	3 (249–261)	1 (145)	1 (193)	unsp.	1 (308)	1 (133)	1 (212)	3 (219–231)	unsp.	unsp.	unsp.	2 (178–190)	1 (141)	3 (202–210)	3 (229–261)	unsp.	6/12
	Pitheciidae	<i>Pithecia pithecia pithecia</i>	1	1 (226)	1 (297)	2 (190–194)	1 (458)	1 (254)	1 (183)	2 (206–210)	1 (249)	2 (254–258)	1 (225)	2 (107–119)	2 (176–188)	2 (155–159)	1 (139)	1 (207)	1 (174)	1 (166)	6/17
		<i>Chiropotes satanas × albinasus</i>	1	—	—	unsp.	1 (183)	unsp.	1 (292)	1 (163)	na	—	—	1 (183)	unsp.	2 (210–230)	2 (149–153)	1 (124)	2 (170–174)	na	3/8
<i>Cacijo calvus</i>		1	1 (226)	1 (285)	na	1 (521)	1 (143)	1 (180)	1 (163)	2 (220–224)	2 (258–270)	1 (118)	unsp.	unsp.	2 (242–246)	1 (160)	unsp.	1 (176)	na	3/12	
<i>Callicebus cupreus</i>	1	2 (400–416)	1 (231)	na	1 (190)	1 (303)	1 (180)	2 (172–176)	2 (213–221)	1 (268)	1 (205)	2 (106–114)	2 (196–208)	1 (138)	unsp.	unsp.	1 (207)	2 (229–241)	6/14		
Atelidae	<i>Alouatta caraya</i>	1	2 (277–289)	1 (266)	1 (123)	1 (482)	1 (114)	1 (315)	1 (137)	1 (222)	2 (209–213)	unsp.	na	unsp.	2 (186–190)	2 (180–188)	1 (212)	2 (258–266)	unsp.	5/13	
	<i>Ateles belzebuth hybridus</i>	1	—	—	2 (142–150)	unsp.	1 (240)	1 (311)	2 (124–140)	2 (212–216)	—	—	1 (450)	unsp.	2 (195–211)	2 (205–217)	1 (204)	1 (246)	na	5/10	
	<i>Ateles fusciceps robustus</i>	11	2 (231–235)	2 (239–243)	3 (138–150)	2 (289–297)	unsp.	2 (297–313)	3 (124–144)	1 (212)	1 (213)	1 (216)	na	unsp.	1 (185)	4 (192–204)	2 (196–204)	1 (246)	unsp.	8/13	
	<i>Lagothrix lagothricha</i>	3	4 (240–268)	2 (201–205)	2 (138–146)	unsp.	unsp.	unsp.	3 (122–134)	3 (204–212)	1 (215)	unsp.	na	1 (179)	3 (170–190)	3 (255–267)	2 (200–212)	unsp.	1 (434)	8/11	

\*Classification according to Groves 2001.

Unspecific amplifications (unsp.) means that clear products are detected, but spread over an unusually large size range. na, no amplification. (—), not tested.

The probability that a pair of siblings share the same composite genotype is extremely low ( $P_{ID_{sibs}} = 0.0001$ ) (Waits *et al.* 2001). No consistent deviations from Hardy–Weinberg equilibrium or linkage disequilibrium were found ( $P < 0.05$ ) (Raymond & Rousset 1985).

Although no evidence of null alleles was found through deviations of allele frequency expectations (Raymond & Rousset 1985; Marshall *et al.* 1998), subsequent pedigree analysis suggested occurrence of a null allele at locus Ceb115 in one group, involving 12 related individuals spanning three generations. The null allele was initially detected by comparisons of the genotypes of one female and two of her known offspring, and the remaining carriers of the null allele were subsequently detected through examination of the parentage analysis records: despite mismatching at a seemingly homozygous locus, the potential parent carrying the putative null allele always had a higher likelihood of parentage than other sampled candidates.

Cross-species amplification tests were done using approximately 200 pg of DNA in 15  $\mu$ L PCRs as mentioned above but under varying magnesium salt concentrations (1.5, 2.0 and 2.5 mM) and two annealing temperatures (2 and 5 °C below the optimal annealing temperature for *Cebus capucinus*).

Between 84 and 100% of the 17 loci amplified in other species of New World primates ( $N = 23$ ), although in some cases reactions yielded unspecific products (Table 2). All species tested were polymorphic (two or more alleles) for at least two loci (mean = 7.0, SD =  $\pm 3.7$ ). The three species of *Cebus* (*C. apella*, *C. olivaceus* and *C. xanthosternos*) showed the largest numbers of potentially useful loci, as expected due to phylogenetic proximity. Results varied among loci, and while locus Ceb115 only amplified or yielded specific products in half of the species tested, Ceb09 successfully amplified in all species, often exhibiting polymorphism.

A set of 18 polymorphic microsatellite markers appropriate for individual identification and analyses of parentage was characterized in white-faced capuchins. The preliminary analysis of cross-species amplification success suggests that the markers reported here may also be useful for assessing reproductive patterns and population genetic structure in a variety of Neotropical primates.

## Acknowledgements

We thank the Centre de Primatologie (CdP), Strasbourg, France, for providing blood samples from *C. capucinus* and C. Roos of the German Primate Center (DPZ), Goettingen, Germany, for providing DNAs from additional New World monkeys. Funding was provided by the Max Planck Society. Technical assistance was kindly provided by H. Siedel, C. Lang, and D. Schwochow. This work is part of an investigation of the genetics of the Lomas Barbudal study groups in collaboration with S. Perry, J. Manson and colleagues, particularly H. Gilkenson.

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