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Heterologous amplification and diversity of microsatellite loci in three owl monkey species (*Aotus azarai, A. lemurinus, A. nancymaae*)

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Genetic support for behavioral studies of primate social systems is fundamental in order to assess reproductive success of individuals. Molecular markers such as microsatellites have found broad application in population genetics as well as conservation assessments and serve as a powerful tool for paternity analysis (Bruford and Wayne 1993). The effort required to characterize highly polymorphic microsatellite loci may be reduced if primers developed for the human genome may be utilized for non-human primates through cross-species amplification (Moore et al. 1991; Coote and Bruford 1996; Morin et al. 1997, 1998). Only a limited number of New World monkey microsatellite primers have been reported to successfully amplify owl monkey DNA. Twenty polymorphic microsatellite loci were characterized in three species of owl monkeys (*Aotus azarai, A. lemurinus, A. nancymaae*) for use in parentage analysis from human and New World monkey genomes. Genetic variation across the three species and within a subset of a wild population of *A. azarai* has been examined.

Fibroblast cells, whole blood, and tissues were obtained from the Center for Reproduction of Endangered Species (CRES) of the Zoological Society of San Diego in California, the DuMond Conservancy in Miami, Florida, and the Province of Formosa, Argentina (Fernandez-Duque et al. 2001). Genomic DNA was extracted from fibroblast cell cultures using a modified protocol of Priest (1997) for dispersion of monolayer cells followed by DNA extraction utilizing a kit (QIAGEN Inc). DNA from blood and tissue was extracted following a modified version of Geyer et al. (1993). A total of 39 individuals from three different species were available for this study. Twelve individuals were from a wild population in Formosa where individuals from different social groups are regarded as unrelated and twenty-seven were from captive groups; 21 belonged to five reference families (one family of *A. azarai* x *A. nancymaae*, one of *A. lemurinus*, and three of *A. nancymaae*).

A total of 215 published human microsatellite primer pairs (GDB, NCBI) and six published primers designed to amplify microsatellites in *Saimiri boliviensis* (Witte and Rogers 1999) and *Cebus apella* (Escobar-Páramo 2000) which had been reported to amplify *Aotus* DNA were screened using a gradient PCR thermal cycler (Eppendorf Scientific, Inc.) to carry out PCR amplification. A 15 μl volume was used and the reaction mix contained 15 ng of genomic DNA, 0.2 μM of each primer, 0.05 mM of each dNTP, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin and 0.5 U of AmpliTaq Gold^{TM} (Zhang et al. 2001). PCR conditions were

Table 1. Microsatellite loci characterized for three species of owl monkeys using human and New World monkey microsatellite primers

Locus	Primer sequences (5′–3′)	Annealing temp °C	*A. azarai* (N = 11)		*A. lemurinus* (N = 4)		*A. nancymaae* (N = 8)		Human[a]	
			No. of alleles	Size range (bp)	No. of alleles	Size range (bp)	No. of alleles	Size range (bp)	Size range (bpt)	Variation type
D2S95	**F:** GACAGAGCAACACCCCAACT **R:** TCATCACTCACCCAGACCAA	60.1	4	150–156	3	150–154	2	137, 139	146	dinucleo-tide repeat
D2S382	**F:** ATGCTAAATCTCAAAGCCAGTCT **R:** CTAATNCTCAATTCAGTGCTCCT	60.1	6	135–159	2	153, 157	7	155–167	160	di-
D4S397	**F:** AGGGAGGTCATCAGTTCATT **R:** TGTTGCAAACTTTGCTTTTC	56.9	3	191–217[10]	1	217	4	211–217	192–214	di-
D4S411	**F:** AGGCTGTCTTGGCAGAAAT **R:** GATGTAATCCTGTGCTATGGC	60.1	8	130–170	6	140–184	11	136–164	135–143	di-
D5S117[b]	**F:** TGTCTCCTGCTGAGAATAG **R:** TAATATCCAAACCACAAAGGT	60.1	5	130–146[10]	3	132–142	6	134–152	147–163	di-
D5S178	**F:** TGGAGAGTCGATTTACATAAG **R:** CAGGAAATAGGGTCTTT	51.6	5	81–89	5	79–91	6	79–95	98–120	di-
D5S353	**F:** ATACACTGGAAATCCACATTGTG **R:** ATCCCACACACAGTGCAGAA	56.9	3	125–129 [10]	3	129–133[3]	7	119–131	133	di-
D5S814	**F:** TGGACTTTTCCAGCACAGAT **R:** CTCTACAAAAGAAGTTAAATCGAGC	51.6	4	176–192 [8]	4	176–196	4	180–192	181	tetra-
D8S260	**F:** AGGCTTGCCAGATAAGGTTG **R:** GCTGAAGGCTGTTCTATGGA	60.1	5	207–215	4	207–215	4	203–211	187–213	di-
D8S275	**F:** AAATCGCTAGAAAATGTCCA **R:** TCACACCTGGGAATTAGAAG	60.1	6	140–164	4	157–169	5	140–152	139–157	di-

D10S2327	**F:** CCCAGAGCAAGTACTCACCT **R:** ATAGTTTTGTGCTTATTGACATGA	56.9	4	173–189	4	173–189	6	155–189	200	tetra-
D12S309	**F:** AGCTCATTCGCACATGG **R:** TCCTATCTTGGATCAGGTTG	60.1	2	128, 130	2	128, 134	2	114, 128	136–146	di-
D13S155	**F:** ACAGCCAGCACATTTATTGA **R:** GGTATATTCTCAGAGCCTGGAT	60.1	5	207–237[10]	4	219–239	7	205–237	204–218	di-
D13S160	**F:** CGGGTGATCTAAGGCTTCTA **R:** GGCAGAGATATGAGGCAAAA **R:** GGCAGAGATATGAGGCAAAA	60.1	7	220–242	6	236–254	9	220–244	229–241	di-
D15S108	**F:** AGGAGAGCTAGAGCTTCTAT **R:** GTTTCAACATGAGTTTCAGA	51.6	6	171–189	5	173–201	8	169–195	185–205	di-
D15S816	**F:** AATAGAAACACAGGAGATCTCTTT **R:** GTCAGGCGAGTTTTTGAAGA	60.1	2	89, 93	1	89	2	89, 93	135	tetra-
D16S417	**F:** CTGTCCAACATGCAGCC **R:** TGAAGTCAATCCCACTTGAA	60.1	5	118–134	4	110–128	8	120–136	124–142	di-
D16S505	**F:** GACTGTGTCTGCCCAA **R:** TCTGCCTCCATACGTG	56.9	5	231–247[10]	5	217–253	9	217–251	239–261	di-
PEPC8[c]	**F:** TTCAGGATGCATCAAATGATT **R:** TAGCAGTCTATTTAGGTGTTAAT	48.7	4	247–257	1	247	2	253, 255	128–168 (in *Cebus apella*)	$(CA)_{16}$
SW34D[d]	**F:** CATCAAAGGATATTATTATC **R:** TACATTTCTGGATACTAGGC	51.6	2	128, 134 [10]	4	142–152	3	122–126	140–144 (in *Saimiri bolivienis*)	$(CA)_{14}$

(N) Number of unrelated individuals tested for this species. Offspring of sampled parents were excluded.[ ] Number of unrelated individuals tested for this species at the particular locus

[a] The Genome Database (GDB).

[b] This locus was characterized for use in the mantled howler monkey (*Alouatta palliata*) Ellsworth and Hoelzer 1998; Winkler et al. 1999), common marmoset (*Callithrix jacchus*) (Nievergelt et al. 1998), and Bolivian squirrel monkey (*Saimiri boliviensis*) (Witte and Rogers 1999).

[c] Escobar-Páramo 2000

[d] Witte and Rogers 1999.

Table 2. Genetic variation among three species of *Aotus* and between captive and wild *A. azarai* populations. The number of unrelated individuals sampled (N), average number of alleles (n), and mean observed heterozygosity (H_o) across 17 loci that are polymorphic for all three species (unless otherwise noted) with 95% confidence interval in parentheses are presented

Species	N	n	Mean H_o (95% interval)
Aotus azarai (captive and wild individuals combined)	11	4.8	0.39(0.36, 0.42)
Captive individuals[a]	4	4.4	0.52(0.47, 0.57)
Wild individuals[a]	7	2.2	0.44(0.40, 0.48)
Aotus lemurinus	4	4.0	0.52(0.48, 0.56)
Aotus nancymaae	8	6.1	0.60(0.57, 0.63)

Offspring of sampled parents were excluded in the analyses.
[a] Only polymorphic loci for the wild population (13 out of the 20) were considered for comparison between captive and wild *A. azarai* individuals.

as follows: initial denaturation at 94 °C for 10 min followed by 40 cycles of 94 °C for 1 min, annealing temperatures ranging from 40 to 60 °C for 1 min, 72 °C for 1 min and 72 °C for 10 min at the end. No attempt was made to adjust $MgCl_2$ concentration or other reaction conditions that might have yielded additional useful primers.

PCR products were electrophoresed on 1.25% TBE agarose gel, stained with ethidium bromide and visualized under ultraviolet light using the AlphaImager™ 2200 Documentation and Analysis System (Alpha Innotech Corp.). Amplified products were sized on an ABI 3100 Genetic Analyzer using the GeneScan Analysis 3.7 program (Applied Biosystems). Characterization was based on the entire sample pool of individuals not known to be related (23) with no attempts made to optimize PCR conditions and therefore assignment of monomorphic microsatellites should be considered tentative. The program Arlequin version 2.000 (Schneider et al. 2000) set at the default parameters was used to calculate the observed heterozygosity (H_o). The mean allele numbers and mean H_o across polymorphic loci (17) were used as measures of genetic diversity. The *t* distribution was used to obtain confidence intervals for gene diversity estimates (Nei 1987; Gutiérrez-Espeleta et al. 2000).

We applied a collection of published human microsatellite primers known to be conserved between humans and Old World primates (Morin et al. 1997, 1998; Rogers et al. 2000) to test for amplification in *Aotus*. Out of 215 analyzed human microsatellite loci, 107 were found to produce amplification products for *Aotus* species. Of these, 81 had the dye chemistry suitable for our ABI 3100 Genetic Analyzer. Of these 81, 18 were both polymorphic and informative for *Aotus* species. Altogether, 20 polymorphic loci were identified based on reliable and reproducible amplification of single bands; 18 derived from human microsatellite primers, one designed for *Cebus apella* (Escobar-Páramo 2000) and one for *Saimiri boliviensis* (Witte and Rogers 1999) (Table 1). Mendelian inheritance was observed in all 20 loci in each of the five families with three exceptions. The existence of null alleles (Callen et al. 1993) would explain the non-Mendelian patterns observed once for each of these loci (D5S178, D5S353, and D16S417). Otherwise, Mendelian inheritance of microsatellite alleles was displayed in the examined pedigrees. Although our findings are consistent with the presence of null alleles at these loci in our captive population samples, confirmation of their existence requires additional investigation. Two or three repeat amplifications were undertaken on all individuals with identical results; offspring that initially genotyped as heterozygotes qualifying to both parents were repeated at least once and individuals scored as homozygotes were repeated at least twice.

Summary data for diversity levels in each species are provided in Table 2. The *A. azarai* individuals were subdivided into captive and wild individuals and the mean allele numbers and mean H_o were calculated with only those loci that were polymorphic (13) for the wild population (Table 2).

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