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Short Communication

Informative microsatellites for genetic population studies of black-faced lion tamarins (*Leontopithecus caissara*)

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Abstract

Leontopithecus caissara is a critically endangered primate species from the Brazilian Atlantic Forest. Nineteen microsatellite loci, previously developed for congeneric species, were tested with 34 *L. caissara* individuals from Superagüi Island. Of the 19 loci, 17 (89.4%) produced robust alleles, nine (47.4%) of these proved to be polymorphic, with a total of 23 alleles and an average of 2.56 alleles per locus. Expected and observed heterozygosity averaged 0.483 and 0.561, respectively. The exclusion power for identifying the first parent of an arbitrary offspring was 0.315 over all loci. The results thus indicate both the usefulness and limitations of these nine microsatellite loci in the genetic analysis of *L. caissara*, as well as their potentiality for genetic investigation in other congeneric species.

Key words: lion tamarins, endangered species, genetic diversity, New World primate, SSR transferability.

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The black-faced lion tamarin (*Leontopithecus caissara*), whose specific status has recently received support from molecular data (Perez-Sweeney *et al.*, 2008), is a critically endangered Neotropical primate (Kierulff *et al.*, 2008). Its distribution range lies in lowland swampy forests of southeastern Brazil (Lorini and Persson, 1994), with a population currently estimated at less than 500 individuals (A. Nascimento, pers comm). This has generated apprehension when considering the impact on such a small population, of barriers hindering gene flow between main populations, to the point of genetic evaluation being considered top priority in any conservation plan involving this primate (Holst *et al.*, 2006).

Microsatellites are useful for investigating behavioral ecology (Di Fiore, 2009) and shedding light on questions concerning biological conservation (Selkoe and Toonen, 2006). They are considered expedient, notably in anticipation of management decisions beneficial to wildlife conservation. Microsatellites present relatively high rates of transferability among mammals (Barbará *et al.*, 2007), which is advantageous, since their development can be time-consuming (Squirrell *et al.*, 2003; Sarre and Georges, 2009). Thus, exploiting microsatellite available for one or more species could be a plausible alternative in the genetic investigation of congeners. Here we investigated feasibility of employing microsatellites previously isolated in other *Leontopithecus* species in *L. caissara*.

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Blood samples were taken from 34 free-ranging black-faced lion tamarins from Superagüi Island, state of Paraná, Brazil. DNA was extracted according to a modified phenol-chloroform method (Sambrook *et al.*, 1999). Nineteen microsatellites, previously developed for *Leontopithecus rosalia* (Grativol *et al.*, 2001), *L. chrysopygus* (Perez-Sweeney *et al.*, 2005) and *L. chrysomelas* (Galbusera and Gillemot, 2008), were tested. A primer for each locus was constructed with an M13 tail. A fluorescently-labeled M13 primer was also used in a three primer-PCR (polymerase chain reaction), following an established protocol (Schuelke, 2000). Microsatellite loci were amplified in a 10 μL reaction volume containing 20 ng of template DNA, 1 μL of each primer, 0.2 mM of dNTP, 1.5 mM of $MgCl_2$, and 1 U *Taq* polymerase (Fermentas). After annealing-temperature optimization, amplifications were carried out in either a Perkin Elmer 2400 thermal cycler or an Eppendorf Gradient Mastercycler, under the following conditions: 5 min at 94 °C, 30 cycles of 30 s denaturation at 94 °C, annealing at 51-61 °C for 45 s, extension for 45 s at 72 °C, and finally 10 cycles of 30 s denaturation at 94 °C, annealing at 53 °C for 45 s, extension for 45 s at 72 °C, followed by a final extension step of 10 min at 72 °C. Amplified fragments were checked on 2% agarose gels. PCR products were analyzed on a MegaBace automatic sequencer, and allele sizes scored using the FRAGMENT PROFILER version 1.2 program (Applied Biosystem®). The GENEPOP version 4.0 program (Raymond and

Table 1 - Characteristics of nine microsatellite loci from *Leontopithecus* spp. tested on 34 individuals of *Leontopithecus caissara*.

Locus	GenBank Accession	Repeat motif	T_a (°C)	Size (bp)	N_A	H_E	H_O	$Pr(Ex_1)$
Leon2[1]	AY706915	$(CA)_{18}$ (CG)$(CA)_3$	55	212	3	0.564	0.676	0.845
Leon3c20[1]	AY706916	$(GT)_{22}$	51	300	2	0.349	0.382	0.940
Leon15c85[1]	AY706920	$(GA)_{17}$	51	281	2	0.507	0.735*	0.875
Leon21c75[1]	AY706922	$(GT)_{19}(NA)_1$ $(GT)_5$	58	282	3	0.465	0.529	0.894
Leon30c73[1]	AY706927	$(TC)_{25}(AA)(TC)(TG)_{16}$	55	269	3	0.327	0.382	0.948
Leon31c97[1]	AY706928	$(GA)_2(CA)_2(GA)_{19}(TT)(GA)$ $(CA)_4$	58	323	2	0.421	0.470	0.913
LrP2BH6[2]	AF320577	$(CA)_{19}$	55	102	2	0.444	0.470	0.904
Lchμ04[3]	DQ979346	$(GATA)_{14}$	61	386	3	0.627	0.617	0.809
Lchμ07[3]	DQ979350	$(TG)_{16}$	54	325	3	0.644	0.794	0.798
All loci					2.56	0.483	0.561	0.315

Annealing temperature (T_a); size of the original *Leontopithecus* spp. clone in base pairs (bp); number of detected alleles per locus (N_A); expected heterozygosity (H_E); observed heterozygosity (H_O); exclusion probability of the first parent [$Pr(Ex_1)$]. [1]: Perez-Sweeney *et al.* (2005); [2]: Grativol *et al.* (2001); [3]: Galbusera and Gillemot (2008). * = Departs significantly from HWE at p = 0.017 after correction (Benjamini and Yekutieli, 2001).

Rousset, 1995) was used to test for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). Genetic-diversity parameters and the non-exclusion probability from parentage were estimated using the CERVUS version 3.0.3 software (Marshall *et al.*, 1998).

From the 19 microsatellite loci tested, 17 (89.4%) produced robust alleles, of which nine (47.4%) were polymorphic and eight (42.1%) monomorphic. The remaining two (10.5%) failed to amplify fragments under all the tested conditions. Analysis using the nine polymorphic microsatellites and the 34 black-faced lion tamarins revealed a total of 23 alleles. The number of alleles per locus ranged from two to three (Table 1), with an average of 2.56 alleles per locus. Among loci, expected heterozygosity varied from 0.327 to 0.644, with an average of 0.483, whereas observed heterozygosity ranged from 0.382 to 0.794, with an average of 0.561. The total exclusion power for identifying an unrelated candidate parent of an arbitrary offspring [$Pr(Ex_1)$] when neither parent was known, was estimated to be 0.315 over all loci. This value indicates that these loci may not be suitable for paternity testing. Only the Leon15c85 locus deviated significantly (p = 0.015 for corrected p = 0.017) from HWE (Table 1). Analysis using MICRO-CHECKER version 2.2.3 software (Van Oosterhout *et al.*, 2004), failed to indicate the presence of null alleles (p = 0.05) at this locus. Four loci pairs (Leon21c75 - LrP2BH6, Leon21c75 - Lchμ04, Leon3c20 - Lchμ04 and LrP2BH6 - Lchμ04) displayed significant LD after Benjamini and Yekutieli (2001) correction.

Our results confirm the usefulness of the nine microsatellite loci in genetic analyses involving *L. caissara*. Lion tamarins have figured as flagship (Dietz *et al.*, 1994) and umbrella species (Simberloff, 1998) favoring wildlife conservation at several sites in the Brazilian Atlantic Forest (Kleiman and Rylands, 2002). The wise management of lion tamarin populations could turn out to be a time saving procedure, in which the successful transferability of microsatellites between congeneric species will be of great assistance.

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