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.

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 MgCl₂, 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...
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. All loci

| Locus  | GenBank Accession | Repeat motif | Tm (°C) | Size (bp) | Na | He   | Ho   | Pr(Ex1) |
|--------|-------------------|--------------|---------|-----------|----|------|------|---------|
| Leon2  | AY706915          | (CA)15(CG)(CA)2 | 55      | 212       | 3  | 0.564 | 0.676 | 0.845   |
| Leon3c20 | AY706916          | (GT)22       | 51      | 300       | 2  | 0.349 | 0.382 | 0.940   |
| Leon15c85 | AY706920         | (GA)17       | 51      | 281       | 2  | 0.507 | 0.735* | 0.875   |
| Leon21c75 | AY706922          | (GT)41(NA)11(GT)6 | 58     | 282       | 3  | 0.465 | 0.529 | 0.894   |
| Leon30c73 | AY706927          | (TC)2(AA)(TC)(TG)16 | 55    | 269       | 3  | 0.327 | 0.382 | 0.948   |
| Leon31c97 | AY706928          | (GA)2(CA)2(GA)2(TT)(GA)2 | 58  | 323       | 2  | 0.421 | 0.470 | 0.913   |
| LrP2BH6  | AF320577          | (CA)10       | 55      | 102       | 2  | 0.444 | 0.470 | 0.904   |
| Lchu04   | DQ979346          | (GATA)14     | 61      | 386       | 3  | 0.627 | 0.617 | 0.809   |
| Lchu07   | DQ979350          | (TG)16       | 54      | 325       | 3  | 0.644 | 0.794 | 0.798   |
| All loci |                   |              |         | 2.56      | 0.483 | 0.561 | 0.315 |

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

procedure, in which the successful transferability of microsatellites between congeneric species will be of great assistance.

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### Internet Resources

**GENEPOP software**, http://kimura.univ-montp2.fr/~rousset/Genepop.htm (April 14, 2009).

**IUCN Red List of Threatened Species**, http://www.iucnredlist.org (May 23, 2010).

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