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Transferability of microsatellites for studies on the social behavior of the tufted capuchin monkey (genus *Sapajus*)

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ABSTRACT. Because of relevant results that indicated that molecular techniques can provide increased knowledge of animal social systems, they usually complement observational field studies. Despite the great utility of microsatellites, they are not available for all species. Gathering genetic information using microsatellites that were originally designed for other species is a time-saving procedure. The aim of this study was to test the transferability of microsatellites and their usefulness in studies of social behavior of black capuchin monkeys (*Sapajus nigritus*). We noninvasively sampled adult and subadult black capuchins of three wild groups in southeastern Brazil. Seventeen microsatellites, which were previously designed for and successfully amplified in multiple Neotropical primate species, were tested. Nine of the 17 microsatellite loci tested produced an average of 6.22 alleles (range 2-12) per locus. The allelic richness and the expected heterozygosity for all loci was 5.93 and 0.70, respectively. The combined non-exclusion probability for one candidate parent across all loci was 0.01. The nine microsatellite loci

optimized in this study have a great potential for application in studies of social structure and dispersal patterns in *S. nigritus* populations and in other Neotropical primate species.

Key words: Microsatellite; Capuchin monkey; *Sapajus*

INTRODUCTION

In the past decade, genetic analyses became an important tool in studies of animal behavior (Di Fiore, 2003) because inferences about attributes that are crucial to understanding animal social dynamics, such as kinship, dispersal, and paternity, are highly constrained by field observations only. Because of relevant results that indicated that molecular techniques can provide increased knowledge about animal sociality, they usually complement observational field studies.

Microsatellites have become feasible molecular markers in the genetic assessment of relatedness and paternity because they are highly polymorphic, selectively neutral, and follow Mendelian inheritance, thus comprising a useful tool in studies of animal behavior (Di Fiore, 2003; Selkoe and Toonen, 2006). Despite the great utility of microsatellites, they are not available for all species. In addition, their development is expensive and time-consuming. The success of transferability of microsatellites is high between congener species, but successful transfer may also be reached between genera and families (Barbará et al., 2007). The aim of this study was to test the transferability of microsatellites and their usefulness in studies of the social behavior of black capuchin monkeys (*Sapajus nigritus*).

Capuchin monkeys include two Neotropical primate genera, *Cebus* and *Sapajus*, that comprise 12 species (Lynch Alfaro et al., 2012). Field studies indicate that capuchin monkey social systems can be highly flexible, with intra- and interspecific variations in social organization and social structure, including sex bias in dispersal and social relationships (Jack and Fedigan, 2009; Izar et al., 2012). Most of these studies were based on behavioral observations only, and our knowledge about the behavioral flexibility of this taxon would improve with the aid of molecular techniques.

MATERIAL AND METHODS

The population studied of black capuchin monkeys lives at Carlos Botelho State Park (PECB) in São Miguel Arcanjo (24°00' to 24°15'S, 47°45' to 48°10'W), State of São Paulo, southeastern Brazil. The PECB comprises an area of about 380 km^2 within the Atlantic Forest domain, and with three other frontier parks, forms a continuous protected forest of more than 1200 km^2. Fecal samples were collected from subadult and adult monkeys (12 females and 9 males) belonging to three different social groups (Pimenta, Testa, and Pitoco). These wild free-ranging animals were habituated to the presence of researchers, and all subadult and adult individuals were individually known. We used the commercial kit QIAamp DNA Stool Mini Kit® (Qiagen, Germany) to extract DNA. Seventeen microsatellites, which were previously designed for and successfully amplified in multiple Neotropical primate species, were tested. Four microsatellites were isolated in at least two *Sapajus* species (PEPC3, PEPC8, PEPC40, and PEPC59: Escobar-Páramo, 2000), one in *Lagothrix lagotricha* (PEPL4: Escobar-Páramo, 2000), eight in *Cebus capucinus* (Ceb3, Ceb8, Ceb9, Ceb11, Ceb119, Ceb120, Ceb121, and

Ceb130: Muniz and Vigilant, 2008), one in *Leontopithecus rosalia* (Lr.P2BH6: Grativol et al., 2001), two in *Leontopithecus chrysopygus* (Leon15c85 and Leon21c75: Perez-Sweeney et al., 2005), and one in *Leontopithecus chrysomelas* (Lchμ07: Galbusera and Gillemot, 2008).

We labeled the forward primer of each locus with an M13 tail (Schuelke, 2000) and added a fluorescently labeled universal M13 primer to the polymerase chain reaction (PCR) after the final extension step, as suggested by de Arruda et al. (2010). Our 10-μL reaction volume contained 2 μL DNA, 5 μL GoTaq Colorless Master Mix (Promega, USA), 1 μL bovine serum albumin (Fermentas, Lithuania), 1 μL forward primer, and 1 μL reverse primer. Amplifications were carried out under the following conditions: an initial denaturation step at 95°C for 3 min; 45 cycles at 95°C for 30 s, annealing at 48-60°C for 30 s, and extension at 72°C for 30 s; and a final extension for 10-15 min at 72°C. Then, 10 cycles at 94°C for 30 s, 53°C for 45 s, and 72°C for 45 s. Amplified fragments were checked on 2% agarose gels. PCR products were analyzed on a MegaBACE (GE Healthcare, USA) automatic sequencer, and allele sizes were scored using the FRAGMENT PROFILER program version 1.2 (Applied Biosystems®, USA). We used the GENEPOP version 4.0 (Raymond and Rousset, 1995) to test for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). We addressed the overall genetic diversity and the exclusion probability of the first parent (Pe_1) using the CERVUS version 3.0 (Kalinowski et al., 2007) and FSTAT version 2.9.3 software (Goudet, 2001). We checked for the presence of null alleles with the MICRO-CHECKER version 2.2.3 (Van Oosterhout et al., 2004).

RESULTS

Ten of the 17 microsatellites (59%) were successfully transferred. We managed to find alleles in four homologous loci at the genus level and in six heterologous loci. The PEPC8 locus was excluded from the analysis because only three individuals (14%) were successfully genotyped. The loci Leon15c85, Lr.P2BH6, Ceb08, Ceb09, Ceb119, Ceb120, and Ceb121 did not amplify.

The number of alleles per locus produced by the 9 microsatellites ranged from 2 to 12, with a mean of 6.22 (Table 1). The average allelic richness was 5.93, the expected heterozygosity was 0.70, and Pe_1 across all loci was 0.01 (Table 1). The loci Leon21c75 and Lchμ07

Table 1. Characteristics of nine microsatellite markers used.

Locus	GenBank accession	Ta (°C)	N	N_A	R_S	Pe_1	H_O	H_E	P_{HWE}
PEPC3[a]	AF103994	51	18	12	11.06	0.36	0.89	0.92	0.07
PEPC40[a]	AF103997	54	19	4	4.00	0.69	0.63	0.75	0.02
PEPC59[a]	AF103998	48	21	5	4.67	0.69	0.48	0.74	0.04
PEPL4[a]	AF104000	51	16	10	9.83	0.48	0.69	0.90	0.03
Ceb3[b]	EU019198	54	21	2	2.00	0.88	0.33	0.51	0.18
Ceb11[b]	EU019204	55	21	2	1.89	0.41	0.10	0.09	1.00
Ceb130[b]	EU019215	60	20	8	7.53	0.54	0.75	0.83	0.02
Leon21c75[c]	AY706922	52	18	5	4.73	0.99	0.44	0.68	0.00*
Lchμ07[d]	DQ979350	53	14	8	8.00	0.76	0.86	0.88	0.00*
All loci				6.22	5.93	0.01	0.57	0.70	

Ta = annealing temperature; N = number of genotyped individuals; N_A = number of alleles; R_S = allelic richness; Pe_1 = non-exclusion probability for one candidate parent; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HWE} = Hardy-Weinberg equilibrium test. *Departs significantly from HWE at P = 0.017 after correction (Benjamini and Yekutieli, 2001). [a]Escobar-Páramo (2000); [b]Muniz and Vigilant (2008); [c]Perez-Sweeney et al. (2005); [d]Galbusera and Gillemot (2008).

showed significant deviations from HWE, but the deviations were not associated with the occurrence of null alleles. In the pooled analysis, we recorded a significant (P = 0.00) departure from HWE. No loci pair exhibited significant LD [P > 0.017 after Benjamini and Yekutieli correction (Benjamini and Yekutieli, 2001)]. The inbreeding coefficient for all loci was 0.18 (P large = 0.01, 180 randomizations), indicating no sign of inbreeding. Null alleles were detected at the loci PEPC59 and PEPL4.

DISCUSSION

The allelic richness and heterozygosity values suggest considerable genetic diversity in the PECB population of *S. nigritus*. The deviations from HWE recorded for the Leon21c75 and Lchμ07 loci may have resulted from a deficit of heterozygotes and, in the pooled analysis, from the presence of null alleles in the two loci. Allele dropout may also lead to HWE deviations. However, in order to minimize this type of error, we genotyped at least 85% of the homozygous samples three times. In addition, we genotyped at least 70% of the heterozygous samples twice. The low value for Pe_I may constrain the reliable identification of fathers in paternity analysis if only candidates from the three studied groups are considered. However, increasing the sample size may offset this.

Because the selection of appropriate microsatellite loci is a crucial step to genetic analyses and because field studies on primate social behavior require extensive data collection, we believe that our efforts are useful for field primatologists interested in applying genetic analyses in studies of social behavior. Many questions about features of primate social systems were only properly answered when genetic analyses were incorporated with field observations, for example, the complex sex-biased dispersal pattern in *Colobus guereza* (Harris et al., 2009) and inbreeding avoidance in *Cebus capucinus* (Muniz et al., 2006). Our results highlight the success of transferability among close and more distantly related species. A higher rate of transferability within and between genera is expected among mammals compared to other vertebrate groups (Barbará et al., 2007). In conclusion, the nine microsatellite loci optimized in this study have a great potential for application in studies of social structure and dispersal patterns of *S. nigritus* populations and in other Neotropical primate species.

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