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Allele frequencies and genetic diversity in two groups of wild tufted capuchin monkeys (*Cebus apella nigritus*) living in an urban forest fragment

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ABSTRACT. There have been numerous studies genetically characterizing Old World Primates using microsatellites. However, few studies have been made of New World species and none on free-ranging *Cebus apella*, even though it is probably the most widely distributed species of monkey in the New World. The paucity of studies is due, in part, to the lack of polymorphisms described for this species. We studied two groups of wild tufted capuchins, *Cebus apella nigritus*, which inhabit Mata Santa Teresa, the Ecological Reserve of Ribeirão Preto, a 158-ha forest fragment in a semi-urban zone of Ribeirão Preto, SP, Brazil. Group 1 had about 60 animals, 35 of which were sampled, and group 2 had about 40 animals, 20 of which were sampled. These group sizes are much larger than the published reports of 6-30 for this species, despite, or perhaps due to the isolation and the size of the forest fragment. Allele PEPC59*1 was the most frequent of all alleles at all loci in both groups (55.7 and 55%), allele PEPC8*1 was the most common allele in group 2 (46.9%) and PEPC8*4 in group 1 (41.1%), allele PEPC3*2 was the most common in group 1 (35.7%) and allele PEPC3*4 in group 2 (31.6%). The genetic diversity, considering each locus in each group, varied from 61.9% at locus PEPC59 to 78.6% at locus PEPC3, both in group 1. The mean genetic diversity (H_S), considering both groups for all of the loci, was 71.1%. The inter-group diversity (F_{ST}) was 1.9%, indicating that these groups belong to the same population. These groups apparently have a high genetic diversity, despite their isolation in a limited forest fragment, although more data are needed to adequately characterize this population.

Key words: *Cebus apella*, DNA polymorphisms, Short tandem repeats, Microsatellites, Population structure, Tufted capuchins

INTRODUCTION

Monkeys of the genus *Cebus* are arboreal Neotropical primates, medium in size, with a robust body, and a semi-prehensile tail. This genus contains seven species and 32 morphotypes (Rylands, 2001); however, this number is provisional, since this taxon is being revised (Silva Jr., 2001). *Cebus apella* Linnaeus 1758 (Cebidae, Platyrrhini, Primates) is possibly the most widely distributed of all the Neotropical primates, being found from the eastern part of the Andes to the east coast of South America, from Colombia to 27° S latitude (Paraguay and northern Argentina), and up to 2,700 m altitude (Freese and Oppenheimer, 1981; Fragaszy et al., 1990). This species generally lives in relatively large groups, with the number of individuals varying from approximately 6-30 (Freese and Oppenheimer, 1981; Di Bitetti, 1997; Izar, 1999; Lynch and Rímoli, 2000). *Cebus apella* is a polygamic species, with various males and females of all ages present in a group (Di Bitetti, 1997).

These monkeys are among the most intensively studied primates in the New World. Natural populations of *C. apella* have been the objective of many behavioral (Di Bitetti, 1997; Izar, 1999; Lynch and Rímoli, 2000; Di Bitetti and Janson, 2001) and ecological (Terborgh, 1983; Brown and Zunino, 1990; Amaral and Polegatto, 1999) studies. Various aspects of the anatomy and physiology of these animals have also been investigated (see Ortiz et al., 2005). Although we know much about the biology of this genus, there is little information about its genetic variability.

Various molecular biology techniques have been useful for resolving genetic problems related to the conservation of the biodiversity of wild animals (Miyaki, 1996). Genetic variability is quite large in most natural populations, which makes genetic polymorphisms common phenomena, and it has been widely studied by various techniques, at both the protein and DNA levels. DNA analysis provides useful data for conservation biologists who are trying to save a species as well as for elucidation on the evolution of populations of socially structured primates.

Information on genetic variability within primate species is available from various regions of the world. Polymorphic genetic markers can be used for paternity testing of colonies of animals in captivity (e.g., Smith et al., 1992) and in wild populations (Morin et al., 1994; Altmann et al., 1997), as well as in analyses of genetic population structure (Nozawa et al., 1982; Melnick et al., 1984; Rogers and Kidd, 1996). Genetic polymorphisms are also used for mapping primate genes (Rogers et al., 1995; Perelygin et al., 1996).

Microsatellites or short tandem repeats (STRs) are a type of DNA polymorphism that has become important in various research contexts (Inoue and Takenaka, 1993; Takenaka et al., 1993; Bruford and Wayne, 1993; Morin et al., 1994, 1997; Rogers et al., 1995; Dib et al., 1996; Altmann et al., 1997). Until now, few microsatellites specific to the Platyrrhine have been described (Ellsworth and Hoelzer, 1998; Witte and Rogers, 1999; Escobar-Paramo, 2000; Nievergelt et al., 2000; Grativol et al., 2001), and many of the markers derived from human studies, which are useful for Old World primates, have limited use in the Platyrrhine.

As few genetic analyses have been made of *C. apella*, estimates of the variability of these loci in New World primates would provide useful information about the genetic variability of this species. Our objective was to determine the allele frequencies of three microsatellite loci described by Escobar-Paramo (2000) and the inter- and intra-group diversity of two groups of *C. apella nigritus* that comprise a wild population living in a semi-urban forest fragment.

MATERIAL AND METHODS

Fifty-five individuals from the two groups of an estimated 100 free-living *C. apella nigritus* (Cebidae) found in an ecological reserve were successfully captured and sampled from September 2003 to May 2005. Group 1, which dominated the better forage areas near a small river, had about 60 members, and group 2, about 40. These monkeys inhabit the semi-urban forest fragment named Mata Santa Teresa, Estação Ecológica de (Ecological Reserve of) Ribeirão Preto, close to the city of Ribeirão Preto, SP, Brazil (21°14'S, 47°55'W; 570 m altitude; Siemers, 2000), which consists of a mesophyllic deciduous forest of about 158.2 ha. This forest fragment is isolated on one side by a major highway, on another by a housing development and on the rest of the margin by various crops, especially sugar cane.

This population of monkeys is divided into two groups, based on their geographic localizations within the forest and on the presence of a dominant male in each group. We were able to capture until now 35 members of group 1 and 20 members from group 2. We have not yet been able to determine if there is reproductive crossing between the two groups, although this is quite likely, based on evidence from other publications on this species (Fedigan et al., 1996; Jack and Fedigan, 2004).

All of the individuals were captured using trap cages containing food as bait, and they were anesthetized by a team of veterinarians with 10 mg/kg ketamine and 2 mg/kg xylazine. Blood samples were collected from the femoral vein and a mouth swab was collected from all of the monkeys. These samples were collected into sterile glass Vacutainer tubes containing EDTA. All samples were maintained in a cooler on ice, but without direct contact with the ice, so that they would not freeze.

DNA was extracted using the methodology described by Higuchi (1989). Amplification was made by PCR, using specific protocols for each primer (Escobar-Paramo, 2000), with some modifications in the reaction mixtures for each STR (Table 1). The amplified product was analyzed with 10% PAGE, with silver nitrate staining. Statistical analyses were performed by GENEPOP software programs, Version 2.0 (Raymond and Rousset, 1995), FSTAT software, Version 2.8 (Goudet, 1999) and GDA software, Version 1.0 (Lewis and Zaykin, 1997).

Table1. Reagents used in the PCR reaction mixture for each short tandem repeat (STR) in the analysis of *Cebus apella nigritus* DNA.

STRs	Sterile deionized water (μL)	10% Reaction buffer (μL)	20 mM dNTP solution (μL)	0.2 μM Primer for each STR (μL)	$MgCl_2$ μL (mM)	1 U/μL DNA polymerase (μL)
PEPC59	16.15	2.5	0.25	1.6	*	0.5
PEPC3	13.3	3.2	0.5	3.5	*	0.5
PEPC8	14.75	2.5	0.25	2.0	1.0 (2.5)	0.5

*None.

RESULTS AND DISCUSSION

This is the first report on the frequencies of these three species-specific microsatellites (PEP59, PEPC8 and PEPC3). Consequently, it was not possible to compare our results on these groups of *C. apella nigritus* with those of other populations of this species.

Allele PEPC59*1 was the most frequent in the two groups (55.7 and 55%, Table 2); allele PEPC8*1 was the most frequent in group 2 (46.9%) and PEPC8*4 in group 1 (41.1%); allele PEPC3*2 was the most frequent in group 1 (35.7%) and allele PEPC3*4 in group 2 (31.6%). Group 1 was in Hardy-Weinberg disequilibrium in the analysis of all the loci, taken together. These deviations are due to a deficit of heterozygotes at the loci PEPC59 and PEPC3, though this could be a consequence of the sample size.

The genetic diversity, considering each locus in each group, varied from 61.9% at locus PEPC59 to 78.6% at locus PEPC3, both in group 1 (Table 3). The mean genetic diversity (H_S), considering all of the populations for all the loci, was 71.1%. The interpopulational difference distance (F_{ST}) was 1.9%, which is not significant, indicating that the two groups probably freely interbreed. Most of the diversity was intra-populational, that is, among the members of each group; differences between the groups were small or undetectable at these loci.

These two groups of monkeys, with about 60 and 40 animals, totalling about 100 in a 158-ha area, comprise a greater than normal population density; these group sizes are also larger than the normal 6-30 reported for this species (Freese and Oppenheimer, 1981; Di Bitetti, 1997; Izar, 1999; Lynch and Rímoli, 2000), despite, or perhaps due to the isolation and the size of the forest fragment. These population peculiarities may have affected the genetic diversity in this area. Although these two groups of monkeys are relatively isolated, they maintain a high genetic diversity. The data on these alleles could be used in comparisons with other groups of this species.

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Table 2. Allele frequencies of the short tandem repeats PEPC59, PEPC8 and PEPC3 in the two groups of *Cebus apella nigritus* that reside in the Ecological Station of Ribeirão Preto, SP, Brazil.

Locus	Populations	
	Group 1 (N = 35)	Group 2 (N = 25)
PEPC59		
1	0.557	0.550
2	0.014	0.000
3	0.000	0.025
4	0.071	0.075
5	0.257	0.300
6	0.100	0.050
PEPC8		
1	0.268	0.469
2	0.107	0.031
4	0.411	0.188
5	0.018	0.094
9	0.036	0.031
10	0.000	0.031
11	0.161	0.156
PEPC3		
1	0.086	0.158
2	0.357	0.289
3	0.214	0.000
4	0.186	0.316
5	0.100	0.184
6	0.057	0.053

Table 3. Genetic diversity (H_S) of the short tandem repeats PEPC59, PEPC8 and PEPC3 in the two groups of *Cebus apella nigritus* that reside in the Ecological Station of Ribeirão Preto, SP, Brazil.

Locus	Group 1 (N = 35)	Group 2 (N = 25)
PEPC59	0.619	0.612
PEPC8	0.734	0.733
PEPC3	0.786	0.778

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