Genetics and Molecular Biology, 31, 1, 166-171 (2008)

www.sbg.org.br

Short Communication

Genetic structure in two northern muriqui populations (*Brachyteles hypoxanthus,* Primates, Atelidae) as inferred from fecal DNA

Valéria Fagundes[1], Marcela F. Paes[1], Paulo B. Chaves[1], Sérgio L. Mendes[1], Carla de B. Possamai[2,3], Jean P. Boubli[4*] and Karen B. Strier[5]

[1]*Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Vitória, ES, Brazil.*
[2]*RPPN Feliciano Miguel Abdala, Caratinga, MG, Brazil.*
[3]*Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, MG, Brazil.*
[4]*Conservation and Research for Endangered Species, Zoological Society of San Diego, San Diego, CA, USA.*
[5]*Department of Anthropology, University of Wisconsin-Madison, Wisconsin, USA.*

Abstract

We assessed the genetic diversity of two northern muriqui (*Brachyteles hypoxanthus* Primata, Atelidae) populations, the Feliciano Miguel Abdala population (FMA, n = 108) in the Brazilian state of Minas Gerais (19°44' S, 41°49' W) and the Santa Maria de Jetibá population (SMJ, n = 18) in the Brazilian state of Espírito Santo (20°01' S, 40°44' W). Fecal DNA was isolated and PCR-RFLP analysis used to analyze 2160 bp of mitochondrial DNA, made up of an 820 bp segment of the gene cytochrome c oxidase subunit 2 (*cox2,* EC 1.9.3.1), an 880 bp segment of the gene cytochrome b (*cytb,* EC 1.10.2.2) and 460 bp of the hypervariable segment of the mtDNA control region (HVRI). The *cox2* and *cytb* sequences were monomorphic within and between populations whereas the HVRI revealed three different population exclusive haplotypes, one unique to the SMJ population and two, present at similar frequencies, in the FMA population. Overall haplotype diversity ($h = 0.609$) and nucleotide diversity ($\pi = 0.181$) were high but reduced within populations. The populations were genetically structured with a high fixation index ($F_{ST} = 0.725$), possibly due to historical subdivision. These findings have conservation implications because they seem to indicate that the populations are distinct management units.

Key words: Brachyteles, conservation genetics, fecal DNA, mtDNA, PCR-RFLP.

Received: April 27, 2007; Accepted: September 25, 2007.

The muriqui or woolly spider monkey (*Brachyteles Spix* 1823: Primates, Atelidae), endemic to Brazil, is the largest Neotropical primate and was once widespread in the southeastern Atlantic Forest of Brazil. Aguirre (1971) estimated a total population of 2,791-3,226 muriquis, contrasting with a population of about 400,000 he reckoned would have existed in 1500. In the last decade this genus has been split into two species (Groves, 2005), the northern muriqui (*Brachyteles hypoxanthus* Kuhl 1820) and the southern muriqui (*Brachyteles arachnoides* É. Geoffroy 1806). Current population estimates for the northern muriqui have indicated at least 864 individuals in the wild and data available for the southern muriqui suggest a minimum population of about 1,300 (Melo and Dias, 2005).

Send correspondence to Valéria Fagundes. Laboratório de Genética Animal, Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Av. Marechal Campos 1468, 29043-900 Maruípe, Vitória, ES, Brazil. E-mail: vfagunde@npd.ufes.br.
*Present address: Department of Anthropology, The University of Auckland, Auckland, New Zealand.

The IUCN Red List cites the northern muriqui as a critically endangered species because only about 900 individuals are known dispersed in 12 populations, five of which contain less than 20 members. Some of the populations are restricted to small, unprotected and isolated forest fragments extending from the south of the state of Bahia throughout Minas Gerais and Espírito Santo states as well as along the Mantiqueira Mountains (Serra da Mantiqueira) on the borders of the states of Minas Gerais, Rio de Janeiro and São Paulo (Rylands *et al.*, 2003b; Mendes *et al.*, 2005). The southern muriqui is distributed along the Serra do Mar from the south of Paraná state to Rio de Janeiro state, is listed as endangered in the IUCN Red List and no more than 1300 are known to occur in relatively large and well protected areas (Melo and Dias, 2005).

The small size and fragmented distribution of northern muriqui populations compromises the ecological viability of this species (Brito and Grelle, 2006). Their advanced age of 9 years at first reproduction and long inter-birth interval of three years, make the small persistent

populations more vulnerable to unfavorable demographic conditions than primates with faster life histories or larger populations (Strier *et al.*, 2006). Habitat reduction and hunting have probably forced surviving northern muriqui populations into bottlenecks and reduced or eliminated opportunities for gene flow through populations, resulting in changes in the frequency of alleles, loss of alleles, or both (Young and Clarke, 2000) similar to those documented in the golden lion tamarin (Grativol *et al.*, 2001).

Population viability analysis (PVA) has suggested that only muriqui populations of at least 700 monkeys would be genetically viable (Strier, 1993/1994; Brito and Grelle, 2006) but no empirical genetic data was included in these analyses. Although a preliminary allozyme analysis comparing one population of each species reported a high fixation index (Pope, 1998), knowledge of genetic structure of more populations is crucial for muriqui conservation planning (Fagundes, 2005).

Standardization of a non-invasive method for DNA extraction from muriqui feces is an important tool for assessing the conservation status and behavioral ecology of these monkeys (Chaves *et al.*, 2006). Furthermore, data on the levels of genetic variability and differentiation in muriqui populations as well as pedigree reconstruction and information regarding the relatedness between individuals and the extent of inbreeding can contribute to the planning of effective conservation strategies for these species.

We used the Polymerase Chain Reaction and Restriction Fragment Length Polymorphism analysis (PCR-RFLP) to evaluate the genetic diversity of two northern muriqui populations and characterize the distribution of genetic variability within and between populations. Fecal samples from 126 free-ranging northern muriquis (*Brachyteles hypoxanthus* Kuhl 1820) were collected immediately after defecation and stored at 4 °C or room temperature (~24-28 °C) in 50 mL polypropylene vials containing a layer, about 1 mm to 4 mm deep, of desiccated silica beads to dehydrate the feces (Chaves *et al.* 2006). Each individual sampled was identified by its natural markings by the experienced field researchers who collected the fecal samples within the ambit of a collecting license (number 363/2001) issued by the Brazilian National Environmental Agency (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA). Two populations, separated by 150 km, were studied (Figure 1). One population is in a private conservation unit (Reserva Particular do Patrimônio Natural Feliciano Miguel Abdala, RPPN-FMA) of 957 hectares (ha) located in the state of Minas Gerais (19°44' S, 41°49' W) and is designated the FMA population. This population is made up of four groups (three mixed-sex and one only-male group) totaling 226 northern muriquis (Strier *et al.*, 2006) of which nearly 50% (n = 108) were sampled between 2001 and 2002, once this population has been monitored over the last 25 years by K. B. Strier and her coworkers and animals are used to the human presence. The other population, designated the SMJ population, is in privately owned forest fragments in the municipality of Santa Maria de Jetibá (SMJ) in the state of Espírito Santo (20°0' S, 40°44' W). The forest patches containing the muriqui range from 60 ha to 350 ha and are highly fragmented due to agricultural activities and unevenly connected to each other by hilltop corridors. This metapopulation has been estimated to be comprised of 115 muriqui (Mendes *et al.*, 2005), although only about 16% (n = 18) could be sampled because monitoring of this population only started in 2001.

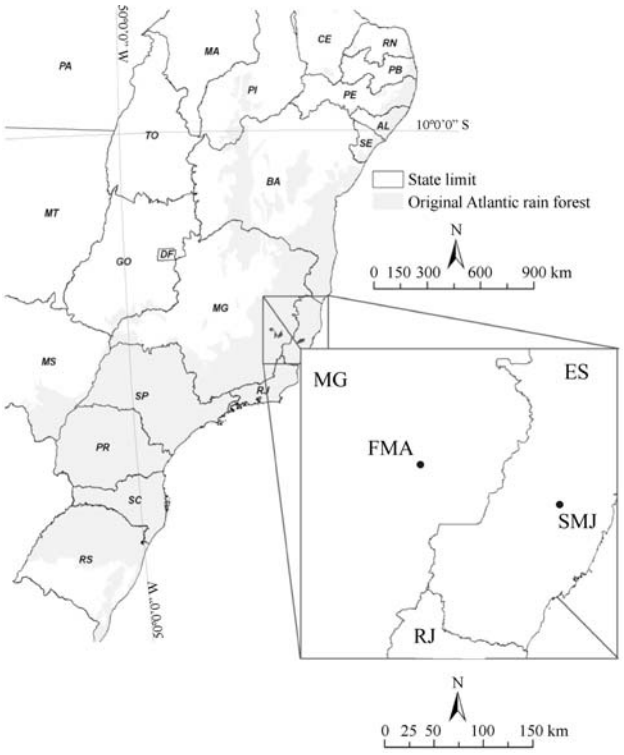

Figure 1 - Map depicting the easternmost portion of Brazil. Sampling sites of the *Brachyteles hypoxanthus* populations are highlighted. The FMA population in Minas Gerais (MG) state is roughly 150 km from the municipality of Santa Maria de Jetibá (SMJ) in Espírito Santo (ES) state.

We extracted DNA from the feces using the QIAamp DNA Stool Minikit (Qiagen) and assessed the quality and amount using 1% (w/v) agarose gels stained with 0.5 µg/mL ethidium bromide. Appropriate primers were used for the PCR amplification of a total of 2160 base pairs (bp) from the following three mitochondrial DNA (mtDNA) segments: 820 bp of the gene *cytochrome c oxidase subunit 2* (*cox2,* EC 1.9.3.1), amplified using the L6955/H7766 primer pair (Ashley and Vaughn, 1995); 880 bp of the gene *cytochrome b* (*cytb,* EC 1.10.2.2), amplified using the MVZ05/MVZ16 primer pair (Smith and Patton, 1993); and the 460 bp hypervariable segment of the mtDNA control region (HVRI), for which we designed a novel specific primer pair consisting of a 5'-CTACTCCCT GAATAACCAAC-3' forward primer (Mono1) and a 5'-AGCGAGAAGAGCGGCAAATG-3' reverse primer

(Mono2), which were based on the *Brachyteles* sequence (GenBank AF213966) with the 3' annealing positions (L15463 for Mono1 and H15890 for Mono2) from the *Cebus albifrons* mtDNA sequence (GenBank AJ309866). The specificity of the HVRI primers was shown by their inability to amplify human DNA, probably due to the 19 mismatches (12 forward and 7 reverse) between the primers, which had been derived for monkey sequences, and human DNA. An *in silico* restriction simulation was performed with *Brachyteles* HVRI sequence from Genbank using BIOEDIT 7.0.5.3 (Hall, 1999) to identify restriction sites and restriction fragment sizes. Analysis of the human HVRI restriction sites excluded cross-contamination of muriqui PCRs with human DNA (Figure 2).

The PCR was carried out in a final volume of 50 µL of 1X Taq buffer containing 3 mM $MgCl_2$, 0.4 mM of each dNTP, 0.4 µM of each primer, 2.5 units of Platinum Taq DNA polymerase (Invitrogen) and 50 ng of DNA (Chaves *et al.*, 2006). Amplifications were conducted at 92 °C for 5 min, followed by 35-37 cycles of 92 °C for 60 s, 47 °C to 52 °C for 30 s to 60 s and 72 °C for 30 s to 60s, with a final extension at 72 °C for 5 min. After amplification the amplicons were electrophoretically sized on 1% (w/p) agarose gel using 1 kb and 100 bp Ladders (Invitrogen). Mock PCR blanks were included to check for contamination.

Estimates of the number of nucleotide substitutions (genetic polymorphisms) took into account the previous *in silico* analysis shown in Figure 2. Three to seven restriction endonucleases (Table 1) digested roughly 20 ng of the PCR amplicons following the manufacturer's protocol. Restriction fragment sizes were determined using polyacrylamide gel electrophoresis (5 to 10% w/v) and ethidium bromide staining, with the gels being photographed under ultraviolet light. Restriction fragment sizes were estimated using 1 kb and 100 bp ladders as reference (detailed procedure is available upon request). Table 1 summarizes the PCR-RFLP results obtained after cutting PCR amplicons.

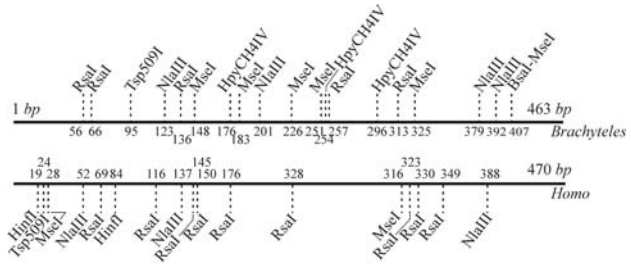

Figure 2 - The *in silico* restriction analysis resulted in the haplotype A?AABAA in the muriqui sequence, which is highly similar to muriqui haplotypes found in the SMJ and FMA populations. However, this haplotype was not in our assessment, possibly because this sequence is from an individual from a different population. The human haplotype is markedly different from all muriqui haplotypes, indicating that if human DNA were amplified due to inadvertent contamination it would be readily identified. The *Bsa*I and *HpyCH4*IV restriction enzymes cannot cut the human segment (GenBank AC_000021/*Homo sapiens*), while the *Hinf*I restriction enzyme cannot cut muriqui DNA.

For the restriction fragment data analysis, one allele was represented by one fragment obtained with an enzyme, and the set of all alleles of a specific digestion (*i.e.*, the cleavage pattern) received a capital letter (Table 1). The composite haplotype panel for each monkey included the cleavage pattern of all the enzymes (Table 2, see Bates, 2002 for details). Genetic diversity analyses were calculated using ARLEQUIN 3.01 (Excoffier *et al.*, 2005) after converting composite haplotypes into a binary matrix (Table 2) based on the presence or absence of alleles (restriction fragments). The population parameters calculated were haplotype frequency, haplotype diversity (*h*), nucleotide diversity (π), mean number of pairwise difference between haplotypes, and Wright's fixation index (F_{ST}, Wright, 1951).

All 80 restriction assays for *cox2* (n = 49) and *cytb* (n = 31) resulted in monomorphic haplotypes for the seven endonucleases (Table 2). Therefore, we concentrated on analyzing all 126 muriquis for the HVRI segment based on the following assumptions. Firstly, amplification success for HVRI was 100% (against 20%-30% for *cox2* and *cytb*), which is amongst the highest rate ever reported for fecal DNA (Broquet *et al.*, 2007 and references therein). This is likely to be due to the specificity of the primers we used, as well as the short-length segment, which are better suited to analyses of highly degraded fecal DNA. Secondly, HVRI seems to be one of the most variable segments within mammalian mtDNA (Aquadro and Greenberg, 1983; Sbisà *et al.*, 1997), and is thus highly suitable for revealing within-population variation. Finally, mtDNA evolves as a single locus (Avise, 2004), thus analyses of the most variable segment can provide a rough estimate of the diversity of the entire mtDNA genome.

The HVRI assay revealed 35 restriction fragments (Table 2), of which 17 (48.6%) were polymorphic. The *Mse*I and *Rsa*I restriction enzymes generated more fragments (n = 8) and *Bsa*I generated fewer fragments (n = 2), while *Hinf*I generated the longest fragment (460 bp, with no restriction site). Based on the restriction site map shown in Figure 2 one polymorphic restriction site was present for *Hinf*I (position 240 or 220), two sites were present for *Rsa*I (positions 56 and 313), and one site each was present for *Mse*I (position 251) and Tsp509I (around position 400 to 420). The *Nla*III and *Hpy*CH4IV sites were invariable. After assembling the single digestion patterns generated by each endonuclease the HVRI, RFLP identified three composite haplotypes, with haplotype SMJ1 being exclusively present in all the 18 monkeys from the SMJ group, while the other two haplotypes (FMA1 and FMA2) were unique to the FMA population and were nearly equal in frequency at 57.4% for FMA1 and 42.6% for FMA2 (Table 2). Differences in pairwise distances between haplotypes (SMJ1/FMA1 = 12, SMJ1/FMA2 = 13 and FMA1/FMA2 = 9) demonstrated the closer relationship between FMA1 and FMA2 than between either of these haplotypes and the

Table 1 - Restriction enzyme cleavage patterns of the genes c*ytochrome c oxidase subunit 2* (*cox2*, 820 bp), *cytochrome b* (*cyt b*, 880 bp) and the hypervariable segment of mtDNA control region (HVRI, 460 bp) of the *Brachyteles hypoxanthus* populations from Santa Maria de Jetibá in the Brazilian state of Espírito Santo (SMJ population, n = 18) and Feliciano Miguel Abdala in the Brazilian state of Minas Gerais (FMA population, n = 108). The total number (n) of muriquis analyzed for each mtDNA segment is shown in parentheses in the first column.

Segment, number of individuals analyzed (n) and restriction enzyme*	Fragment sizes (bp)	Cleavage pattern†	Frequency (%)	Population
cox2 (n = 49)				
*Alu*I	230, 190, 170, 130, 100	A	100	SMJ and FMA
*Hinf*I	390, 430	A	100	SMJ and FMA
*Mbo*I	280, 270, 140, 130	A	100	SMJ and FMA
*Msp*I	620, 200	A	100	SMJ and FMA
*Rsa*I	270, 220, 200, 130	A	100	SMJ and FMA
cyt b (n = 31)				
*Hae*III	410, 200, 150, 120	A	100	SMJ and FMA
*Hinf*I	480, 290, 60, 50	A	100	SMJ and FMA
*Msp*I	560, 270, 50	A	100	SMJ and FMA
HVRI (n = 126)				
*Hinf*I	460	A	100	SMJ
*Hinf*I	460	A	42.6	FMA
*Hinf*I	240, 220	B	57.4	FMA
*Rsa*I	200, 120, 70, 55,15*	A	100	SMJ
*Rsa*I	150, 120, 70, 55, 55, 15*	B	57.4	FMA
*Rsa*I	150, 120, 70, 65, 55	C	42.6	FMA
*Mse*I	150, 80, 75, 60, 40, 35, 25*	A	100	SMJ
*Mse*I	150, 80, 75, 60, 40, 35, 25*	A	42.6	FMA
*Mse*I	150, 95, 80, 60, 40, 35	B	57.4	FMA
*Tsp509*I	355, 105	A	100	FMA
*Tsp509*I	300, 105, 55	B	100	SMJ
*HpyCH4*IV	165, 90,75, 70, 60	A	42.6	FMA
*HpyCH4*IV	165, 160, 75, 60	B	57.4	FMA
*HpyCH4*IV	165, 160, 75, 60	B	100	SMJ
*Nla*III	180, 125, 80, 75	A	100	SMJ and FMA
*Bsa*I	430, 30	A	100	SMJ and FMA

*The restriction enzyme 5'→3' sites were as follows: *Alu*I = AG/CT; *Hinf*I = G/ANTC; *Mbo*I = /GATC; *Msp*I = C/CGG; *Rsa*I = GT/AC; *Hae*III = GG/CC; *Hinf*I = G/ANTC; *Mse*I = T/TAA; *Tsp509*I = /AATT; *HpyCH4*IV = A/CGT; *Nla*III = CATG/; and *Bsa*I = GGTCTCN/N_4.
†A, B and C are the cleavage patterns (set of restriction fragments) for each enzyme.
¯These fragments (15 bp and 25 bp) were not visualized in the gels, probably because they were lost.

SMJ1 haplotype. This finding suggests that the SMJ and FMA populations have been isolated long enough to prevent gene flow and haplotype sharing.

Overall haplotype ($h = 0.609 \pm 0.022$) and nucleotide diversity ($\pi = 0.181 \pm 0.095$) were relatively high. Within-population diversity indices were slightly lower in the FMA population ($h = 0.494 \pm 0.016$; $\pi = 0.127 \pm 0.070$) than in the SMJ population, which were both zero. The low number of haplotypes homogeneously distributed is an unfavorable scenario for the genetic diversity of the FMA population, but it is better than the single haplotype found in the SMJ population.

We also found a high fixation index of $F_{ST} = 0.725$ ($p < 0.001$), which is strongly indicative of genetic distinctiveness between the SMJ and FMA populations. This F_{ST} is considerably higher than the $F_{ST} = 0.413$ for allozyme polymorphisms of 12 muriqui (two from a what is now considered a *B. arachnoides* population and ten from a *B. hypoxanthus* population different to that studied by us) reported by Pope (1998), who used this data to recommended the elevation of the southern and northern forms of the muriqui into separate species (*B. arachnoides* and *Brachyteles hypoxanthus* respectively).

Populations containing mtDNA haplotypes at significantly different frequencies or, in more extreme cases, pre-

Table 2 - Composite haplotypes obtained after PCR-RFLP analysis of HVRI in 126 northern muriqui monkeys from Santa Maria de Jetibá in the Brazilian state of Espírito Santo (SMJ population, n = 18) and Feliciano Miguel Abdala in the Brazilian state of Minas Gerais (FMA population, n = 108). FMA1 and FMA2 represent the two haplotypes from FMA population and SMJ1 the one from SMJ population. The binary code should be read from left to right, a '1' indicating the production of a restriction fragment of the specified size (in bp) and a '0' indicating that the fragment was not detected (see footnote for key).

Haplotype	Composite haplotype*	Composite haplotypes converted to binary code†	Frequency %	Population
SMJ1	AAABBAA	1001011010110111111011111010111111	100	SMJ
FMA1	BBBABAA	0110111011111101110101011010111111	57.4	FMA
FMA2	ACBAAAA	1000111110011101110101010111111111	42.6	FMA

*Letters represent the cleavage pattern resulted of digestion with *Hinf*I, *Rsa*I, *Mse*I, *Tsp509*I, *HpyCH4*IV, *Nla*III and *Bsa*I, respectively. For details see Table 1.

†Starting from left (1) to right (35) in the binary code, the 35 restriction enzyme fragments used were as follows: 1. *Hinf* I (460 bp); 2. *Hinf* I (240 bp); 3. *Hinf* I (220 bp); 4. *Rsa*I (200 bp); 5. *Rsa*I (150 bp); 6. *Rsa*I (120 bp); 7. *Rsa*I (70 bp); 8. *Rsa*I (65 bp); 9. *Rsa*I (55 bp); 10. *Rsa*I (55 bp); 11. *Rsa*I (15 bp); 12. *Mse*I (150 bp); 13. *Mse*I (95 bp); 14. *Mse*I (80 bp); 15. *Mse*I (75 bp); 16. *Mse*I (60 bp); 17. *Mse*I (40 bp); 18. *Mse*I (35 bp); 19. *Mse*I (25 bp); 20. *Tsp509*I (355 bp); 21. *Tsp509*I (300 bp); 22. *Tsp509*I (105 bp); 23. *Tsp509*I (55 bp); 24. *HpyCH4*IV (165 bp); 25. *HpyCH4*IV (160 bp); 26. *HpyCH4*IV (90 bp); 27. *HpyCH4*IV (75 bp); 28. *HpyCH4*IV (70 bp); 29. *HpyCH4*IV (60 bp); 30. *Nla*III (180 bp); 31. *Nla*III (125 bp); 32. *Nla*III (80 bp); 33. *Nla*III (75 bp); 34. *Bsa*I (430 bp); and 35. *Bsa*I (30 bp).

senting population-exclusive haplotypes, have been referred as management units and seem to have been historically separated during evolutionary time (Moritz, 1994). In our study, since the FMA and SMJ northern muriqui populations did not share haplotypes, it is reasonable to assume that they should be given management unit status and actively managed. In addition, the FMA and SMJ populations are connected by low levels of gene flow, are functionally independent and carriers of a portion of the species evolutionary legacy. As a caveat, caution must be exercised with this conclusion since broadening our sampling could reveal a different scenario with admixture of haplotypes.

High genetic diversity and F_{ST} seem to be intrinsic to *Brachyteles*, and may be an important factor contributing to the persistence of small, isolated populations. Relatively few genetic studies involving fecal DNA have been conducted on New World primates as compared to Old World primates (Oklander *et al.*, 2004). Nonetheless, as our results demonstrate, noninvasive genetic studies of wild muriqui populations can provide important insights for conservation.

Acknowledgments

This work was funded by Brazilian Environment Ministry (Ministério do Meio Ambiente, PROBIO-MMA) and Critical Ecosystem Partnership Fund (CEPF). Scholarships for MFP and PBC were provided by the Brazilian National Counsel for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq), CBP was supported by the Brazilian Higher Education Training Program (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES) and JPB received a fellowship from the Zoological Society of San Diego. We are indebted to the Brazillian organizations the Institute for Atlantic Forrest Research (Instituto de Pesquisas da Mata Atlântica, IPEMA) and the Dryad Institute (Instituto Dríades) for assistance and logistic support. A. Grativol, L. Oliveira, Y. Leite and two anonymous reviewers provided invaluable improvements on early versions of the manuscript. We also thank everyone who helped with this project, especially A.S. Perrone, I.D. Louro, R. Nunes, L. Cajaiba, R. Santos, H. Dazilio, A. Araújo, G. Picoretti, G. Schulz, A. Santos, L. Carmo, F. Paim, M. Iurck, K. Tolentino, V. Souza, D. Guedes, J. Oliveira, M. Tokuda, F. Ferreira, W. Silva and L.G. Dias.

References

Aguirre AC (1971) O Mono *Brachyteles arachnoides* (E. Geoffroy). Situação Atual da Espécie no Brasil. Academia Brasileira de Ciências, Rio de Janeiro, 53 pp.

Aquadro CF and Greenberg BD (1983) Human mitochondrial DNA variation and evolution: Analysis of nucleotide sequences from seven individuals. Genetics 103:287-312.

Ashley MV and Vaughn JL (1995) Owl monkeys (*Aotus*) are highly divergent in cytochrome c oxidase (*COII*) sequences. Int J Primatol 16:793-806.

Avise JC (2004) Molecular Markers, Natural History, and Evolution. University of Georgia, Athens, pp 684.

Bates JM (2002) The genetic effects of forest fragmentation on five species of Amazonian birds. J Avian Biol 33:276-294.

Brito D and Grelle CEV (2006) Estimating minimum area of suitable habitat and viable population size for the northern muriqui (*Brachyteles hypoxanthus*). Biodivers Conserv 15:4197-4210.

Broquet T, Ménard N and Petit E (2007) Noninvasive population genetics: A review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. Conserv Genet 8:249-260.

Chaves PB, Paes MF, Mendes SL, Strier KB, Louro ID and Fagundes V (2006) Noninvasive genetic sampling of endangered muriqui (Primates, Atelidae): Efficiency of fecal DNA extraction. Genet Mol Biol 29:750-754.

Excoffier L, Laval G and Schneider S (2005) ARLEQUIN ver. 3.0: An integrated software package for population genetics data analysis. Evol Bioinform Online 1:47-50.

Fagundes V (2005) Conservation genetics of muriqui: Past, present and future. Neotrop Primates 13(suppl):85-91.

Grativol AD, Ballou JD and Fleischer RC (2001) Microsatellite variation within and among recently fragmented populations of the golden lion tamarin (*Leontopithecus rosalia*). Conserv Genet 2:1-9.

Groves CP (2005) Order primates. In: Wilson DE and Reeder DM (eds) Mammal Species of the World: A Taxonomic and Geographic Reference. 3rd ed. The Johns Hopkins University Press, Maryland, pp 111-184.

Hall TA (1999) BIOEDIT: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98.

Melo FR and Dias LG (2005) Muriqui populations reported in the literature over the last 40 years. Neotrop Primates 13(suppl):19-24.

Mendes SL, Melo FR, Boubli JP, Dias LG, Strier KB, Pinto LPS, Fagundes V, Cosenza B and de Marco Jr P (2005) Directives for the conservation of the northern muriqui, *Brachyteles hypoxanthus* (Primates, Atelidae). Neotrop Primates 13(suppl):7-18.

Moritz C (1994) Defining 'evolutionarily significant units' for conservation. Trends Ecol Evol 9:373-375.

Oklander LI, Marino M, Zunino GE and Corach D (2004) Preservation and extraction of DNA from faeces in howler monkeys (*Alouatta caraya*). Neotrop Primates 12:59-63.

Pope TR (1998) Genetic variation in remnant populations of the woolly spider monkey (*Brachyteles arachnoides*). Int J Primatol 19:95-109.

Rylands AB, Bampi MI, Chiarello AG, da Fonseca GAB, Mendes SL and Marcelino M (2003a) *Brachyteles arachnoides*. In: IUCN 2006. 2006 IUCN Red List of Threatened Species. Downloaded on 18 February 2007. www.iucnredlist.org.

Rylands AB, Bampi MI, Chiarello AG, da Fonseca GAB, Mendes SL and Marcelino M (2003b) *Brachyteles hypoxanthus*. In: IUCN 2006. 2006 IUCN Red List of Threatened Species. Downloaded on February 18, 2007. www.iucnredlist.org.

Sbisà E, Tanzarillo F, Reyes A, Pesole G and Saccone C (1997) Mammalian mitochondrial D-loop region structural analyses: Identification of new conserved sequences and their functional and evolutionary implications. Gene 205:125-140.

Smith MF and Patton JL (1993) The diversification of South American murid rodents: Evidence from mitochondrial DNA sequence data for the akodontine tribe. Biol J Linn Soc 50:149-177.

Strier KB (1993/1994) Viability analyses of an isolated population of muriqui monkeys (*Brachyteles arachnoides*): Implications for primate conservation and demography. Primate Conserv 15:43-52.

Strier KB, Boubli JP, Possamai CB and Mendes SL (2006) Population demography of Northern muriquis (*Brachyteles hypoxanthus*) at the Estação Biológica de Caratinga/Reserva particular do Patrimônio Natural-Feliciano Miguel Abdala, Minas Gerais, Brazil. Am J Phys Anthropol 130:227-237.

Wright S (1951) The genetical structure of populations. Ann Eugen 15:323-354.

Young AG and Clarke GM (2000) Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, Cambridge, pp 456.

Internet Resource

GenBank, http://www.ncbi.nih.gov/Genbank/ (February 18, 2007).

Associate Editor: Fábio de Melo Sene