CONSERVATION GENETICS OF THE MURIQUI: PAST, PRESENT AND FUTURE

Valéria Fagundes

Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo. Av. Marechal Campos, 1468, Vitória 29.040-090, Espírito Santo, Brazil, e-mail: <vfagundes@npd.ufes.br>

Abstract

Our understanding of the genetics of the muriqui have increased in recent decades. In the mid 1980's the first data was obtained from polymorphisms of allozymes of 10 individuals of *B. hypoxanthus* (northern muriqui) from Minas Gerais, and two individuals of *B. arachnoides* (southern muriqui) from São Paulo. All specimens were considered to be of a single species. The DNA was extracted from blood samples, which required capture and anesthesia of animals. We can now extract mitochondrial DNA from feces samples. Analyzing more than 120 individuals of the northern muriqui from two populations, we are now able make inferences about genetic variability, population distinctiveness as well as intra- and interpopulation gene flow. DNA sampling through feces is reliable, efficient, and economic, and does not risk the physical integrity of the animals, and furnishes enough DNA that is easily reproducible for PCR amplification. Using this method it is possible to sample a greater number of individuals in nature than would be possible if live capture were necessary. A muriqui feces and DNA bank has been set up, and currently has samples of 230 individuals from seven of the twelve known populations of northern muriqui. The samples resulted from field studies, but more coordinated and systematic efforts among fieldworkers at the different muriqui sites are needed to improve representation across populations and species. Future perspectives include the use of new genetic markers (nuclear and mitochondrial DNA) to identify parents, offspring, and closely related individuals in captive and wild populations; to define units for conservation and the gene flow between them; to quantify genetic variability in the populations; to assess the rate at which genetic variation has been lost over time; to estimate the degree of inbreeding in the population; and to understand better the genetic differentiation of the two species.

Key Words – conservation genetics, muriqui, *Brachyteles*, fecal DNA, genetic variability.

General Issues of Genetic Conservation

A major goal of conservation biology is to preserve genetic diversity, based on the assumption that higher heterozygosity (the measure of the within-population component of genetic variation) increases the probability of a population's survival in the event of ecological or evolutionary changes. Under this concept, concerns about the conservation of biodiversity represent concerns about the conservation of genetic diversity.

For threatened species, hunting and reduction of habitat are associated with a reduction of population size. In extreme cases, a significant percentage of a population is killed or otherwise prevented from reproducing (bottleneck); the most frequent cause of loss of heterozygosity. When a large percentage of a population is lost, only a subset of the genes of the original gene pool will survive. Some alleles will be disproportionately represented, while other alleles may be gone forever. If one survivor has a rare allele, then that allele may come to be represented in the resulting population (a few generations later) in greater numbers than was the case prior to the population crash (a founder effect). In small populations, the effects of genetic drift on loss of genetic variation (stochastic events that alter allele frequencies among members of a population and change the diversity of the group over time) are much greater than they are in larger populations. Some examples of the effects of habitat reduction on loss of genetic variation have been reported for both the Florida panther (Roelke *et al*., 1993) and the cheetah (O'Brien, 1994).

A critical parameter for the management and conservation of natural populations, therefore, is the effective population size, which affects the rate at which genetic diversity is lost in the population by genetic drift (Franklin, 1980). The longer the species experiences a bottleneck, the higher the effects of genetic drift (Futuyma, 1992). Delays in promoting a species' population recovery can be highly prejudicial in terms of loss of genetic diversity, and measures that can rapidly bring about an increase in population size are paramount.

Moreover, due to reduction of population size, most of the individuals will not mate randomly within the population, and the effect of inbreeding is that the alleles are prone to become homozygous and the hybrid heterozygous advantage is lost. With inbreeding, the chance of unfavorable recessive genes turning up in the offspring is greatly enhanced. Thus, the presence of recessive alleles in the population may lead to inbreeding depression, which means impaired fitness with the reduction in evolutionary potential,

and ultimately an increase in the probability of a population going extinct (Frankham *et al.*, 2002).

The viability of species that survive short-term demographic and environmental threats may depend upon the genetic variability and its spatial distribution prior to the population crash. The spatial pattern of the population crash also has important consequences for the resulting genetic variability (or lack of it). Thus, small and isolated populations are at a higher risk of extinction than large and well-connected populations (Frankham, 1995; Meffe and Carroll, 1997; Ebert *et al.*, 2002; Frankham *et al.*, 2002).

Over the short term, most endangered species are threatened by extrinsic, environmental factors, but effective long-term conservation planning must also incorporate genetic factors. Conservation planning operates at many levels, from whole ecosystems and communities to individual organisms. At each of these levels, molecular genetic techniques may provide appropriate tools to evaluate processes and to develop management strategies. However, it is necessary to understand how molecular analyses can address questions about levels of threats and extinction.

First, threats must be identified clearly, and effective steps taken to alleviate them (Caughley, 1994). Next, it is necessary to identify priorities for conservation action — an understanding of where measures are needed most urgently to avoid the situation deteriorating rapidly and irrevocably. In both cases, molecular genetic analyses can play an important role.

Management of genetic heterozygosity is the challenge of conservation genetics. In captive populations, controlling inbreeding to maintain minimal population sizes and adequate levels of heterozygosity is crucial (Queiroz *et al.*, 2000). In natural populations, measures must be taken not only to guarantee the maintenance of the habitat, but also to facilitate gene flow among individuals of local populations in order to avoid inbreeding and loss of heterozygosity.

The general importance of genetic factors can be summarized in two points. First, knowledge of genetic variation allows an understanding of the current status and the historical evolutionary processes that generated biodiversity patterns. Second, future persistence of populations may depend upon the preservation of important remnant components of genetic diversity.

In the pre-molecular era, most of the studies involved phenotypic data amenable to observation (morphology, physiology, behavior) to estimate kinship and phylogeny (Avise, 2004). However, rapid advances in the development of molecular markers over the last two decades have facilitated gaining a better knowledge of the genetic structure of natural populations. Molecular genetic approaches have been used to identify parents, offspring, and closely related individuals in captive and wild populations; to define units for conservation, and the gene flow between such units; to quantify genetic variability of historical populations; to assess the rate at which genetic variation has been lost over time in fragmented landscapes; to estimate the degree of inbreeding in the population; to determine limits that distinguish a species, and so on. All of these areas are important in the conservation genetics of endangered species.

Conservation Genetics of the Muriqui

Interspecific differentiation

The genus *Brachyteles*, the muriqui or mono-carvoeiro, belongs to the Family Atelidae. In the past, only one species was recognized, *Brachyteles arachnoides*, distributed from southern Bahia to northern Paraná, along the Brazilian Atlantic rainforest. In 1971, Aguirre (1971) estimated that the total number of individuals had been about 400,000 individuals.

Vieira (1944) it was who recognized two subspecies of *Brachyteles*. Some morphological differences such as facial skin pigmentation and the presence or absence of vestigial thumbs suggested that this monotypic genus should be separated into *arachnoides* É. Geoffroy, 1806 (in the states of Rio de Janeiro, São Paulo and Paraná along the Serra do Mar) and *hypoxanthus* Kuhl, 1820, occurring in southern Bahia, Minas Gerais, and Espírito Santo south to the Serra da Mantiqueira. Lemos de Sá *et al.* (1990, 1993), Fonseca *et al.* (1991) and Lemos de Sá and Glander (1993) indicated that Vieira's (1944) standing was valid, but that differentiation is even more extreme and justifies the classification of the two forms as separate species (Coimbra-Filho *et al.*, 1993; Groves, 2001), the northern muriqui, *B. hypoxanthus* (Kuhl, 1820), and the southern muriqui, *B. arachnoides* (É. Geoffroy, 1806). Whether the presence or absence of vestigial thumbs is a good morphological marker to separate the species remains questionable, however (S. L. Mendes, pers. comm.).

The first question, therefore, about muriqui genetics involves whether the southern specimens, *B. arachnoides*, possess enough genetic diversification from northern specimens, *B. hypoxanthus*, to be designated as a separate species under phylogenetic approaches, and whether, in an *a priori*, non-discriminated analysis, specimens from the south group together separately from the ones from northern areas.

In general, mitochondrial DNA allows for analysis of phylogenetic relationships reflecting the history of maternal lineages within a population, requiring only one-fourth of the effective population size when compared to autosomal nuclear genes. The use of mtDNA is inappropriate for detecting paternal gene flow between populations. On the other hand, mtDNA is only a single genetic locus, lacking recombination, which reduces its power to detect the structure and the genetic history of the population at spatial and temporal levels. DNA sequence data allow both a

phylogenetic and an allele-frequency approach to the analysis of population structure and patterns of population variation. Because effective population size calculated under mitochondrial loci in a given population is one-fourth of nuclear loci, genetic drift is more powerful at mitochondrial loci; populations separated recently can show mitochondrial divergence unlike nuclear loci (Neigel and Avise, 1986). Thus, the mtDNA analyses involving samples from several localities of northern and southern muriqui would seem to be sufficient to address this question.

Genetic structure of populations

The historical deforestation of the Brazilian Atlantic forest since the 1500s has reduced it to small fragments with little or no connection between them. This has resulted in few, small and widely separated muriqui populations which represent genetic bottlenecks, and has eliminated opportunities for gene flow through the migration of individuals between these populations.

Currently, about 1,000 southern muriquis occur in relatively large and well protected areas. The situation of the northern muriquis is, however, more dramatic with fewer than 900 individuals in 12 separate populations, a large number restricted to unprotected forest fragments (Rylands *et al*. 2003; Mendes *et al*., 2005a). *Brachyteles hypoxanthus* is considered one of the world's 25 most endangered primates (Strier *et al*., 2006a).

The ecology, behavior, reproduction, and demography of a population of northern muriquis have been investigated by Karen B. Strier and her students since 1982 at the Caratinga Biological Station (EBC), also called the RPPN Feliciano Miguel Abdala (EBC/RPPN-FMA), in Minas Gerais (Strier, 1999). The population at the EBC has been growing steadily since long-term monitoring began, increasing from about 50–70 individuals in the 1980's to about 226 individuals as of January 2005 (Strier *et al*., 2006b). Since 2001, some new populations have been studied by Sérgio Mendes in the municipality of Santa Maria de Jetibá, in the state of Espírito Santo (Mendes *et al*., 2005b). Since 2003, Luis G. Dias has also initiated studies of muriqui populations in other areas of the state of Minas Gerais (Rio Doce and Serra do Brigadeiro state parks, and the Mata do Sossego private reserve).

In large populations with multiple groups, female muriquis leave their natal groups and move into others before reaching reproductive maturity, reducing the risks of close inbreeding (Printes and Strier, 1999; Strier and Ziegler, 2000). However, in smaller populations living in highly fragmented and disconnected areas, dispersal is restricted, and females with nowhere to go have been observed at the periphery of their natal groups (S. L. Mendes, pers. comm.). Research into the genetic variation and gene flow among muriqui populations and the relationship of population size to within-population genetic variability is critically needed for the formulation and implementation of a management plan to ensure the long-term persistence of remaining muriqui populations.

The first study involving muriqui genetics was conducted by Pope (1998), based on polymorphisms of allozymes of 10 individuals of *B. hypoxanthus* from Fazenda Esmeralda, Minas Gerais, and two individuals of *B. arachnoides* from Fazenda Barreiro Rico, São Paulo. The DNA for these genetic analyses was extracted from blood, with an invasive method that depended on the capture of live specimens (Lemos de Sá and Glander, 1993). At that time, Pope analyzed all individuals as the same species, *B. arachnoides* (the possibility of two species was not generally recognized). Pope observed a high level of genetic divergence using allozyme investigations, despite the small size and isolation of the populations. The variability observed was probably large due to between-species rather than within-population genetic diversity.

The first study using 126 individuals from two northern muriqui populations (Estação Biológica de Caratinga – EBC, Minas Gerais, and Santa Maria do Jetibá – SMJ, Espírito Santo) investigated the polymorphisms of restriction fragments (PCR-RFLP) of 480 base pairs (bp) of a hypervariable region of mtDNA or D-loop (Paes, 2005; Fagundes *et al*., in press). DNA was obtained from feces samples, a noninvasive method useful for endangered species (Chaves *et al*., 2006). The overall level of genetic differentiation between these muriqui populations was high. The test of genetic differentiation (F_{ST}), which characterizes genetic differentiation among populations, indicated that there was extensive genetic differentiation (F_{ST} = 0.635, *P*-value = 0.000) between the EBC and SMJ populations (Fagundes *et al*., in press). Values of *Nm* [$Nm = (1/F_{ST}-1)/4$] indicated less than 1 migrant per generation between SMJ and EBC, and related F_{ST} data revealed an absence of gene flow and high genetic distinction between these two populations.

Also, Chaves (2005) studied the sequence of 280 bp from the hypervariable internal segment of D-loop of 84 individuals from EBC and SMJ, and also observed high genetic diversity among these populations (F_{ST} = 0.56). Despite the distinction in mtDNA, the alleles did not cluster by geographic origin. Alleles from EBC and SMJ are interspersed in both clades, which mean that these populations are not completely distinct at this locus. Chaves (2005) also observed that values of nucleotide diversity of *B. hypoxanthus* are considerably lower when compared to other endangered species of mammals, including the gorilla (Garner and Ryder, 1996), chimpanzee (Deinard and Kidd, 2000), and the giant panda (Lu *et al*., 2001).

Our previous studies have shown that the loss and fragmentation of once contiguous habitat has caused the loss of genetic variation in the muriqui, and that genetic variation in the populations is among the lowest reported for any species of primate. This substantial loss of genetic variation

has contributed to extensive genetic differentiation among populations.

The next steps to measure genetic variability among populations of northern muriquis will require field efforts to sample new populations and individuals. Currently, our collection of feces is from individuals of seven of the twelve populations (Table 1). Some populations are still poorly sampled, however, and not yet representative of the total population. Just a few genetic samples for southern muriquis are available from blood, which makes it difficult to conduct comparative analysis of genetic variability between *B. arachnoides* and *B. hypoxanthus*. New approaches to evaluate genetic variability, including nuclear loci, are needed to better understand the consequences of fragmentation, reduction of population size and genetic drift in muriquis, and to assess the degree of genetic variation between the northern and southern populations.

The genetic markers used in the last two decades have changed, and there is an improved understanding of their potential to answer the most important questions in conservation genetics. They include either mitochondrial (mtDNA) or nuclear loci analyses, each with their advantages and disadvantages (Avise 2004). Analyses of nuclear DNA microsatellite loci have become an important approach for the conservation geneticist. This class of highly variable repeated sequences, polymorphic and single locus markers is routinely used to assess levels of genetic variability in small, endangered populations using non-invasive samples, with important implications in conservation biology. Because of their high mutation rates, microsatellites can be excellent markers for studying genetically depauperate populations, which show little or no variation at allozyme loci or mitochondrial DNA. Moreover, because of their short sequences, microsatellite loci can be amplified from degraded DNA, as often occurs when DNA is obtained non-invasively from hair and scat samples.

Table 1. Samples of feces collected from individuals of northern muriqui populations.

Population	Minimum population size	Total of sampled individuals	Percentage of sampled population
Espírito Santo			
Santa Maria do Jetibá	115	43	37.4
Caparaó National Park	82	2	2.4
Minas Gerais			
Caratinga Biological Station	226	130	57.5
Serra do Brigadeiro State Park	285	20	7.0
Rio Doce State Park	132	22	16.7
Serra do Ibitipoca	5	3	60.0
RPPN Mata do Sossego	42	10	23.8

Which methodology and DNA segments are more appropriate to study is always subject to doubt. To answer this question, it is necessary to look at the evolutionary properties of each class of DNA segment (mitochondrial or nuclear, single or multiple copy, coding or non-coding sequence, functional or non-functional) to evaluate the mutation rate and forces that drive the evolutionary patch of each segment. In general, most molecular data have proved to support rather than contradict previous statements based on other molecular markers. Nonetheless, the use of multiple molecular markers allows for more accurate conclusions.

Genetic approaches to the study of reproductive and social behavior

Developments in techniques of molecular genetics have been widely used to address important issues in the biology and behavioral ecology of mammal species (Woods *et al.*, 1999; Aitken *et al.*, 2004). In particular, knowledge of the current level of genetic variability and differentiation, relatedness between individuals, extent of inbreeding, and pedigree reconstructions are required to develop effective strategies for the conservation of endangered populations.

Many of the demographic features of *Brachyteles*, such as small and isolated populations, long inter-birth intervals, slow maturation rates, and long and overlapping generations, make them vulnerable to the effects of genetic drift and inbreeding, with the risks of lowering genetic variability and viability (Strier, 2000; Strier *et al.*, 2002). Nonetheless, demographic data on the muriquis have not yet revealed any evidence of deleterious signs of close inbreeding. Reproductive rates have remained stable, and infant mortality has been low in one group (Matão group) that has been monitored since 1982 (Strier, 2000). Females typically disperse from their natal groups prior to the onset of puberty or sexual activity, thereby reducing the risks of close inbreeding between siblings or father-daughter pairs (Strier, 1996; Printes and Strier, 1999; Strier and Ziegler, 2000).

The mating patterns of northern muriquis at the EBC are based on a system in which each female copulates with several males during their peri-ovulatory periods (Strier, 1997). Although females generally mate with multiple partners, copulations between mothers and their adult sons are very rarely observed (Strier, 1997). Also, behavioral observations have revealed a high tolerance of adult males to all infants (Guimarães and Strier, 2001). It is possible that behavioral mechanisms for inbreeding avoidance have contributed to the growth of the EBC muriqui population, which has more than quadrupled in the past 24 years (Strier *et al.*, 2006b).

Because of the muriquis' promiscuous mating system, it is impossible to determine the paternity of infants without

direct genetic data. Results from paternity studies can provide new insights into understanding muriqui mating behavior, and the relationship between adult male tolerance toward infants and male-infant genetic relatedness.

Known as Short Tandem Repeats (STR) sequences, microsatellites have the potential to evaluate inter-individual genetic variability. Microsatellite loci have been extensively used to genotype samples of primates. Many of them, isolated from the closely-related spider monkey (*Ateles*), can also be used to genotype the northern muriqui (Di Fiore and Fletcher, 2004). The use of heterologous primers is a good strategy to initiate new investigations on endangered species because the development of new primers of microsatellite DNA demands training, time and heavy financial investments. Heterologous primers were successful in amplifying the DNA of black and brown bears (Lorenzini *et al.*, 2004). Also, tests of several human microsatellite loci were positive and compatible in baboons (Morin *et al.*, 1998) and chimpanzees (Constable *et al.*, 2001), and may also be an option in muriqui studies.

Paternity and relatedness among members of northern muriqui populations can be obtained from analyses of genetic variability of STR sequence data. Analyses could be conducted on infants and juveniles, their mothers and possible fathers. However, this approach is only possible in a population such as that at the EBC, where data on paternity and genetic variation can be used to evaluate hypotheses derived from long-term behavioral data.

Monitoring and Management of Populations

The Management Plan for Muriquis, proposed by Mendes *et al.* (2005a), includes recommendations for monitoring unknown populations, conducting long-term studies of ecology and behavior, the reforestation of habitats in order to facilitate dispersion of females, and reductions in hunting and deforestation. Isolated groups and females in muriqui populations would seem to be more frequently observed in areas of highly fragmented forest. Translocation of reproductive individuals into potential receptor groups is a difficult challenge, but it is necessary in order to rescue the genetic variation represented by these individuals, which would otherwise die without leaving descendants. Due to the relatively recent history of translocations in muriqui (S. L. Mendes, pers. comm.), it is unknown what effects they can have on the genetic structure of the populations.

The problem of preserving the remaining wild populations is pertinent to concerns about the most appropriate conservation and management units, such as Evolutionary Significant Units (ESUs) and Management Units (MUs) as defined by Waples (1991) and Moritz (1994). Distinct MUs can be defined as populations connected by little or no contemporary gene flow, but not separated historically for very long periods of time (Waples, 1991).

The population viability of endangered species can be estimated based on demographic data (such as birth rates, death rates, longevity, reproductive rates, effective population size). Nonetheless, genetic studies of small populations can provide fundamental evidence of how observed patterns of mating and dispersion affect the loss of rare alleles within and between groups. In addition, comparative genetic studies can help to identify populations that are at such high risk of extinction due to the loss of heterozygosity that interventions (such as translocations) might be necessary.

The Muriqui Feces Bank

Most of the molecular genetic analyses of primates have been restricted to DNA extracted from blood or tissue samples (Surridge *et al.*, 2002), limiting the number of samples and risking the individuals due to anesthesia, capture and handling. The quantity of material required for DNA analyses may be infinitesimal using noninvasive methods, which are essential to studies of endangered species. Hairs, archeological or museum samples of pelts and bones, or feces from wild subjects, for example, can be obtained noninvasively. The first successful effort in isolating human shed epithelial cells mixed with feces was performed by Iyengar *et al.* (1991), and since then, studies in conservation genetics using DNA from faecal samples have been carried out on many threatened species.

The non-invasive technique of obtaining DNA from feces to investigate the genetic variability of northern muriquis has been applied in the past few years. DNA sampling through feces is a reliable, efficient, and economic method, which does not risk the physical integrity of the animals, and furnishes enough DNA that is easily reproducible for PCR amplification of fragments up to 800 bp in length (Chaves *et al.*, 2006). Also, it is a way of sampling a greater number of individuals in nature than would be possible if live captures to obtain blood were necessary.

At the same time, intensive fieldwork is needed to be able to obtain reliable fecal samples from individuals in nature. This may require long-term investment to habituate wild animals to human presence. An extensive study involving monitoring populations is the limiting factor in enabling the collection of feces from individually-known animals.

Currently, the feces bank comprises samples of 230 individuals from seven out of twelve populations of northern muriqui, all the result of extensive field studies (Table 1). The bank represents still a minor percentage of the northern populations, and a more coordinated and systematic effort among fieldworkers at the different muriqui sites is needed to achieve better representation across populations and species. Although the present studies are not as complete as would be desirable, they represent a landmark in the conservation genetics of free-living populations of muriqui, since the lack of completeness of the data is

a situation that confronts all population geneticists and behavioral ecologists studying endangered species.

Acknowledgments

I thank the Brazilian Institute for the Environment (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA*) for the collecting license (363/2001), and S. L. Mendes, P. B. Chaves, M. F. Paes, K. B. Strier, J. P. Boubli, C. B. Possamai, L. G. Dias, and the fieldwork teams for making this work possible; L. P. Costa, K. B. Strier and S. L. Mendes kindly reviewed this manuscript. The Brazil Science Research Council (*Conselho Nacional do Desenvolvimento Científico e Tecnológico – CNPq*), the Ministry of the Environment (MMA-PROBIO), Critical Ecosystem Partnership Fund (CEPF), and the Brazilian Higher Education Authority (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES*) provided financial support.

References

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

Aitken, N., Smith, S., Schwarz, C. and Morin, P. A. 2004. Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. *Molec. Ecol.* 13: 1423–1431.

Avise, J. C. 2004. *Molecular Markers, Natural History and Evolution*. 2nd Ed. Sinauer Associates Inc., Sunderland, MA.

Caughley, G. 1994. Directions in conservation biology. *J. Anim. Ecol.* 63: 215–244.

Chaves, P. B. 2005. Diversidade e Estrutura Genética de *Brachyteles hypoxanthus* (Primates: Atelidae). Undergraduate thesis, Departamento de Ciências Biológicas Universidade Federal do Espírito Santo, Vitória, Espírito Santo. 54pp.

Chaves, P. B., Paes, M. F., Strier, K. B., Mendes, S. L., Louro, I. D. and Fagundes, V. 2006. Non-invasive DNA extraction method optimization for the analysis of the endangered species of primate *Brachyteles*. *Genet. Molec. Biol*. 29: 750–754.

Coimbra-Filho A. F., Pissinatti A. and Rylands A. B. 1993. Experimental multiple hybridism and natural hybrids among *Callithrix* species from eastern Brazil. In: *Marmosets and Tamarins: Systematics, Behaviour, and Ecology*, A. B. Rylands (ed.), pp. 95–120. Oxford University Press, Oxford.

Constable, J. L., Ashley, M. V., Goodall, J. and Pusey, A. E. 2001. Noninvasive paternity assingnment in Gombe chimpanzees. *Molec. Ecol.* 10: 1279–1300.

Deinard, A. S. and Kidd, K. K. 2000. Identifying conservations units within captive chimpanzee populations. *Am. J. Phys. Anthropol.* 111: 25–44.

Di Fiore, A. and Fleischer R. C. 2004. Microsatellite markers for woolly monkeys (*Lagothrix lagotricha*) and their amplification in other New World primates (Primates: Platyrrhini). *Molec. Ecol. Notes* 4: 246–249.

Ebert, D., Haag, C., Kirkpatrick, M., Riek, M., Hottinger, J. W. and Pajunen, V. I. 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295: 485–488.

Fagundes, V., Paes, M. F., Mendes, S. L., Strier, K. B., Possamai, C. B., Boubli, J. P. In press. Genetic structure within the northern muriqui (*Brachyteles hypoxanthus*, Atelinae, Primates) inferred by fecal DNA and PCR-RFLP analysis. *Genet. Molec. Biol.*

Fonseca, G. A. B. da, Lemos de Sá, R. M., Pope, T. R., Glander, K. E. and Struhsaker, T. T. 1991. A pilot study of genetic and morphological variation in the muriqui (*Brachyteles arachnoides*) as a contribution to a long-term conservation management plan. Report, World Wildlife Fund – US, Washington, DC. 58pp.

Frankham, R. 1995. Inbreeding and extinction: a threshold effect. *Conserv. Biol*. 9: 792–799.

Frankham, R., Ballou, J. and Briscoe, D. 2002. *Introduction to Conservation Genetics*. Cambridge University Press, New York.

Franklin, I. R. 1980. Evolutionary change in small populations In: *Conservation Biology: An Evolutionary–Ecological Perspective*, M. E. Soulé and B. A. Wilcox, (eds.), pp.135–149. Sinauer Associates, Sunderland, MA.

Futuyma, D. J. 1992. *Biologia Evolutiva*. Sociedade Brasileira de Genética. 2nd ed. Ribeirão Preto, São Paulo.

Garner, K. J. and Ryder, O. A. 1996. Mitochondrial DNA diversity in gorillas. *Molec. Phylog. Evol.* 6: 39–48.

Groves, C. P. 2001. *Primate Taxonomy*. Smithsonian Institution Press, Washington, DC.

Guimarães, V. O. and Strier, K. B. 2001. Adult male-infant interactions in wild muriquis (*Brachyteles arachnoides hypoxanthus*). *Primates* 42: 395–399.

Iyengar, V., Albaugh, G. P., Lohani, A. and Nair, P. P. 1991. Human stools as a source of viable colonic epithelial cells. *Faseb J.* 5: 2856–2859.

Lemos de Sá, R. M. and Glander, K. E. 1993. Capture techniques and morphometrics for the woolly spider monkey, or muriqui (*Brachyteles arachnoides* E. Geoffroy, 1806). *Am. J. Primatol.* 29: 145–153.

Lemos de Sá, R. M., Pope, T. R., Glander, K. E., Struhsaker, T. T. and Fonseca, G. A. B. da. 1990. A pilot study of genetic and morphological variation in the muriqui (*Brachyteles arachnoides*). *Primate Conserv.* (11): 26–30.

Lemos de Sá, R., Pope, T. R., Struhsaker, T. T., Glander, K. E. 1993. Sexual dimorphism in canine length of woolly spider monkeys (*Brachyteles arachnoides*, E. Geoffroy 1806). *Int. J. Primatol*. 14: 755–762.

Lorenzini, R., Posillico, M., Lovari S. and Petrella, A. 2004. Non-invasive genotyping of the endangered Apennine brown bear: a case study not to let one's hair down. *Anim. Conserv.* 7: 199–209.

Lu, Z., Johnson, W. E., Menotti-Raymond, M., Yuhki, N., Martenson, J. S., Mainka, S., Shi-Qiang, H., Zhihe, Z., Li, G., Pan, W., Mao, X. and O'Brien, S. J. 2001. Patterns

of genetic diversity in remaining giant panda populations. *Conserv. Biol.* 15: 1596–1607.

Meffe, G. K., and Carroll, C. R. 1997. *Principles of Conservation Biology*. Sinauer, Sunderland, MA.

Mendes, S. L., Melo, F. R., Boubli, J. P., Dias, L. G., Strier, K. B., Pinto, L. P. S., Fagundes, V., Cosenza, B. and De Marco Jr, P. 2005a. Directives for the conservation of the northern muriqui, *Brachyteles hypoxanthus* (Primates, Atelidae). *Neotrop. Primates* 13(suppl.): 7–18.

Mendes, S. L., Santos, R.R. and Carmo, L. P. 2005b. Conserving the northern muriqui in Santa Maria de Jetibá, Espírito Santo. *Neotrop. Primates* 13(suppl.): 31–35.

Morin, P. A., Mahboubi, P., Wedel, S. and Roger, J. 1998. Rapid screening and comparison of human microsatellite markers in baboons: allele size in conserved, but allele number is not. *Genomics* 53: 12–20.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molec. Ecol.* 3: 401–411.

Neigel, J. E. and Avise, J. C. 1986. Phylogenetic relationship of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory*, E. Nevo and S. Karlin (eds.), pp. 515–534. Academic Press, New York.

O'Brien, S. J. 1994. A role for molecular genetics in biological conservation. *Proc. Natl. Acad. Sci. USA.* 91: 5748–5755.

Paes, M. F. 2005. Análise da Variabilidade Genética de *Brachyteles hypoxanthus*: Implicações na Conservação. Master's Thesis. Universidade Federal do Espírito Santo, Espírito Santo, Brazil.

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

Printes, R. C. and Strier, K. B. 1999. Behavioral correlates of dispersal in female muriquis (*Brachyteles arachnoides*). *Int. J. Primatol*. 20: 941–960.

Queiroz, S. A., Albuquerque, L. G. and Lanzoni, N. A. 2000. Efeito da endogamia sobre características de crescimento de bovinos da raça Gir no Brasil. *Rev. Bras. Zootec*. 29: 1014–1019.

Roelke, M. E., Martenson, J. S. and O'Brien S. J. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* 1: 340–350.

Rylands, A. B., Bampi, M. I., Chiarello, A. G., da Fonseca, G. A. B., Mendes, S. L., and Marcelino, M. 2003. *Brachyteles arachnoides*. In: *2004 IUCN Red List of Threatened Species*. Website: <www.redlist.org>. Accessed 2 August 2005.

Strier, K. B. 1996. Reproductive ecology of female muriquis (*Brachyteles arachnoides*). In: *Adaptive Radiations of Neotropical Primates*, M. A. Norconk, P. A. Garber and A. L. Rosenberger (eds.), pp.511–532. Plenum Press, New York.

Strier, K. B. 1997. Mate preferences of wild muriqui monkeys (*Brachyteles arachnoides*): reproductive and social correlates. *Folia Primatol.* 68: 120–133.

Strier, K. B. 1999. *Faces in the Forest: The Endangered Muriqui Monkeys of Brazil.* Harvard University Press, Cambridge, MA.

Strier, K. B. 2000. Population viabilities and conservation implications for muriquis (*Brachyteles arachnoides*) in Brazil's Atlantic Forest. *Biotropica* 32: 903–913.

Strier, K. B. and Ziegler, T. E. 2000. Lack of pubertal influences on female dispersal in muriquis monkeys, *Brachyteles arachnoides*. *Anim. Behav*. 59: 849–860.

Strier, K. B., Boubli, J. P., Guimarães, V. O. and Mendes, S. L. 2002. The muriqui population of the Estação Biológica de Caratinga, Minas Gerais, Brazil: updates. *Neotrop. Primates* 10: 115–118.

Strier, K. B., Mendes, S. L., Boubli, J. P. and Dias, L. G. 2006a. The northern murqiui, *Brachyteles hypoxanthus* (Kuhl, 1820). In: Primates in Peril: The World's 25 Most Endangered Primates, 2004–2006, R. A Mittermeier, C. Valladares-Pádua, A. B. Rylands, A. A. Eudey, T. M. Butynski, J. U. Ganzhorn, R. Kormos, J. M. Aguiar, and S. Walker (eds.), pp.10, 23. *Primate Conserv.* (20): 1–28.

Strier, K. B., Boubli, J. P., Possamai, C. B. and Mendes, S. L. 2006b. Population demography of northern muriquis (*Brachyteles hypoxanthus*) at the Estação Biológica de Caratinga/Reserva Particular de Patrimônio Natural–Felìciano Miguel Abdala, Minas Gerais, Brazil. *Am. J. Phys. Anthropol.* 130: 227–237.

Surridge, A. K., Smith, A. C., Buchanan-Smith, H. M. and Mundy, N. I. 2002. Single-copy nuclear DNA sequences obtained from noninvasively collected primate feces. *Am. J. Primatol*. 56**:** 185–190.

Vieira, C. da C. 1944. Os símios do Estado de São Paulo. *Pap. Avuls. Zool., São Paulo* 4: 1–31.

Waples, R. S. 1991. Pacific salmon, *Oncorhynchus* spp. and the definition of "species" under the endangered species act. *Mar. Fish. Rev.* 53: 11–22.

Woods, J. G., Paetkau, D., Lewis, D., McLellan, B. N., Proctor, M. and Strobeck, C. 1999. Genetic tagging of free-ranging black and brown bears. *Wildl. Soc. Bull*. 27: 616–627.