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ORIGINAL ARTICLE

Population genetics of the California National Primate Research Center's (CNPRC) captive *Callicebus cupreus* colony

Adrian Mendoza · Jillian Ng · Karen L. Bales · Sally P. Mendoza · Debra A. George · David Glenn Smith · Sree Kanthaswamy

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Abstract The California National Primate Research Center maintains a small colony of titi monkeys (*Callicebus cupreus*) for behavioral studies. While short tandem repeat (STR) markers are critical for the genetic management of the center's rhesus macaque (*Macaca mulatta)* breeding colony, STRs are not used for this purpose in the maintenance of the center's titi monkey colony. Consequently, the genetic structure of this titi monkey population has not been characterized. A lack of highly informative genetic markers in titi monkeys has also resulted in scant knowledge of the species' genetic variation in the wild. The purpose of this study was to develop a panel of highly polymorphic titi monkey STRs using a cross-species polymerase chain reaction (PCR) amplification protocol that could be used for the genetic management of the titi monkey colony. We screened 16 STR primer pairs and selected those that generated robust and reproducible polymorphic amplicons. Loci that were found to be highly polymorphic, very likely to be useful for parentage verification, pedigree assessment, and studying titi monkey population genetics, were validated using Hardy–Weinberg equilibrium and linkage disequilibrium analyses. The genetic data generated in this study were also used to assess directly the impact on the colony's genetic diversity of a recent adenovirus outbreak. While the adenovirus epizootic disease caused significant mortality (19 deaths among the 65 colony animals), our results suggest that the disease exhibited little or no influence on the overall genetic diversity of the colony.

Keywords Genetic management · Population genetic structure · Captive populations · Closed colony · Paternity · Cross-species amplification · Red or coppery titi monkey

A. Mendoza · D. G. Smith · S. Kanthaswamy
University of California Graduate Program in Forensic Science, University of California, One Shields Ave, Davis 95616, CA, USA

J. Ng · D. A. George · D. G. Smith · S. Kanthaswamy
Department of Anthropology, University of California, One Shields Ave, Davis 956163, CA, USA

K. L. Bales · S. P. Mendoza
Department of Psychology, University of California, One Shields Ave, Davis 95616, CA, USA

K. L. Bales · S. P. Mendoza · D. G. Smith · S. Kanthaswamy
California National Primate Research Center, University of California, One Shields Ave, Davis 95616, CA, USA

S. Kanthaswamy
Department of Environmental Toxicology, University of California, One Shields Ave, Davis 95616, CA, USA

S. Kanthaswamy (✉)
303 Young Hall, Davis 95616, CA, USA
e-mail: skanthaswamy@ucdavis.edu

Introduction

Titi monkeys (*Callicebus* spp.) are small new world monkeys (NWMs) that belong to a large, diverse genus of platyrrhines that are widely distributed in tropical regions in South America. They are longhaired, brown-colored, and lack prehensile tails, with adults of the species weighing about 1 kg (Hershkovitz 1988). Field studies of titi monkeys have focused on various aspects of their social organization and behavioral ecology. They are socially monogamous and live in groups typically consisting of an adult pair and one or two young (Valeggia et al. 1999). Laboratory studies have also broadened our knowledge of them by providing some insights into their mating and

parenting behavior. Experimental findings with mating pairs of titis have shown that both sexes strongly prefer each other over strangers in choice tests, even after the mates have been separated for days (Fernandez-Duque et al. 2000). Adult males exhibit parental behavior, which includes marked interaction with infants starting from the first few days of the infants' lives (Valeggia et al. 1999; Mendoza and Mason 1986). Although much has been discovered about the social structure and behavior of titi monkeys, no study of their genetic relationships, including the confirmation of parentage via genetic tests, has been conducted in wild or captive titi monkeys.

Approximately 30 species of *Callicebus* are currently recognized, constituting a considerable proportion of total platyrrhine species richness (Gualda-Barros et al. 2012; Becker et al. 2013). The genus is one of the best examples of karyological diversity in NWMs because the diploid numbers among the 30 species range from $2n = 16$ to $2n = 50$ (Stanyon et al. 2003). Despite this karyological diversity, the genetic variability at the population level in titi monkeys is still poorly described relative to that of other species of NWMs. In addition to the importance of understanding evolutionary processes, knowledge of the genetic variability of any species is important for the analysis of population dynamics, including long-term viability in the context of factors such as inbreeding and genetic drift (Menescal et al. 2009).

In the wild, the coppery, or red, titi monkey (*C. cupreus*) is found in Brazil, Colombia, Ecuador, and Peru (Kinzey 1981; Hershkovitz 1990; van Roosmalen et al. 2002). According to van Roosmalen et al. (2002), *C. cupreus* and *C. moloch* groups are not only allopatric but sympatric with *C. torquatus* groups. The Delta Regional Primate Center began the first significant breeding program for the *C. cupreus* in 1965 (Lorenz and Mason 1971). In 1971, the California National Primate Research Center (CNPRC) established a closed colony of 84 *C. cupreus* individuals descended from founders of the Delta Regional Primate Center (Covington, LA, USA) colony, which was augmented by other introductions in 1990 (Lorenz and Mason 1971; Becker et al. 2013). The CNPRC titi monkeys are housed in small social groups (Mason 1966) and used in research primarily involving behavioral and neurobiological studies. Because differences in genetic structure and composition of captive-bred colonies influence their suitability in research, understanding these population genetic characteristics will support the development of appropriate animal models for research. Moreover, colony management employing sound genetic management approaches enhances the production of nonhuman primates (NHPs) for research within the National Primate Research Center system (Kanthaswamy et al. 2012).

This study was initiated to assess the genetic structure and diversity among titi monkeys housed in the CNPRC using a panel of short tandem repeats (STRs) for cross-species polymerase chain reaction (PCR) amplification. STR loci are suitable for population studies because of their abundance in eukaryotes and their highly polymorphic nature (Hughes and Queller 1993). STRs have been used to perform parentage analyses and to assess population genetic variability in many NHP populations (Moore et al. 1998; Rogers et al. 2005; Kanthaswamy et al. 2006) and are extremely useful in genetic management of captive primate colonies (Kanthaswamy et al. 2010, 2012).

Cross-species amplification uses loci characterized in one species to genotype representatives of another, usually closely related, species. Because the identification of novel species-specific STR markers is time and labor intensive, cross-species amplification has been widely used in NHPs (Chambers et al. 2004). To minimize ascertainment bias that can lead to underestimates of some genetic parameters, such as genetic diversity, these polymorphic markers are typically selected among those identified in as closely a related species as possible. The titi monkey colony at the CNPRC is ideal for validating a panel of STRs developed using cross-species amplification, because their pedigrees have been categorized based on multigenerational colony records and banked blood and DNA samples from members of these pedigrees.

Using samples from the CNPRC's titi monkey biomaterial repository, this study evaluated 16 candidate STR primer pairs (12 of which were originally identified in NWMs) that have been developed for PCR amplification and fragment analysis. Markers that exhibited Mendelian properties were used to compute allele frequencies, as well as observed and expected heterozygosity. These data were then used to characterize the genetic structure of the colony.

Since the titi monkeys studied experienced a deadly outbreak of fulminant pneumonia and hepatitis that struck the CNPRC in May 2009 (Chen et al. 2011), we investigated the levels of genetic diversity, genetic structure, and inbreeding both prior to and after the viral epizootic disease. The outbreak, which lasted 3 months, was attributed to a novel adenovirus that infected 23 of 65 titi monkeys in the colony. Most animals that developed symptoms that progressed to fulminant pneumonia and hepatitis were random individuals that were housed in cages located close to those few that developed the acute respiratory illness earlier during the outbreak. Subsequently, 19 of the 23 affected animals died or were euthanized due to complications from infection (Chen et al. 2011). Because infectious diseases and their demographic consequences influence the genetic composition of populations, we used the genetic information from this study to determine the

Table 1 The 16 short tandem repeat (STR) loci screened in this study

Reported use in NHP species	Type of repeat	Locus	Result	Annealing temperature (°C)	Mg^{2+} concentration (Mm)	Reference
Lagothrix lagotricha	Dinucleotide	1110	No amplification	N/A	N/A	Di Fiore and Fleischer (2004)
Lagothrix lagotricha	Dinucleotide	1115	Polymorphic	56.7	1.5	Di Fiore and Fleischer (2004)
Lagothrix lagotricha	Dinucleotide	157	Polymorphic	56.7	2	Di Fiore and Fleischer (2004)
Lagothrix lagotricha	Trinucleotide	311	Dimorphic	56.7	2.5	Di Fiore and Fleischer (2004)
Aotus azarai	Dinucleotide	D13s160	Poor amplification	57.8	2.5	Babb et al. (2011)
Aotus azarai	Dinucleotide	D15s108	No amplification	N/A	N/A	Babb et al. (2011)
Aotus azarai	Dinucleotide	D4s411	Polymorphic	56.7	2	Babb et al. (2011)
Aotus azarai	Dinucleotide	D8s275	Polymorphic	56.7	2.5	Babb et al. (2011)
Aotus azarai	Dinucleotide	PEPC3	Poor amplification	56.7	2	Babb et al. (2011)
Aotus azarai	Dinucleotide	PEPC40	No amplification	N/A	N/A	Babb et al. (2011)
Aotus azarai	Dinucleotide	PEPC59	No amplification	N/A	N/A	Babb et al. (2011)
Aotus azarai	Dinucleotide	PEPC8	Polymorphic	56.7	2	Babb et al. (2011)
Alouatta belzebul	Tetranucleotide	Ab13	Poor amplification	55.8	2.5	Goncalves et al. (2004)
Cebus capucinus	Tetranucleotide	Ceb19	No amplification	N/A	N/A	Muniz and Vigilant (2008)
Cebus capucinus	Tetranucleotide	Ceb10	Polymorphic	60.4	2	Muniz and Vigilant (2008)
Cebus capucinus	Tetranucleotide	Ceb130	Polymorphic	59.1	1.5	Muniz and Vigilant (2008)

NHP nonhuman primate, Mg^{2+} magnesium, *N/A* not available

impact of the outbreak on the colony's genetic diversity. While the loss of only between one quarter and one third of a population does not constitute a dramatic genetic bottleneck, the random-sampling effects associated with such loss yields unpredictable effects on genetic diversity that require empirical documentation.

Methods

The 16 STRs described in Table 1 were screened and PCR amplification conditions optimized using 50 DNA samples from the CNPRC's titi monkey biomaterial repository. These samples were obtained from animals that were housed at the CNPRC before ($N = 49$) and after ($N = 39$) the adenovirus outbreak in the colony. The STR loci were previously amplified in closely related species of NWMs, including *Alouatta belzebul* (Goncalves et al. 2004; Menescal et al. 2009), *Lagothrix lagotricha* (Di Fiore and Fleischer 2004), *Cebus capucinus* (Muniz and Vigilant 2008), and *Aotus azarai* (Babb et al. 2011), minimizing the effect of ascertainment bias. Screening and subsequent genotype analysis was performed at the Molecular Anthropology Laboratory, Department of Anthropology,

University of California at Davis. Primers for potential STR markers underwent gradient tests to optimize annealing temperatures and magnesium sulfate ($MgSO_4$) concentrations and to assess the success of amplification in titi monkeys.

Primer pairs were prepared and tested at three different $MgSO_4$ concentrations varying from 1.5 to 2.5 mM. Each reaction contained 1.25 µl of 3 ng/µl titi DNA and 11.25 µl of PCR primer cocktail containing the following: 0.25 µl deoxyribonucleotide triphosphate (dNTP) (10 mM), 1.25µl 10× ammonium (NH_4) PCR buffer (Bioline, Taunton, MA, USA), 0.2 mM of each forward and reverse primer (Integrated DNA Technologies, Inc., San Diego, CA, USA), 0.024 U Taq polymerase (Invitrogen™ Platinum® Taq DNA Polymerase, Life Technologies, Grand Island, NY, USA), $MgSO_4$ (amount varied with desired salt concentration), and double-distilled water (ddH_2O). PCR conditions for the gradient were as follows: 1 cycle at 94 °C for 2 min, 40 cycles at 94 °C for 30 s, annealing temperature gradient range 55.0–64.9 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 5 min. Two microliters of PCR product was electrophoresed in a 2 % agarose gel (40 mM Tris-acetate, 1.0 mM ethylenediaminetetraacetate (EDTA), pH 8.0) and visualized with ethidium bromide to evaluate the success of amplification.

STR alleles were genotyped using a genetic analyzer (model Applied Biosystems® 3130xL; Life Technologies) and the sizes determined through comparison with an internal size standard (Applied Biosystems® GeneScan™ 500 LIZ™; Life Technologies). All reactions were run with negative controls to monitor contamination. Only markers that generated robust, reliable, and reproducible amplicons were used in subsequent analyses.

Genotypes were examined for probable scoring errors due to stutter or large allele dropout, and incidences of null alleles were examined using Micro-Checker version 2.2 (van Oosterhout et al. 2004). GENEPOP 4.2 was used to test for adherence to Hardy–Weinberg equilibrium (HWE) and to detect linkage disequilibrium (LD) among pairs of markers at the 0.05 level of probability using Fisher's exact tests (Raymond and Rousset 1995). Unbiased *P* values were generated by the Markov Chain Monte Carlo (MCMC) approach (Guo and Thompson 1992) with 5,000 iterations per batch. Banked DNA samples from 17 known family trios (sire, dam, and offspring) housed in the colony prior to the adenovirus outbreak were used to confirm Mendelian segregation of alleles across all STR loci.

Arlequin version 3.5.1.3 (Excoffier et al. 2005) was used to calculate genetic diversity among the study animals by calculating allele numbers (N_a), observed heterozygosity (OH), and estimating gene diversity [expected heterozygosity (EH)]. The same software was used to compute Wright's (Wright 1978) *F* statistics: F_{IS} (inbreeding coefficient), F_{ST} (total coancestry or population subdivision coefficient), and F_{IT} (total fixation coefficient). These parameters estimate the combined loss of heterozygosity due to nonrandom mating. In addition, an analysis of molecular variance (AMOVA) was conducted to estimate population differentiation.

Fisher's exact probability test using the Markov chain analysis (10,000 dememorizations; 10,000 iterations per batch, 1,000 batches) was carried out to establish whether there were differences in genetic diversity between titi monkeys that survived the adenovirus outbreak of 2009 (Chen et al. 2011) and those that succumbed to it. Under a null hypothesis postulating significant allelic differentiation between survivors ($N = 36$) and the dead ($N = 10$), the homogeneity test was performed using GENEPOP 4.2 (Raymond and Rousset 1995). Because of the small samples compared with the level of genotypic variability (all individuals were genotypically unique), comparisons of allelic differentiation across loci used genic rxc (rows times columns) tables rather than genotypic tables, as recommended by Goudet et al. (1996) for a more robust analysis. Differences in genetic diversity between the surviving and deceased titi monkeys was also assessed using a one-sided Mann–Whitney *U* test.

Although it has been claimed that different species of titi monkeys can be distinguished based on differences in pelage, body color, and pattern (Hershkovitz 1988), body length and weight *C. cupreus* and *C. moloch* exhibit significant phenotypic overlap, making it difficult to differentiate the two species (Hershkovitz 1990). While earlier studies on the CNPRC titi colony reported that these individuals represent the species *C. moloch* (Valeggia et al. 1999), it is not known whether or not the founding stock of the titi colony at the CNPRC represents a diversity of species or subspecies. To determine whether allele frequencies and distributions are representative of two or more species or subspecies of titi monkeys, we used STRUCTURE 2.3.4 (Pritchard et al. 2000) to calculate the assignment index and determine the relative probabilities of assignment of an animal to *K* or more genetically discrete clusters based on that animal's genotypes. The analysis was run at sweeps of 5×10^5 iterations after a burn-in period of 10^5 with and without a priori population information for *K* values from 1 to 5. *K* values were graphed with their respective posterior probability Ln *P*(*D*); the *K* value with the highest Ln *P*(*D*) and smallest variance between runs was selected as the true *K* value.

In addition to determining parentage visually by match comparison, CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007) was used to confirm assignments of parentage based on categorical allocation (Jones et al. 2010) to verify the parentage for titi monkeys probabilistically. The natural logarithm of the likelihood odds ratio

Table 2 Primer sequences, repeat motifs, allele size ranges, and estimates of observed (OH) and expected (EH) heteozygosity and allele numbers (N_a) per locus

Name/ strand	Primer sequence	Repeat motif	Allele range	OH	EH	N_a
1115F	GCTCATATTCATACATCCCTTGG	$(GT)_3(GA)_1(GT)_5$	206–220	0.61	0.5	5
1115R	TTTGCTTGCTCATTCATTGC					
157F	TGGCAAGTCTGGTTTCAAGC	$(GA)_4(GT)_4(CT)_1$	204–230	0.98	0.83	8
157R	TTCCAGACTGAGCTAGGATGC					
311F	CTTCCGAAAGCCATTTCTCC	$(GAA)_3(GAG)_9$	183–186	0.20	0.19	2
311R	TTAATGCCAGATGATTTTGG					
D4S411F	AGGCTGTCTTGGCAGAAAT	$(TA)_{20}$	121–155	1	0.88	11
D4S411R	GATGTAATCCTGTGCTATGGC					
D8S275F	AAATCGCTAGAAAATGTCCA	$(TC)_{20}$	129–149	0.91	0.86	10
D8S275R	TCACACCTGGGAATTAGAAG					
PEPC8F	TTCAGGATGCATCAAATGATT	$(CT)_{16}$	251–259	0.68	0.75	5
PEPC8R	TAGCAGTCTATTTAGGTGTTAAT					
Ceb10F	TTGCTGATGCTTGCCTTC	$(AGAT)_{13}$	215–227	0.68	0.61	4
Ceb10R	TGGCAGATTGTGGACTTCTC					
Ceb130F	CAAAGTCCACTCACTTAACCAC	$(ATCT)_9$	225–253	0.82	0.78	6
Ceb130R	AGAAGACCCTGCCTCAAG					

(LOD) was calculated for parentage assignments involving every adult in the colony. An individual was assigned paternity or maternity if his or her likelihood was sufficiently higher than the second most likely adult male or female, respectively (Walling et al. 2010).

Results

Eight of the 16 screened loci amplified robustly and provided complete and reproducible genotype profiles for 49 of the 50 samples and yielded multiple alleles per locus. Of the 49 samples, 39 were from survivors of the outbreak. The eight STR loci were statistically unlinked and in HWE. Micro-Checker confirmed that these markers did not exhibit any null alleles. Primer sequences, repeat motif, and allele size ranges of each of these eight STR markers are provided in Table 2, as are allelic richness (N_a), observed heterozygosity (OH), and gene diversity or expected heterozygosity (EH) for each of the eight loci. Alleles observed at each locus fell within variable size ranges. The eight markers exhibited an average of approximately six alleles per locus, an average OH of 0.735 (range 0.15–1.00), and an average estimate of EH) of 0.67 (0.14–0.87). OH estimates exceeded EH estimates for seven of the eight loci.

Fisher's exact test using the Markov chain algorithm did not reveal any significant differences in allelic composition between the 36 surviving and the ten deceased animals (P value > 0.05; Table 3). Furthermore, based on a one-tailed Mann–Whitney U test where the critical value of U was computed to be 15 at P value ≤ 0.05 and n_1 and $n_2 = 8$, respectively, there were no significant differences between measurements of N_a (U value = 29.0), OH (U value = 31.0), or EH (U value = 31.0) between animals that survived the viral outbreak ($N = 39$) and those that died ($N = 10$) (Table 3). A match comparison of genotypes between 17 known family trios (offspring, dam, and sire) revealed no deviations from Mendelian inheritance. Parentage based on colony breeding records was confirmed for 16 (94.12 %) of the 17 offspring in this study. The highest trio LOD scores for these 16 cases ranged from 0.584 to 12.443. All parentage assignments were additionally supported at the 95 % confidence level by the maximum likelihood method calculated by CERVUS. The single exception that resulted in unresolved parentage was due to failed amplification of STRs in the sample of the offspring of one trio.

Estimates of both F_{IS} and F_{ST} were negative whether the sample set was divided into: (1) titi monkey colony ($N = 49$) before and after ($N = 39$), or (2) animals living after the viral outbreak ($N = 39$) and those that perished ($N = 10$). Negative F_{IS} estimates result when the number of OH exceeds expectation (Wright 1978), suggesting that the CNPRC titi colony is not highly inbred. As estimates of F_{ST} were also negative, the two pairs of groups that were compared are genetically homogeneous or undifferentiated.

AMOVA showed that the estimate of within-group diversity is much larger than the estimate obtained from variance between groups (Table 4). In fact, the additional variance component due to variance among groups (i.e., the F_{ST}) is zero, and all genetic variation ($\sim$100 %) stemmed from differences between individual samples within each of the two data sets (Table 4). This is consistent with

Table 3 Genetic diversity estimates [allele numbers (N_a, observed heterozygosity (OH), and estimated heterozygosity (EH) of the titi monkey colony before ($N = 49$) and after (i.e., survivors) ($N = 39$) the adenovirus outbreak of 2009 and monkeys that succumbed to the disease ($N = 10$)

Locus	N_a				OH				EH				[a]*P*er locus *P* value
	Before	Survivors	Dead	Mean	Before	Survivors	Dead	Mean	Before	Survivors	Dead	Mean	
311	2	2	2	2	0.20	0.10	0.20	0.17	0.19	0.09	0.19	0.14	0.50
157	8	8	6	7.33	0.98	1.00	0.90	0.96	0.83	0.83	0.86	0.85	0.94
1115	5	4	5	4.67	0.61	0.62	0.70	0.64	0.54	0.53	0.57	0.55	0.90
D4s411	10	10	8	9.33	1.00	1.00	1.00	1	0.88	0.89	0.91	0.90	0.10
D8s275	10	9	8	9	0.91	0.86	1.00	0.92	0.86	0.87	0.88	0.88	0.10
PEPC8	5	5	5	5	0.68	0.71	0.50	0.63	0.75	0.72	0.79	0.76	0.87
CEB10	4	3	3	3.33	0.68	0.67	0.60	0.65	0.61	0.54	0.61	0.58	0.72
CEB130	6	6	6	6	0.82	0.90	0.90	0.87	0.78	0.79	0.82	0.81	0.99
Mean	6.25	5.88	5.4		0.74	0.73	0.73		0.68	0.66	0.70		
SD	2.87	2.90	2.13		0.26	0.30	0.28		0.23	0.27	0.24		

[a] Genic differentiation: Fisher's exact probability test: $\chi^2 = 2.7003$, ($df = 16$) and unbiased *P* value across all loci = 0.999917

Table 4 Analysis of molecular variance (AMOVA) for the titi monkey colony before and after the adenovirus outbreak of 2009

Source of variation	Sum of squares	Variance component	Variance percentage
Between populations	0.70 (0.91)	−0.03 (−0.06)	−1.15 (−2.15)
Among individuals within populations	153.14 (70.69)	−0.25 (−0.24)	−9.56 (−9.19)
Within individuals	191.00 (90.50)	2.94 (2.92)	110.71 (111.34)
Total	344.84 (162.08)	2.65 (2.62)	

Results of the AMOVA analysis among those that survived and those that died are in parentheses

estimates of F_{ST}, confirming the absence of any influence of the viral outbreak on the genetic structure of the titi monkey population.

Results of the STRUCTURE analysis for $K = 1$ with no prior population information exhibited the highest posterior probability and the least variance compared with those for $K = 2$ (or greater). All individuals included in the analysis were assigned to a single cohesive and discrete genetic cluster (data not shown).

Discussion

The genetic structure of captive colonies of NHPs must be characterized and monitored to preserve genetic variability and minimize genetic subdivision (Kanthaswamy et al. 2006). The identification of parentage, which allows pedigree construction and consanguinity level estimations within the small captive titi monkey colony will facilitate maintenance of its genetic variation. The panel of eight STRs allowed us to determine accurately the sires and dams of all but three offspring born each year since 1971 at the 95 % confidence level. As such, the panel meets the standards for parentage testing for genetic management of the titi monkey colony at the CNPRC. Mean heterozygosity estimates (OH and EH) for all but two markers, 311 and 1115, were at least comparable with those in other NWMs in which they were identified (Di Fiore and Fleischer 2004; Goncalves et al. 2004; Muniz and Vigilant 2008; Menescal et al. 2009; Babb et al. 2011), reflecting the absence of significant ascertainment bias. The mean estimates of OH and EH of the captive titi monkey population (0.74 and 0.70, respectively) were greater than those estimated for a sample set of 23 animals from a wild population of red-bellied titis (*C. moloch*) in eastern Amazonia (0.33 and 0.63, respectively) (Menescal et al. 2009). While the low level of genetic variability in the wild population could have been due to sampling error from the use of only four STR loci or the small size of the wild population, it is also plausible that breeding-colony management has resulted in producing more heterozygotes by minimizing mating between relatives, or by the avoidance of inbreeding by the monkeys themselves, which would be consistent with their negative F_{IS} estimate. Greater variability among study animals, based on N_a, OH, and EH estimates, has been observed in other species of captive NHPs, such as rhesus and pigtail macaques, compared with their wild counterparts (Kanthaswamy et al. 2010, 2012). This difference is probably due in part to the acquisition of founders of captive colonies from multiple geographic sources.

Since emerging infectious agents remain a threat to laboratory-reared NHPs, it is important to study the effect

that a specific zoonotic disease has on both genetic heterogeneity and colony health (Bailey and Mansfield 2010). A comparison of genetic diversity and genetic subdivision before and after the viral outbreak and between survivors and nonsurvivors of the outbreak revealed no significant differences, implying that no loss of variability occurred among colony animals that could be directly attributed to the viral epizootic disease.

The estimates of F statistics (F_{IS}, F_{ST}, F_{IT}) for the captive titi monkey colony suggest that there are no significant deviations from panmixia (random mating). As colony breeding and pedigree records confirm, three incidences of mating between parents and offspring occurred more than a decade ago, notwithstanding the negative F_{IS} values. Given the small size of the colony and panmixia, more instances of consanguineous matings would be expected than were observed, suggesting that colony managers have become more effective in minimizing mating between relatives. Moreover, these animals represent a small closed colony in which mating between close relatives is generally avoided. The excess of heterozygosity responsible for the negative estimate of F_{IS} is probably a result of consanguineous mating prevention by the colony managers and the avoidance of inbreeding by the colony animals themselves.

STRUCTURE failed to distinguish more than one genetic cluster among the animals tested, implying that there is a marked genetic homogeneity of *C. cupreus* monkeys without any evidence of interspecies admixture or population structure (genetic subdivision). Therefore, based on the current data, no gene flow from *C. moloch* or *C. torquatus* is evident. However, without reference DNA samples from these three species, it is not possible to verify this observation. Lacking data to indicate otherwise, it must be assumed that all founders of the titi monkey colony represent the species *C. cupreus*.

It is worth noting that this study is limited in scope, and it is necessary to increase both sample size and the number of loci to illustrate the advantages of such comparisons as those made in this study. Other studies have shown that cross-species amplification using common STR markers may pose a greater challenge than was previously appreciated (Smith et al. 2000; Chambers et al. 2004). In those studies, cross-species amplification was proven to be successful for a majority of markers tested, although allele numbers and frequencies of some loci in the new population of interest are likely to be lower and contribute less statistical power to the analysis of the new species than to analyses of populations in which the loci were originally identified (Chambers et al. 2004). With the increasing efficiency of microsatellite discovery (Zane et al. 2002) and the lower success rate of cross-species amplification in new world primates (Ellsworth and Hoelzer 1998), it may be worthwhile to consider establishing species-specific primers for titi monkeys. However, results of our study provide both a baseline by which to continue investigating this species and rationale for further exploring these and additional cross-species STRs for studying mating systems and population genetic structure in titi monkeys.

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References

Babb PL, McIntosh AM, Fernandez-Duque E, Di Fiore A, Schurr TG (2011) An optimized microsatellite genotyping strategy for assessing genetic identity and kinship in Azara's owl monkeys (*Aotus azarai*). Folia Primatol 82:107–117. doi:10.1159/000330564

Bailey C, Mansfield K (2010) Emerging and reemerging infectious diseases of nonhuman primates in the laboratory setting. Vet Pathol 47:462–481. doi:10.1177/0300985810363719

Becker J, Baker AJ, Frampton T, Pullen PK, Bales KL, Mendoza SP, Mason WA (2013) Pitheciines in captivity: challenges and opportunities, past, present and future. In: Veiga LM, Barnett AA, Ferrari SF, Norconk MA (eds) Evolutionary biology and conservation of Titis, Sakis and Uacaris. Cambridge University Press, New York, pp 344–349

Chambers KE, Reichard UH, Moller A, Nowak K, Vigilant L (2004) Cross-species amplification of human microsatellite markers using noninvasive samples from white-handed gibbons (*Hylobates lar*). Am J Primatol 64:19–27. doi:10.1002/ajp.20058

Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP, Canfield DR, Maninger N, Rosenthal A, Spinner A, Bales KL, Schnurr DP, Lerche NW, Chiu CY (2011) Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. PLoS Pathog 7:e1002155. doi:10.1371/journal.ppat.1002155

Di Fiore A, Fleischer RC (2004) Microsatellite markers for woolly monkeys (*Lagothrix lagotricha*) and their amplification in other new world primates (Primates: Platyrrhini). Mol Ecol Notes 4:246–249. doi:10.1111/j.1471-8286.2004.00631.x

Ellsworth JA, Hoelzer GA (1998) Characterization of microsatellite loci in a new world primate, the mantled howler monkey (*Alouatta palliata*). Mol Ecol 7:657–658. doi:10.1046/j.1365-294X.1998.00340.x

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50. doi:10.4137/ebo.s0

Fernandez-Duque E, Valeggia CR, Mason WA (2000) Effects of pair-bond and social context on male-female interactions in captive titi monkeys (*Callicebus moloch*, Primates: Cebidae). Ethology 106:1067–1082. doi:10.1046/j.1439-0310.2000.00629.x

Goncalves EC, Silva A, Barbosa MSR, Schneider MPC (2004) Isolation and characterization of microsatellite loci in Amazonian red-handed howlers *Alouatta belzebul* (Primates, Plathyrrini). Mol Ecol Notes 4:406–408. doi:10.1111/j.1471-8286.2004.00667.x

Goudet J, Raymond M, de Meeus T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144:1931–1938

Gualda-Barros J, Nascimento FOd, Amaral MKd (2012) A new species of *Callicebus Thomas*, 1903 (Primates, Pitheciidae) from the states of Mato Grosso and Pará, Brazil. Papéis Avulsos de Zoologia (São Paulo) 52:261–279

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372. doi:10.2307/2532296

Hershkovitz P (1988) Origin, speciation, and distribution of South American titi monkeys, genus *Callicebus* (Family Cebidae, Platyrrhini). Proc Acad of Natl Sci Phila 140:240–272. doi:10.2307/4064927

Hershkovitz P (1990) Titis, new world monkeys of the genus *Callicebus* (Cebidae, Platyrrhini): a preliminary taxonomic review. Fieldiana Zool n.s.:1–109

Hughes CR, Queller DC (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. Mol Ecol 2:131–137. doi:10.1111/j.1365-294X.1993.tb00102.x

Jones AG, Small CM, Paczolt KA, Ratterman NL (2010) A practical guide to methods of parentage analysis. Mol Ecol Resour 10:6–30. doi:10.1111/j.1755-0998.2009.02778.x

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106. doi:10.1111/j.1365-294X.2007.03089.x

Kanthaswamy S, von Dollen A, Kurushima JD, Alminas O, Rogers J, Ferguson B, Lerche NW, Allen PC, Smith DG (2006) Microsatellite markers for standardized genetic management of captive colonies of rhesus macaques (*Macaca mulatta*). Am J Primatol 68:73–95. doi:10.1002/ajp.20207

Kanthaswamy S, Satkoski J, Kou A, Malladi V, Smith DG (2010) Detecting signatures of inter-regional and inter-specific hybridization among the Chinese rhesus macaque specific pathogen-free (SPF) population using single nucleotide polymorphic (SNP) markers. J Med Primatol 39:252–265

Kanthaswamy S, Ng J, Penedo MC, Ward T, Smith DG, Ha JC (2012) Population genetics of the Washington National Primate Research Center's (WaNPRC) captive pigtailed macaque (*Macaca nemestrina*) population. Am J Primatol 74:1017–1027. doi:10.1002/ajp.22055

Kinzey WG (1981) The Titi Monkeys, Genus *Callicebus*. In: Coimbra-Filho AF, Mittermeier RA (eds) Ecology and behavior of Neotropical Primates, vol 1. Academia Brasileira de Ciencias, Rio de Janeiro, pp 241–276

Lorenz R, Mason WA (1971) Establishment of a colony of Titi monkeys. International Zoo Yearbook 11:168–174. doi:10.1111/j.1748-1090.1971.tb01896.x

Marshall TC, Slate J, Kruuk LE, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655. doi:10.1046/j.1365-294x.1998.00374.x

Mason WA (1966) Social organization of the South Ameican monkey, *Callicebus moloch*: a preliminary report. Tulane stud zool 13:23–28

Mendoza SP, Mason WA (1986) Contrasting responses to intruders and to involuntary separation by monogamous and polygynous new world monkeys. Physiol Behav 38:795–801

Menescal LA, Goncalves EC, Silva A, Ferrari SF, Schneider MP (2009) Genetic diversity of red-bellied Titis (*Callicebus moloch*) from Eastern Amazonia based on microsatellite markers. Biochem Genet 47:235–240. doi:10.1007/s10528-008-9220-4

Moore CM, Leland MM, Brzyski RG, McKeand J, Witte SM, Rogers J (1998) A baboon (*Papio hamadryas*) with an isochromosome for the long arm of the X. Cytogenet Cell Genet 82:80–82

Muniz L, Vigilant L (2008) Permanent genetic resources: isolation and characterization of microsatellite markers in the white-faced capuchin monkey (*Cebus capucinus*) and cross-species amplification in other new world monkeys. Mol Ecol Resour 8:402–405. doi:10.1111/j.1471-8286.2007.01971.x

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rogers J, Bergstrom M, Garcia Rt, Kaplan J, Arya A, Novakowski L, Johnson Z, Vinson A, Shelledy W (2005) A panel of 20 highly variable microsatellite polymorphisms in rhesus macaques (*Macaca mulatta*) selected for pedigree or population genetic analysis. Am J Primatol 67:377–383. doi:10.1002/ajp.20192

Smith KL, Alberts SC, Bayes MK, Bruford MW, Altmann J, Ober C (2000) Cross-species amplification, non-invasive genotyping, and non-Mendelian inheritance of human STRPs in Savannah baboons. Am J Primatol 51:219–227. doi:10.1002/1098-2345(200008)51:4<219:AID-AJP1>3.0.CO;2-G

Stanyon R, Bonvicino CR, Svartman M, Seuanez HN (2003) Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number ($2n = 16$) known in primates. Chromosoma 112:201–206. doi:10.1007/s00412-003-0261-5

Valeggia CR, Mendoza SP, Fernandez-Duque E, Mason WA, Lasley B (1999) Reproductive biology of female titi monkeys (*Callicebus moloch*) in captivity. Am J Primatol 47:183–195. doi:10.1002/(SICI)1098-2345(1999)47:3<183:AID-AJP1>3.0.CO;2-J

van Roosmalen MGM, van Roosmalen T, Mittermeier RA (2002) A taxonomic review of the titi monkeys, genus *Callicebus Thomas*, 1903, with the description of two new species, *Callicebus bernhardi* and *Callicebus stephennashi*, from Brazilian Amazonia. Neotrop Primates 10:1–52

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. doi:10.1111/j.1471-8286.2004.00684.x

Walling CA, Pemberton JM, Hadfield JD, Kruuk LE (2010) Comparing parentage inference software: reanalysis of a red deer pedigree. Mol Ecol 19:1914–1928. doi:10.1111/j.1365-294X.2010.04604.x

Wright S (1978) Evolution and the genetics of populations : a treatise in four volumes. University of Chicago Press, Chicago, London

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16. doi:10.1046/j.0962-1083.2001.01418.x