

Polymorphism of visual pigment genes in the mურიკი (Primates, Atelidae)

M. G. TALEBI,* T. R. POPE,† E. R. VOGEL,‡ M. NEITZ§ and N. J. DOMINY‡

*Department of Biological Anthropology, University of Cambridge, Downing Street, Cambridge CB2 3DZ, UK, †Department of Biological Anthropology and Anatomy, Box 90383, Biological Sciences Building, Duke University, Durham, North Carolina 27708, USA, ‡Department of Anthropology, 1156 High Street, University of California, Santa Cruz, California 95064, USA, §Medical College of Wisconsin, Department of Cell Biology, Neurobiology and Anatomy, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA

Abstract

Colour vision varies within the family Atelidae (Primates, Platyrrhini), which consists of four genera with the following cladistic relationship: {*Alouatta* [*Ateles* (*Lagothrix* and *Brachyteles*)]}. Spider monkeys (*Ateles*) and woolly monkeys (*Lagothrix*) are characteristic of platyrrhine monkeys in possessing a colour vision polymorphism. The polymorphism results from allelic variation of the single-locus middle-to-long wavelength (M/L) cone opsin gene on the X-chromosome. The presence in the population of alleles coding for different M/L photopigments results in a variety of colour vision phenotypes. Such a polymorphism is absent in howling monkeys (*Alouatta*), which, alone among platyrrhines, acquired uniform trichromatic vision similar to that of Old World monkeys, apes, and humans through opsin gene duplication. Dietary and morphological similarities between howling monkeys and mურიკის (*Brachyteles*) raise the possibility that the two genera share a similar form of colour vision, uniform trichromacy. Yet parsimony predicts that the colour vision of *Brachyteles* will resemble the polymorphism present in *Lagothrix* and *Ateles*. Here we test this assumption. We obtained DNA from the blood or faeces of 18 mურიკის and sequenced exons 3 and 5 of the M/L opsin gene. Our results affirm the existence of a single M/L cone opsin gene in the genus *Brachyteles*. We detected three alleles with predicted λ_{\max} values of 530, 550, and 562 nm. Two females were heterozygous and are thus predicted to have different types of M/L cone pigment. We discuss the implication of this result towards understanding the evolutionary ecology of trichromatic vision.

Keywords: *Brachyteles*, opsin, primates, trichromatic vision, woolly spider monkey

Received 7 August 2005; revision accepted 31 October 2005

Introduction

The mურიკი, or woolly spider monkey, is an endangered primate endemic to the Atlantic Forest of Brazil. Fewer than 700 individuals survive in 15 remnant, geographically isolated populations (Mittermeier *et al.* 1987; Martuscelli *et al.* 1994). On the basis of recent morphological assessments, two former subspecies of mურიკი, *Brachyteles arachnoides arachnoides* and *B. arachnoides hypoxanthus*, are now recognized as species, *Brachyteles arachnoides* (southern mურიკი) and *Brachyteles hypoxanthus* (northern mურიკი).

The genus is placed in the family Atelidae, along with howling monkeys (*Alouatta*), spider monkeys (*Ateles*), and woolly monkeys (*Lagothrix*).

Cladistic relationships within the Atelidae have been debated. Morphological studies provide conflicting results on the sister-group relationships among the four genera. Rosenberger (1984) used an unspecified number of morphological characters to group *Ateles* and *Brachyteles* closest to each other, followed by *Lagothrix* and *Alouatta*. Ford (1986) used 205 dental and postcranial characters to group *Ateles*, *Brachyteles*, and *Lagothrix* into an unresolved tritomy, with *Alouatta* as sister to this clade. Kay (1990) used 117 dental characteristics to produce two sister subclades, one grouping *Alouatta* with *Brachyteles* and the other

Correspondence: Nathaniel J. Dominy, Fax: 1-831-459-5900; E-mail: njdominy@ucsc.edu

grouping *Ateles* with *Lagothrix*. In contrast to these results, studies using DNA sequences have yielded congruent sister-group relationships: *Brachyteles* sister groups with *Lagothrix* followed by *Ateles* and the basal *Alouatta* (Harada *et al.* 1995; Schneider *et al.* 1996; Porter *et al.* 1997; Horovitz *et al.* 1998; Hugot 1998; Meireles *et al.* 1999; Cortès-Ortiz *et al.* 2003; Fig. 1).

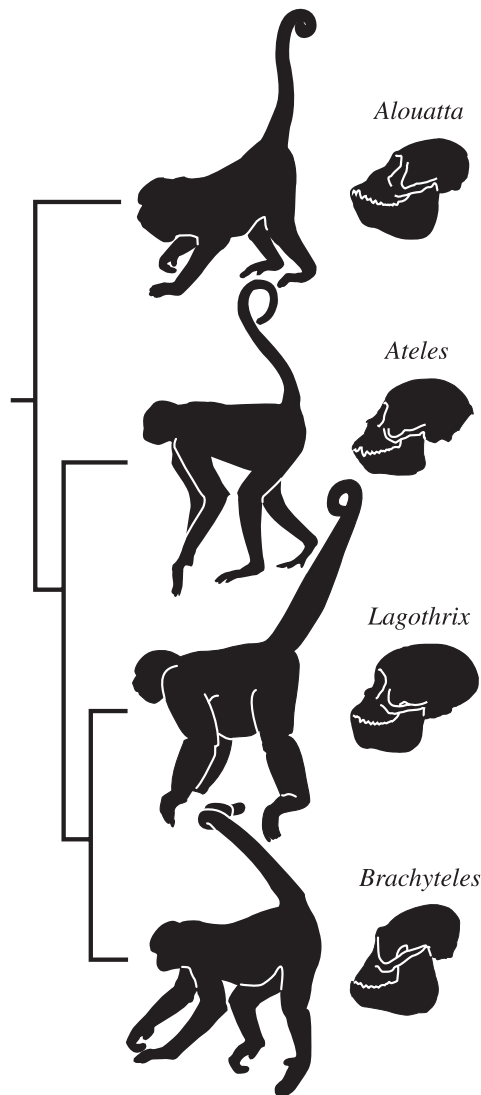


Fig. 1 Phyletic relationships of the Atelidae. The cranial and postcranial morphologies of each genus are depicted. The skulls of *Ateles* and *Lagothrix* are similar: the braincase is globular, the mandible is relatively shallow, and the face is relatively orthogonal. The skulls of *Alouatta* and *Brachyteles* are distinguished by their relatively small cranial capacity and lack of cranial flexion. Compared to *Ateles* and *Lagothrix*, the faces of *Alouatta* and *Brachyteles* are more prognathic and the mandibular rami are expanded and deep (Zingesser 1973). The traits that distinguish *Ateles* and *Lagothrix* from *Alouatta* and *Brachyteles* have been associated with adaptations to frugivory and folivory, respectively (Anthony & Kay 1993). Drawn by C. Underwood.

The foraging ecology of the miquis

The *Alouatta-Brachyteles* subclade of Kay (1990) reflects dental similarities between the two genera; both possess relatively small lower incisors and molars with well-developed shearing crests (Zingesser 1973; Anthony & Kay 1993). The dentition of *Brachyteles*, together with its gnathic and digestive system characteristics, has been viewed as an adaptation to a folivorous diet (Hill 1962; Zingesser 1973; Anthony & Kay 1993). Yet miquis travel by means of suspensory locomotion, a trait that has been associated with specialized frugivory in the closely related *Ateles* and *Lagothrix* (Cant 1986). Behavioural observations of miquis confirm that young leaves and fruits are important food items, but they are consumed to different extents in different populations (Milton 1984; Strier 1991; de Carvalho *et al.* 2004; Talebi *et al.* 2005). Importantly, *Brachyteles* tends to rely on young leaves more than *Lagothrix* and *Ateles* (Table 1). This tendency, coupled with the morphological traits that miquis share with *Alouatta*, raises questions regarding the evolution of their colour vision.

Colour vision in the Atelidae

Variation exists in the photopigments and colour vision of atelid monkeys. Like most platyrrhine monkeys, *Ateles* and *Lagothrix* possess a colour vision polymorphism (Jacobs & Deegan 2001). The polymorphism results from allelic variation of the single-locus middle-to-long wavelength (M/L) cone opsin gene on the X-chromosome (Mollon *et al.* 1984; Jacobs *et al.* 1993). The presence in the population of two alleles coding for different M/L photopigments results in a variety of colour vision phenotypes. Females that are heterozygous for the M/L opsin gene possess trichromatic vision. All other individuals possess dichromatic vision. Such a colour vision polymorphism is absent among howling monkeys. Alone among known platyrrhines, *Alouatta* acquired uniform trichromatic vision similar to that of Old World monkeys and apes (including humans) through opsin gene duplication (Jacobs *et al.* 1996; Boissinot *et al.* 1997; Hunt *et al.* 1998; Kainz *et al.* 1998).

The selective factors that favoured the evolution of uniform trichromatic vision have been debated. Authors have stressed the importance of detecting food targets against a background of mature foliage, but there has been disagreement concerning the importance of particular foods. Some authors have argued that ripe fruits are critical, whereas others have emphasized the importance of detecting tender young leaves (SurrIDGE *et al.* 2003). Understanding the colour vision of miquis may help refine these hypotheses. Ecological and morphological similarities between *Brachyteles* and *Alouatta* raise the possibility that the two genera share a similar form of colour vision, uniform trichromacy. Yet parsimony predicts that the colour vision of miquis

Table 1 Summary of dietary behaviour in Brazilian howling monkeys and other atelid monkeys

	Feeding records (%)				Reference
	Fruit	Leaves	Flowers	Other	
Howling monkeys					
<i>Alouatta belzebul</i>	59.0	13.3	27.6	0.0	Bonvicino (1989)
	43.1	45.6	10.8	0.5	Pinto <i>et al.</i> (2003)
<i>A. caraya</i>	28.9	60.9	2.7	0.0	Bicca-Marques & Calegari-Marques (1994)
<i>A. fusca</i>	13.9	71.5	8.6	6.0	Mendes (1989)
	5.0	73.0	12.0	0.0	Chiarello (1994)
	12.0	72.0	10.0	0.0	Limeira (1997)
<i>A. seniculus</i>	47.3	45.5	1.5	5.7	Queiroz (1995)
Mean = 29.9	54.4	10.5	1.7		
Spider monkeys					
<i>Ateles belzebul</i>	83.0	7.0	< 0.1	10.0	Klein & Klein (1977)
	88.5	8.3	0.0	3.2	Nunes (1998)
	72.0	12.0	5.0	11.0	Stevenson <i>et al.</i> (2000)
<i>A. geoffroyi</i>	77.7	11.1	9.8	1.3	Chapman (1988)
<i>A. paniscus</i>	82.9	7.9	6.4	2.8	van Roosmalen (1985)
	85.4	9.5	2.5	2.6	Simmen & Sabatier (1996)
Mean = 81.6	9.3	4.7	5.2		
Woolly monkeys					
<i>Lagothrix lagothrica</i>	74.5	16.2	0.5	8.8	Peres (1994)
	55.0	16.0	1.0	29.0	Stevenson <i>et al.</i> (2000)
	83.2	11.4	0.1	5.3	Defler & Defler (1996)
Mean = 70.9	14.5	0.5	14.4		
Muriquis					
<i>Brachyteles arachnoides</i>	21.0	67.0	12.0	0.0	Milton (1984)
	59.2	33.2	4.1	3.6	de Carvalho <i>et al.</i> (2004)
	73.2	21.7	1.7	3.1	Talebi <i>et al.</i> (2005)
<i>B. hypoxanthus</i>	32.0	51.0	11.0	6.0	Strier (1991)
	32.9	60.0	4.5	2.6	Rimoli & Ades (1997)
Mean = 43.7	46.6	6.7	3.1		

will resemble that of *Lagothrix* and *Ateles*; that is, muriquis are predicted to possess a colour vision polymorphism. Here we evaluate this prediction.

Methods

Study populations and sample collection

Four populations of *Brachyteles* were sampled. They represent the extremes in geographic range and visible phenotype for the genus (Aguirre 1971). At Fazenda Esmeralda, eastern Minas Gerais, Brazil, Lemos de Sá & Glander (1993) captured and anaesthetized five individuals of *Brachyteles hypoxanthus*. At Fazenda Barreiro Rico, eastern São Paulo State, Lemos de Sá & Glander (1993) captured and anaesthetized two individuals of *B. arachnoides*. One of us, T.R.P., collected blood from the anaesthetized animals. The blood was separated by centrifugation and frozen in liquid nitrogen. The samples remained frozen until analysis at Duke University (Pope 1998). At Carlos Botelho State Park,

eastern São Paulo State, one of us, M.G.T., collected 5–10 g of faeces from seven free-ranging individuals of *B. arachnoides* (Talebi 2005). M.G.T. collected three additional faecal samples of *B. arachnoides* from the semicaptive environment of Curitiba Zoological Park, Curitiba, Paraná, Brazil. The samples were stored in 5–10 mL 92% ethanol in the field at 4 °C for up to 3 months and transported to the University of California, Santa Cruz, under CITES permit no. 02001.006912/2002. The Institutional Animal Care and Use Committee of Duke University and the Chancellor's Animal Research Committee of UC Santa Cruz approved the sampling protocols. Lastly, a sample of *B. arachnoides* genomic DNA was obtained from the laboratory of M. Goodman, Wayne State University (Meireles *et al.* 1999).

Genomic DNA was obtained from blood or faeces using a phenol–chloroform extraction or QIAamp DNA Stool Mini Kit per manufacturer instructions (QIAGEN). Faecal samples were placed in a fume hood overnight to evaporate the ethanol prior to DNA extraction (SurrIDGE *et al.* 2002). Amplification and sequencing of the M/L

cone opsin genes was performed at the Medical College of Wisconsin.

Amplification and sequencing of the M/L cone opsin genes

The genes encoding the opsins of M/L photopigments consist of six exons. Of these, exons 2 to 5 specify the membrane spanning regions of the opsin (Nathans *et al.* 1986). Peak absorption spectra (λ_{\max}) of the M/L pigment can be predicted from the amino acid composition of three sites: site 180 encoded on exon 3 and sites 277 and 285 encoded on exon 5 (Neitz & Neitz 1998). Amino acid changes from Ser to Ala at site 180 (denoted Ser180Ala), Tyr277Phe, and Thr285Ala shift the λ_{\max} values by -7 , -8 , and -15 nm, respectively, although estimates of spectral positioning may vary somewhat depending on the measurement methodology employed (Neitz *et al.* 1991; Merbs & Nathans 1992; Asenjo *et al.* 1994; Yokoyama & Radlwimmer 2001).

Polymerase chain reaction (PCR) was used to amplify exons 3 and 5 of the M/L opsin gene (Neitz & Neitz 1995). Exons 3 and 5 were amplified separately using the AmpliTaq Gold PCR kit per manufacturer instructions (ABI). Each 50- μ L reaction contained a final concentration of 1 \times Buffer, 1 mM MgCl₂, 600 nM of each primer, 50 μ M each of dATP, dCTP, dTTP, and dGTP, and 1.25 U of AmpliTaq Gold (ABI). Exon 3 was amplified using the forward primer 5'-CGTCTGCTGCTCTCCCCTA, and the reverse primer 5'-TTGCCTCAGGGTCACAGAGT. One or two rounds of PCR were done in an ABI 2700 thermal cycler using cycling conditions of 94 °C for 9 min for one cycle, followed by 37 cycles of 94 °C for 45 s, 61 °C for 45 s, 72 °C for 45 s. Reactions were then incubated at 72 °C for 7 min and stored at 4 °C. Exon 5 was amplified using the forward primer 5'-GTGGCAAAGCAGCAGAAAG and the reverse primer 5'-CTGCCGGTTCATAAAGACATAG; or using the forward primer 5'-TCCACCCCCCGACTCACTATCC and the reverse primer 5'-ACGGTATTTTGAGTGGGATCTGCT. The thermal cycling conditions were the same as those used to amplify exon 3 except that the 61 °C step was done at 59 °C. PCR products were directly sequenced using the same primers that were used to amplify the exons and the BigDye Terminator 3.1 kit (ABI). Sequencing reactions were analysed on an ABI 3100 Avante.

Results

Variation in muriqui M/L opsin genes

A total of 18 muriquis served as subjects for this analysis. Table 2 shows the variation in the residues detected at positions 180, 277, and 285 for these animals. At position 180, 12 animals had Ser, 2 had Ala, and 4 could not be determined because exon 3 failed to amplify. At position

277, 14 animals had Tyr and 2 had Phe. The remaining two animals, MTG3 and MTG6, had both residues. At position 285, 16 animals had Thr and 2 animals had Ala. Variation at these positions predicts corresponding pigment variation. Those predictions are also listed in Table 2. Two female subjects, MTG3 and MTG6, were heterozygous and are thus predicted to have different types of M/L cone pigment. Table 3 summarizes the distribution of M/L opsin alleles among ateline X-chromosomes.

Discussion

Here we report the existence of a single M/L cone opsin gene in the genus *Brachyteles*. The result is consistent with genetic examinations of *Ateles* and *Lagothrix* (Hagstrom *et al.* 1993; Boissinot *et al.* 1997; Hiramatsu *et al.* 2005). We detected varying residues at positions 180, 277, and 285 of the M/L cone opsin gene that are predicted to yield pigments with λ_{\max} values of 530, 550, and 562 nm. Two of 11 female subjects were heterozygous for the M/L cone opsin gene and are predicted to possess allelic trichromatic vision. The spectral separation of the 550 and 562 nm pigments is equivalent to human anomalous trichromacy (Neitz *et al.* 1991), and is consistent with the performance of female *Ateles* in colour-discrimination tasks (Grether 1939; Blakeslee & Jacobs 1982).

The spectral positions of the M/L opsin pigments in *Ateles* and *Lagothrix* has been determined with electroretinogram (ERG) flicker photometry. In contrast to other platyrrhine species, which possess three types of M/L pigment, evidence exists for only two pigment types in *Ateles* and *Lagothrix* (Jacobs & Deegan 2001). Among 47 spider monkeys, the pigments had a λ_{\max} value of 550 and 562 nm; among 9 woolly monkeys, the pigments had a λ_{\max} value of 548 and 563 nm. These differences in spectral tuning could reflect opsin sequence variation at one of the tuning sites known to yield small shifts in the spectral positioning of primate M/L cone photopigments (Shyue *et al.* 1998). The absence of a third pigment type in *Ateles* and *Lagothrix* is a potentially important distinction between atelines and other platyrrhines. Jacobs & Deegan (2001) suggested that a third pigment likely exists, but that the allele specifying the pigment is rare in ateline populations. Recently, Riba-Hernández *et al.* (2004) reported unpublished sequence data for the 530 nm allele in a population of *Ateles*. Here we confirm the existence of a 530 nm allele in *Brachyteles*. Although sample sizes are relatively small, the infrequency (1.3%) of the allele across ateline X-chromosomes indicates that it is in fact rare (Table 3).

Under the Hardy-Weinberg equilibrium, the existence of two alleles in population should result in a 50% frequency of heterozygous females, whereas the existence of three alleles should result in a 67% frequency. The rarity of the 530 nm allele among atelines has a major practical

Table 2 Variation in residues detected at positions 180, 277, and 285 of the M/L opsin gene and the predicted pigment absorbances for *Brachyteles arachnoides* and *Brachyteles hypoxanthus**

monkey ID	Sex	Exon 3	Exon 5		λ_{\max} (nm) genotype prediction
		Residue at position 180	Residue at position 277	Residue at position 285	
<i>B. arachnoides</i>					
Carlos Botelho					
MGT1	Male	Ser	Tyr	Thr	562
MGT2	Female	Ser	Tyr	Thr	562
MGT3	Female	Ser	Tyr/Phe	Thr	550/562
MGT5	Female	Ser	Tyr	Thr	562
MGT6	Female	Ser	Tyr/Phe	Thr	550/562
MGT8	Male	Ser	Tyr	Thr	562
GIGI	Female	Ser	Tyr	Thr	562
Curitiba Zoological Park					
MGT7	Female	Ser	Tyr	Thr	562
MGT9	Female	Ser	Tyr	Thr	562
MGT10	Female	Ser	Tyr	Thr	562
Fazenda Barreiro Rico					
TRP10	Female	—	Tyr	Thr	562†
TRP11	Male	Ser	Tyr	Thr	562
Goodman Laboratory					
No identification	—	—	Phe	Thr	550
<i>B. hypoxanthus</i>					
Fazenda Esmeralda					
TRP2	Male	Ala	Phe	Ala	530
TRP3	Male	Ser	Tyr	Thr	562
TRP5	Female	—	Tyr	Thr	562†
TRP6	Male	Ala	Phe	Ala	530
TRP7	Female	—	Tyr	Thr	562†

*GenBank (www.ncbi.nlm.nih.gov/GenBank/) Accession numbers for the nucleotide sequences generated for this paper are DQ218051–DQ218055.

†The failed amplification of exon 3 leaves the spectral tuning of this pigment in some doubt. The detection of Ala at position 180 would yield a photopigment with a predicted λ_{\max} of *c.* 555 nm. Although the pigment exists in the family Cebidae, which includes squirrel monkeys, capuchin monkeys, and owl monkeys, it has yet to be found in an atelid monkey. Accordingly, we infer that these three animals possess an M/L pigment with λ_{\max} of 562 nm, although such an assumption should be treated with caution.

Genus	N individuals	X-chromosomes	M/L alleles (nm)			Reference
			~530	~550	~562	
<i>Ateles</i>	47	76	0	26	50	Jacobs & Deegan (2001)
	20	37	0	15	22	Hiramatsu <i>et al.</i> (2005)
<i>Brachyteles</i>	18	27	2	2	23	This study*
<i>Lagothrix</i>	9	9	0	5	4	Jacobs & Deegan (2001)
Totals	94	149	2	48	99	

*The animal of unknown sex presented in Table 2 is not included in this summary.

Table 3 Distribution of M/L opsin alleles among ateline X-chromosomes

implication: the number of heterozygous females (and, hence, the number of trichromats in the population) will be relatively low compared to species that maintain three allelic versions of the gene. This difference has been

observed: 18% (2 of 11) of the female *Brachyteles* examined here and 52% (24 of 46) of female *Ateles* possess, or are predicted to possess, two M/L pigments (Jacobs & Deegan 2001; Hiramatsu *et al.* 2005). In contrast, *c.* 63% of female

squirrel monkeys (*Saimiri*) have been found to possess two M/L pigments (Jacobs *et al.* 1993). Even more strikingly, the proportion of heterozygous females can reach 83% among titi monkeys (*Callicebus moloch*), a species that maintains five alleles (Jacobs & Deegan 2005).

This result demonstrates the difficulty of linking the photopigments of primates, particularly those of atelid monkeys, with aspects of their foraging ecology. Both *Ateles* and *Lagothrix* are specialized frugivores, with fruits comprising 55–89% of their annual diet (Table 1). Colour vision would appear to be an intuitive sensory modality for guiding fruit selection. Accordingly, fruits have figured prominently in discussions of primate trichromacy. It has long been an article of faith that the detection and discrimination of ripe fruit against a foliage background favoured the evolution of allelic and uniform trichromatic vision (Smith *et al.* 2003; Surridge *et al.* 2003). On the surface, this hypothesis might suggest that *Ateles* and *Lagothrix* would be best served by possessing uniform trichromatic vision, rather than *Alouatta*, the atelid genus with the lowest annual consumption of fruit (Table 1). The fruit-selection hypothesis also predicts that the 530 nm allele should exist at a higher frequency in the population, as a wide spectral separation between M/L pigments is advantageous for discriminating fruit (Osorio & Vorobyev 1996; Regan *et al.* 1998, 2001; Sumner & Mollon 2000; Riba-Hernández *et al.* 2004; Rowe & Jacobs 2004). The fitness of the 530 nm allele among atelines might therefore depend on a balance between the demands of trichromacy, which favours widely separated pigments and equal allele frequencies, and an advantage for long-wavelength pigments in dichromat eyes (Osorio *et al.* 2004). Although it is estimated that dichromatic phenotypes of *Ateles* can detect 94–97% of fruit species detectable to trichromatic phenotypes (Riba-Hernández *et al.* 2004), any specific advantages associated with dichromatic vision have remained theoretical or confined to psychophysical experiments (Morgan *et al.* 1992; Williams *et al.* 1993; Osorio *et al.* 1998; Dominy *et al.* 2003; Yokoyama & Takenaka 2005).

Compared to other platyrrhine genera, the colour vision polymorphism of *Ateles*, *Lagothrix*, and *Brachyteles* appears poorly suited for detecting fruits that reflect long wavelengths. But neither is the polymorphism well suited for detecting tender young leaves. Behavioural observations of catarrhine primates and *Alouatta* have shown that leaf colour is an important cue during periods of folivory (Lucas *et al.* 1998, 2003; Dominy & Lucas 2001, 2004). It was hypothesized from these studies that a seasonal reliance on young leaves was the selective pressure favouring the evolution of uniform trichromatic vision. The absence of uniform trichromatic vision in *Brachyteles*, a seasonal folivore possessing a suite of morphological characteristics associated with leaf eating, obscures the adaptive importance of trichromatic vision for any particular food item (cf. Cropp

et al. 2002; Surridge *et al.* 2005). Instead, trichromatic colour vision may allow for a range of visual advantages that could potentially serve to maintain the adaptation.

Acknowledgements

We are grateful for the intellectual and material contributions of A. Almquist, A. Bastos, D. Conklyn, M. Goodman, S. Roy-Choudari, K. B. Strier, P. C. Lee, W.-H. Li, P. W. Lucas, B. L. W. Miller, V. Pessa, D. M. Snodderly, P. P. Soares, J. N. Thompson, R. H. Tuttle, D. E. Wildman, S. Yi, and the UC-Santa Cruz Molecular Ecology and Evolutionary Genetics Facility. We received permission to conduct this study from the Forestry Institute of Sao Paulo State, CNPq (CITES permit no. 02001.006912/2002), and the US Fish and Wildlife Service. The Institutional Animal Care and Use Committee of Duke University and the Chancellor's Animal Research Committee of UC-Santa Cruz approved our sampling protocols. We received funding from Conservation International USA, the Leakey Foundation, the Margot Marsh Biodiversity Foundation, a Ruth L. Kirchenstein NIGMS National Service Research Award, and the World Wildlife Fund-Brazil.

References

- Aguirre AC (1971) *O mono Brachyteles arachnoides (E. Geoffroy). situação atual da espécie no Brasil*. Academia Brasileira de Ciências, Rio de Janeiro, Brazil.
- Anthony MRL, Kay RF (1993) Tooth form and diet in ateline and alouattine primates: reflections on the comparative method. *American Journal of Science*, **293A**, 356–382.
- Asenjo AB, Rim J, Oprian DD (1994) Molecular determinants of human red/green color discrimination. *Neuron*, **12**, 1131–1138.
- Bicca-Marques JC, Calegari-Marques C (1994) Activity budget and diet of *Alouatta caraya*: an age-sex analysis. *Folia Primatologica*, **63**, 216–220.
- Blakeslee B, Jacobs GH (1982) Color vision in the spider monkey (*Ateles*). *Folia Primatologica*, **38**, 86–98.
- Boissinot S, Zhou Y-H, Qui L *et al.* (1997) Origin and molecular evolution of the X-linked duplicate color vision genes in howler monkeys. *Zoological Studies*, **36**, 360–369.
- Bonvicino CR (1989) Ecologia e comportamento de *Alouatta belzebul* (Primates: Cebidae) na Mata Atlântica. *Revista Nordestina de Biologia*, **6**, 149–179.
- Cant JGH (1986) Locomotion and feeding postures of spider monkeys and howling monkeys: field study and evolutionary interpretation. *Folia Primatologica*, **46**, 1–14.
- de Carvalho O Jr, Ferrari SF, Strier KB (2004) Diet of a miquiri group (*Brachyteles arachnoides*) in a continuous primary forest. *Primates*, **45**, 201–204.
- Chapman CA (1988) Patterns of foraging and range use by three species of Neotropical primates. *Primates*, **29**, 177–194.
- Chiarello AG (1994) Diet of the brown howler monkey *Alouatta fusca* in a semi-deciduous forest fragment of southeastern Brazil. *Primates*, **35**, 25–34.
- Cortès-Ortiz L, Bermingham E, Rico C *et al.* (2003) Molecular systematics and biogeography of the Neotropical monkey genus *Alouatta*. *Molecular Phylogenetics and Evolution*, **26**, 64–81.
- Cropp S, Boinski S, Li W-H (2002) Allelic variation in the squirrel monkey X-linked color vision gene: biogeographical and behavioral correlates. *Journal of Molecular Evolution*, **54**, 734–745.

- Defler TR, Defler SB (1996) Diet of a group of *Lagothrix lagothrica* in southeastern Colombia. *International Journal of Primatology*, **17**, 161–190.
- Dominy NJ, Lucas PW (2001) Ecological importance of trichromatic vision to primates. *Nature*, **410**, 363–366.
- Dominy NJ, Lucas PW (2004) Significance of color, calories, and climate to the visual ecology of catarrhines. *American Journal of Primatology*, **62**, 189–207.
- Dominy NJ, Svenning J-C, Li W-H (2003) Historical contingency in the evolution of primate color vision. *Journal of Human Evolution*, **44**, 25–45.
- Ford SM (1986) Systematics of the New World monkeys. In: *Comparative Primate Biology*, Vol. 1: Systematics, Evolution and Anatomy (eds Swindler DR Erwins J), pp. 73–135. A.R. Liss, New York.
- Grether WF (1939) Color vision and color blindness in monkeys. *Comparative Psychology Monographs*, **15**, 1–38.
- Hagstrom SA, Teunissen DL, Neitz M *et al.* (1993) Cone pigment genes from two species of New World monkey. *Investigative Ophthalmology and Visual Science*, **34**, 749.
- Harada ML, Schneider H, Schneider MPC *et al.* (1995) DNA evidence on the phylogenetic systematics of the New World monkeys: support for the sister grouping of *Cebus* and *Saimiri* from two unlinked nuclear genes. *Molecular Phylogenetics and Evolution*, **4**, 448–456.
- Hill WC (1962) *Primates: Comparative Anatomy and Taxonomy*, Vol. 5. Edinburgh University Press, Edinburgh, UK.
- Hiramatsu C, Tsutsui T, Matsumoto Y *et al.* (2005) Color vision polymorphism in wild capuchins (*Cebus capucinus*) and spider monkeys (*Ateles geoffroyi*) in Costa Rica. *American Journal of Primatology*, in press.
- Horovitz I, Zardoya R, Meyer A (1998) Platyrrhine systematics: a simultaneous analysis of molecular and morphological data. *American Journal of Physical Anthropology*, **106**, 261–281.
- Hugot J-P (1998) Phylogeny of Neotropical monkeys: the interplay of morphological, molecular, and parasitological data. *Molecular Phylogenetics and Evolution*, **9**, 408–413.
- Hunt DM, Dulai KS, Cowing JA *et al.* (1998) Molecular evolution of trichromacy in primates. *Vision Research*, **38**, 3299–3306.
- Jacobs GH, Deegan JF II (2001) Photopigments and colour vision in New World monkeys from the family Atelidae. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 695–702.
- Jacobs GH, Deegan JF II (2005) Polymorphic New World monkeys with more than three M/L cone types. *Journal of the Optical Society of America A*, **22**, 2072–2080.
- Jacobs GH, Neitz M, Deegan JF, Neitz J (1996) Trichromatic colour vision in New World monkeys. *Nature*, **382**, 156–158.
- Jacobs GH, Neitz J, Neitz M (1993) Genetic basis of polymorphism in the color vision of platyrrhine monkeys. *Vision Research*, **33**, 269–274.
- Kainz PM, Neitz J, Neitz M (1998) Recent evolution of uniform trichromacy in a New World monkey. *Vision Research*, **38**, 3315–3320.
- Kay RF (1990) The phyletic relationships of extant and fossil Pitheciinae (Platyrrhini, Anthropoidea). *Journal of Human Evolution*, **19**, 175–208.
- Klein LL, Klein DB (1977) Feeding behaviour of the Colombian spider monkey. In: *Primate Ecology: Studies of Feeding and Ranging Behaviour in Lemurs, Monkeys and Apes* (ed. Clutton-Brock TH), pp. 152–179. Academic Press, London.
- Lemos de Sá RM, Glander KE (1993) Capture techniques and morphometrics for the woolly spider monkey, or muriqui (*Brachyteles arachnoides*, E. Geoffroy, 1806). *American Journal of Primatology*, **29**, 145–153.
- Limeira VLA (1997) Behavioral ecology of *Alouatta fusca clamitans* in a degraded Atlantic Forest fragment in Rio de Janeiro. *Neotropical Primates*, **5**, 116–117.
- Lucas PW, Darvell BW, Lee PK *et al.* (1998) Colour cues for leaf food selection by long-tailed macaques with a new suggestion for the evolution of trichromatic colour vision. *Folia Primatologica*, **69**, 139–152.
- Lucas PW, Dominy NJ, Riba-Hernandez PR *et al.* (2003) Evolution and function of routine trichromatic vision in primates. *Evolution*, **57**, 2636–2643.
- Martuscelli P, Petroni LM, Olmos F (1994) Fourteen new localities for the muriqui *Brachyteles arachnoides*. *Neotropical Primates*, **2**, 12–15.
- Meireles CM, Czelusniak J, Schneider MPC *et al.* (1999) Molecular phylogeny of Ateline New World monkeys (Platyrrhini, Atelinae) based on γ -globin gene sequences: evidence that *Brachyteles* is the sister group of *Lagothrix*. *Molecular Phylogenetics and Evolution*, **12**, 10–30.
- Mendes SL (1989) Estudo ecologico de *Alouatta fusca* (Primates: Cebidae) na estacao biologica de caratinga, MG. *Revista Nordestina de Biologia*, **6**, 71–104.
- Merbs SL, Nathans J (1992) Absorption spectra of human cone pigments. *Nature*, **356**, 433–435.
- Milton K (1984) Habitat, diet, and activity patterns of free-ranging woolly spider monkeys (*Brachyteles arachnoides* E. Geoffroy, 1806). *International Journal of Primatology*, **5**, 491–514.
- Mittermeier RA, Valle CMC, Alves MC *et al.* (1987) Current distribution of the muriqui in the Atlantic Forest region of eastern Brazil. *Primate Conservation*, **8**, 143–149.
- Mollon JD, Bowmaker JK, Jacobs GH (1984) Variations of colour vision in a New World primate can be explained by polymorphism of retinal photopigments. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **222**, 373–399.
- Morgan MJ, Adam A, Mollon JD (1992) Dichromats detect colour-camouflaged objects that are not detected by trichromats. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **248**, 291–295.
- Nathans J, Thomas D, Hogness DS (1986) Molecular genetics of inherited variation in human color vision. *Science*, **232**, 203–222.
- Neitz M, Neitz J (1995) Numbers and ratios of visual pigment genes for normal red-green color vision. *Science*, **267**, 1013–1016.
- Neitz M, Neitz J (1998) Molecular genetics and the biological basis of color vision. In: *Color Vision: Perspectives from Different Disciplines* (eds Backhaus WGK Kliegl R Werner JS), pp. 101–119. Walter de Gruyter, Berlin.
- Neitz M, Neitz J, Jacobs GH (1991) Spectral tuning of pigments underlying red-green color vision. *Science*, **252**, 972–974.
- Nunes A (1998) Diet and feeding ecology of *Ateles belzebuth* at Maraca Ecological Station, Roraima, Brazil. *Folia Primatologica*, **69**, 61–76.
- Osorio D, Ruderman DL, Cronin TW (1998) Estimation of errors in luminance signals encoded by primate retina resulting from sampling of natural images with red and green cones. *Journal of the Optical Society of America A*, **15**, 16–22.
- Osorio D, Smith AC, Vorobyev M, Buchanan-Smith HM (2004) Detection of fruit and the selection of primate visual pigments for color vision. *American Naturalist*, **164**, 696–708.
- Osorio D, Vorobyev M (1996) Colour vision as an adaptation to frugivory in primates. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **263**, 593–599.

- Peres CA (1994) Diet and feeding ecology of gray woolly monkeys (*Lagothrix lagotricha cana*) in Central Amazonia: comparisons with other atelines. *International Journal of Primatology*, **15**, 333–372.
- Pinto ACB, Azevedo-Ramos C, de Carvalho O Jr (2003) Activity patterns and diet of the howler monkey *Alouatta belzebul* in selectively-logged and unlogged forest in eastern Amazonia. *Animal Biodiversity and Conservation*, **26**, 39–49.
- Pope TR (1998) Genetic variation in remnant populations of woolly spider monkeys (*Brachyteles arachnoides*). *International Journal of Primatology*, **19**, 95–109.
- Porter CA, Page SL, Czelusniak J *et al.* (1997) Phylogeny and evolution of selected primates as determined by sequences of the ϵ -globin locus and 5' flanking regions. *International Journal of Primatology*, **18**, 261–295.
- Queiroz HL (1995) *Preguiás e guaribas – os mamíferos folívoros arborícolas do Mamirauá*. Sociedade Civil Mamirauá/MCT-CNPq, Brasília.
- Regan BC, Julliot C, Simmen B *et al.* (1998) Frugivory and colour vision in *Alouatta seniculus*, a trichromatic platyrrhine monkey. *Vision Research*, **38**, 3321–3327.
- Regan BC, Julliot C, Simmen B *et al.* (2001) Fruits, foliage and the evolution of primate colour vision. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **356**, 229–283.
- Riba-Hernandez P, Stoner KE, Osorio D (2004) Effect of polymorphic colour vision for fruit detection in the spider monkey *Ateles geoffroyi*, and its implications for the maintenance of polymorphic colour vision in platyrrhine monkeys. *Journal of Experimental Biology*, **207**, 2465–2470.
- Rimoli J, Ades C (1997) Estratégias de forrageamento de um grupo de muriquis (*Brachyteles arachnoides*, Primates, Cebidae) da Estação Biológica de Caratinga-MG. In: *A Primatologia no Brasil-5* (eds Ferrari SF Schneider H), pp. 39–57. Sociedade Brasileira de Primatologia, Belém.
- Rosenberger AL (1984) Fossil New World monkeys dispute the molecular clock. *Journal of Human Evolution*, **13**, 737–742.
- Rowe MP, Jacobs GH (2004) Cone pigment polymorphism in New World monkeys: are all pigments created equal? *Visual Neuroscience*, **21**, 217–222.
- Schneider H, Sampaio I, Harada ML *et al.* (1996) Molecular phylogeny of the New World monkeys (Platyrrhini, Primates) based on two unlinked nuclear genes: IRBP intron 1 and ϵ -globin sequences. *American Journal of Physical Anthropology*, **100**, 153–179.
- Shyue SK, Boissinot S, Schneider H *et al.* (1998) Molecular genetics of spectral tuning in New World monkey color vision. *Journal of Molecular Evolution*, **46**, 697–702.
- Simmen B, Sabatier D (1996) Diets of some French Guianan primates: food composition and food choices. *International Journal of Primatology*, **17**, 661–693.
- Smith AC, Buchanan-Smith HM, Surridge AK, Osorio D, Mundy NI (2003) The effect of colour vision status on the detection and selection of fruits by tamarins (*Saguinus* spp.). *Journal of Experimental Biology*, **206**, 3159–3165.
- Stevenson PR, Quiñones MJ, Ahumada JA (2000) Influence of fruit availability on ecological overlap among four Neotropical primates at Tinigua National Park, Colombia. *Biotropica*, **32**, 533–544.
- Strier KB (1991) Diet in one group of woolly spider monkeys, or muriquis (*Brachyteles arachnoides*). *American Journal of Primatology*, **23**, 113–126.
- Sumner P, Mollon JD (2000) Catarrhine photopigments are optimised for detecting targets against a foliage background. *Journal of Experimental Biology*, **203**, 1963–1986.
- Surridge AK, Osorio D, Mundy NI (2003) Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution*, **18**, 198–205.
- Surridge AK, Smith AC, Buchanan-Smith HM, Mundy NI (2002) Single-copy nuclear DNA sequences obtained from noninvasively collected primate feces. *American Journal of Primatology*, **56**, 185–190.
- Surridge AK, Suárez SS, Buchanan-Smith HM, Mundy NI (2005) Non-random association of opsin alleles in wild groups of red-bellied tamarins (*Saguinus labiatus*) and maintenance of the colour vision polymorphism. *Biology Letters*, **1**, 465–468.
- Talebi MG (2005) *Factors affecting food choice of the endangered southern muriquis (Brachyteles arachnoides, Primates, E. Geoffroy, 1806) in the Brazilian Atlantic Forest*. PhD Thesis, University of Cambridge, UK.
- Talebi MG, Bastos A, Lee PC (2005) Diet of southern muriquis in continuous Brazilian Atlantic Forest. *International Journal of Primatology*, **26**, 1193–1205.
- van Roosmalen MGM (1985) Habitat preferences, diet, feeding strategy and social organization of the black spider monkey (*Ateles paniscus paniscus* Linnaeus, 1758) in Surinam. *Acta Amazonica*, **15**(Suppl. 3/4), 1–238.
- Williams D, Sekiguchi N, Brainard D (1993) Color, contrast sensitivity, and the cone mosaic. *Proceedings of the National Academy of Sciences, USA*, **90**, 9770–9777.
- Yokoyama S, Radlwimmer FB (2001) The molecular genetics and evolution of red and green color vision in vertebrates. *Genetics*, **158**, 1697–1710.
- Yokoyama S, Takenaka N (2005) Statistical and molecular analyses of evolutionary significance of red-green color vision and color blindness in vertebrates. *Molecular Biology and Evolution*, **22**, 968–975.
- Zingesser MR (1973) Dentition of *Brachyteles arachnoides* with reference to Alouattine and Ateline affinities. *Folia Primatologica*, **20**, 351–390.

M.G.T. is interested in the ecology, behavioural biology, and conservation of neotropical fauna, with an emphasis on primates and the co-evolution of animal plant interactions. T.R.P. is interested in the ecological correlates of different types of mating systems and dispersal tendencies. E.R.V. is interested in the role that food sources play in structuring the behaviour and social organization of animals. N.J.D. is interested in the foraging and sensory ecology of primates.
