RESEARCH ARTICLE

Polymorphic Color Vision in Captive Uta Hick's *Cuxiús*, or Bearded Sakis (*Chiropotes Utahickae*)

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The pitheciines (*Chiropotes*, *Pithecia*, and *Cacajao*) are frugivorous Neotropical primates that specialize on the predation of seeds from unripe fruits, usually cryptic against the foliage. However, little is known about the color vision distribution within this taxon, and even less about the abilities shared by these animals regarding discrimination of chromatic targets. The aim of this study was to evaluate the color vision perception of captive Uta Hick's *cuxiús*, or bearded sakis (*Chiropotes utahickae*) through a behavioral paradigm of color visual discrimination, as well as to estimate, by genetic studies, the number and kinds of medium to long wavelength cone photopigment (opsins) encoded by this species. Among 12 *cuxiús* (7 males and 5 females) studied only 1 female was diagnosed as a trichromat. Results from genotyping were in line with our behavioral data and showed that *cuxiús* carried one (dichromat) or two (trichromat) medium to long wavelength pigments alleles, demonstrating a color vision polymorphism in *C. utahickae* similar to the majority of Neotropical Primates. Am. J. Primatol.

Key words: pitheciines; genotyping; behavior; Munsell color system; discrimination

INTRODUCTION

Color vision is achieved through comparison of photoreceptors activities with spectral peaks in the short (blue cones), middle (green cones), and long (red cones) wavelengths. Among mammals, primates have evolved a unique ability for three-dimensional color vision (trichromacy) via allelic differentiation (polymorphic color vision) or gene duplication (uniform trichromacy) of the middle to long wavelength-sensitive (M/LWS, or red-green) opsin gene [Kawamura et al., 2012]. Polymorphic color vision is a peculiar and characteristic condition of Neotropical primates [Jacobs, 1993, 2007] and some prosimians [Leonhardt et al., 2009; Tan & Li, 1999], although *Alouatta* and *Aotus*, two acknowledged exceptions, present uniform trichromacy [Araújo et al., 2008; Jacobs et al., 1996] and monochromacy [Jacobs, 1993], respectively. The M/LWS (middle/long wavelength) gene is located in a single locus on the X chromosome, which is responsible for coding cone photopigments (opsins) that are maximally sensitive to the green-red spectral range [Jacobs, 2007; Neitz et al., 1991]. On the other hand, the SWS (short wavelength) gene is located on an autosomal chromosome and is responsible for coding opsins that are highly sensitive to blue [Jacobs et al., 1996]. Such arrangement results in dichromatic or trichromatic vision in homozygous or heterozygous females, respectively, while hemizygous males are mandatorily dichromats [Mollon et al., 1984; Neitz et al., 1991].

Color vision is the result of active processes carried out by the nervous system as a whole, which starts in the retina and continues all the way to different areas of the visual cortex [Gegenfurtner &

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Kiper, 2003; Zeki, 1999]. Similar to humans, the processing of color vision in non-human primates occurs through two chromatic opponent channels, yellow-blue (common to dichromats and trichromats) and red-green (solely in trichromats), which operate in a parallel way [Dominy & Lucas, 2001; Regan et al., 2001]. Thus, despite the accuracy and objectivity of the genetic methods in color vision studies, the dimensionality of an animal's color perception can only be demonstrated accurately through behavioral tests [Jacobs et al., 1999] and further correlation with molecular data [Altavini et al., 2012; Caine & Mundy, 2000; Melin et al., 2007; Leonhardt et al., 2009; Saito et al., 2005; Tovée et al., 1992]. Furthermore, external factors might be considered, as they may also influence the perception of color. These include chromaticity, stimulus size and luminosity [Caine et al., 2003; Freitag & Pessoa, 2012; Gomes et al., 2005; Perini et al., 2009].

Several ecological and behavioral factors have been suggested to be instrumental in maintaining and increasing the adaptive value of color perception, such as the discrimination of potential predators [Caine, 2002; Pessoa et al., 2014; Sumner & Mollon, 2003], sexual partners [Bradley & Mundy, 2008; Caro, 2005], and social status in males [Bergman et al., 2009; Setchell & Wickings, 2005]. However, there seems to be a consensus among most researchers that the feeding ecology of primates has acted strongly to improve discrimination of conspicuous targets (red-green spectral range) against a green background [Caine & Mundy, 2000; Smith et al., 2003]. Both the detection of ripe fruit (frugivory hypothesis) [Allen, 1879; Mollon, 1989] and young leaves (folivory hypothesis) [Dominy & Lucas, 2001; Lucas et al., 1998] among the green forest foliage could have been important in the evolution of trichromacy in primates. Trichromats, when compared to dichromats, have higher performance in detecting targets in the yellow-red range against green backgrounds at high light intensity [De Araujo et al., 2006; Dominy & Lucas, 2001; Melin et al., 2009, 2013; Mollon, 1989; Osorio & Vorobyev, 1996; Perini et al., 2009; Sumner & Mollon, 2000], and can also discriminate between green and orange surfaces [Araújo et al., 2008] that are indistinguishable to dichromats [Gomes et al., 2002]. On the other hand, under low levels of luminosity, because of an interaction between cones and rods, dichromats are able to discriminate camouflaged chromatic stimuli (break camouflage), which confers a selective advantage in detecting cryptic targets (e.g., insect and green fruit foraging) against the background [Freitag & Pessoa, 2012; Saito et al., 2005; Caine et al., 2003]. Recently, Melin et al. [2013] demonstrated that under high luminosity human trichromats had superior accuracy in discriminating both cryptic fruits (green) scattered among the foliage and conspicuous fruits (red and yellow), in comparison to dichromats. Although human experimental models in naturalistic conditions may allow a better understanding of di- and trichromats performance in detecting food, the evidence found by Melin et al. [2013] remains to be validated in non-human primates.

The *cuxiús* or bearded sakis are representatives of the genus *Chiropotes* [Barnett et al., 2012], and part of the sub-family Pitheciinae (Pitheciidae: Platyrrhini), along with sakis (*Pithecia*) and uacaris (*Cacajao*) [Groves, 2001]. The pitheciines differ from other Neotropical primates in that they specialize in seeds predation of hard-husked unripe fruits of the family Lecythidaceae [Ayres, 1989; Norconk, 2007; Rosenberger, 1992]. *Chiropotes* and *Cacajao* have dental adaptations and a distal intestine, to assist in opening, chewing and digestion of immature seeds [Norconk, 1996]. Both live in large social groups and have further adaptations in their skeleton and in their locomotion which allows the frequent use of middle to upper canopy in the forest [Walker, 1996]. In contrast, when *Pithecia* occurs in sympatry with one of the other pitheciines or other primates, it occupies mid-canopy in the forest, climbing vertically between the trunks of tall trees, and forming less numerous social groups, which constitute adaptive characteristics to avoid competition for food resources [Walker, 1996]. In the pitheciines, more than half of the species are threatened with extinction [IUCN, 2013], particularly *Chiropotes albinasus* [Veiga et al., 2008a], *C. utahickae* [Veiga et al., 2008b] (both endangered), and *C. satanas* (critically endangered) [Veiga et al., 2008c].

In the past two decades, information about ecology, behavior, taxonomy, and conservation of pitheciids have been collected [Veiga et al., 2013], although their sensory ecology and particularly their visual perception is barely known. To the best of our knowledge, there is no information regarding color vision in *Chiropotes* and *Cacajao* [Jacobs, 2007]. Regarding other pitheciines, *Pithecia* has been shown to have three photopigment alleles, 535, 550, and 562 nm, inferred by molecular genetics [Boissinot et al., 1998], while *Callicebus* (sub-family Callicebinae, family Pitheciidae) has five different alleles, 530, 536, 542, 551, and 562 nm, an exception among Neotropical primates [Jacobs & Deegan II, 2001]. Although these pigments have been demonstrated only in captive *Callicebus*, alleles with sensitivity at wavelengths within the 530 and 542 nm range remain to be found in wild populations [Bunce et al., 2011]. It is important to note that the visual polymorphism of *Pithecia* and *Callicebus* has not been yet confirmed behaviorally.

Therefore, our aims were: (a) to evaluate the color perception of captive Uta Hick's *cuxiús* (*Chiropotes utahickae*) through a behavioral paradigm of color discrimination, and (b) to estimate, through a genetic approach, the number of types of M/L cone pigments carried by males and females of *cuxiús* sampled in

this study. To the best of our knowledge, this is the first study to investigate pitheciine color vision through behavioral and molecular approaches.

METHODS

Study Sites

The study was conducted in the *C. utahickae* colony located at Centro Nacional de Primatas (CENP), Ananindeua, Pará, Brazil. All infrastructure, management, feeding, and health of the captive primates at CENP complied with Brazilian laws. CENP conducts captive breeding of approximately 20 non-human primate species (mostly Neotropical monkeys), for biomedical research. However, in the last few decades it has housed endangered species for conservation purposes, including *C. utahickae*. The molecular analyses were conducted at the Laboratory of DNA Polymorphism, Federal University of Pará, Brazil.

This research complied with the American Society of Primatologists Principles for the Ethical Treatment of Primates. The procedures for data collection and analyses were approved by the environmental agencies (ICMBio No. 28427-1 and 34476-1) and by the Animal Care Committees of the Evandro Chagas Institute (IEC-CEPAN No. 011/2011) and of the Federal University of Pará (CEPAE-UFPA No. BIO082-12).

Behavioral Study

Subjects

Eight subjects (5 females and 3 males), from a total of 12 *cuxiús* (3 females and 3 males adults, 2 females and 1 male juvenile, and 3 infants) that were grouped in 2 separate enclosures (3.8 m width × 2.3 m length × 2.4 m height), were selected for the behavioral experiments. The remaining (3 infants and 1 adult male) were only sampled in the genetic study.

Stimuli and apparatus

We used Munsell Color chips as visual stimuli. In the Munsell system, the color notation is represented by its hue (a number and capital letters) and a fraction which stands for brightness over saturation (e.g. 2.5YR 4/6 corresponds to a yellow-red 2.5, with brightness 4 and saturation 6). For our experiments, we used the following hues: red (R), yellow-red (YR), green-yellow (GY), blue (B), purple-blue (PB), and purple (P). Most of the hue categories varied in the spectrum points 2.5, 5, 7.5, and 10. Each color was presented in four different levels of brightness (4–7) and with a fixed saturation (6).

The Munsell chips were presented in pairs. The degree of difficulty of each pair had been previously estimated by tests on human subjects [Gomes et al., 2002] and corroborated by experiments on several Neotropical primates such as *Sapajus apella* [Gomes et al., 2002], *Saguinus midas niger* [Pessoa et al., 2003], *Callithrix penicillata* [Pessoa et al., 2005a], *Leontopithecus chrysomelas* [Pessoa et al., 2005b], *Saimiri ustus* [Prado et al., 2008], and *Alouatta caraya* [Araújo et al., 2008]. In fact, these same tests have been successfully adapted and used in a South American marsupial (*Didelphis albiventris*) for evaluating its color discrimination abilities [Gutierrez et al., 2011]. In these studies, the pairs were classified as: (1) easy pairs or positive control, easily discriminated by dichromats and trichromats; (2) difficult pairs or diagnostic test, easily discriminated by trichromats and poorly discriminated by dichromats; and (3) impossible pairs or negative control, poorly discriminated by trichromats and dichromats.

Here, we used six positive controls (2.5 YR vs. 5B, 2.5 YR vs. 2.5PB, 2.5 YR vs. 7.5P, 10YR vs. 5R, 10YR vs. 5PB, and 10YR vs. 5P) which do not fall on the confusion lines for dichromatic humans (protanopes and deuteranopes) (Fig. 1). We also presented two diagnostic tests (2.5 YR vs. 7.5 GY and 10YR vs. 5GY) that fall on the confusion lines for protanopes and deuteranopes (Fig. 1). Finally, to confirm that the subjects were not using non-visual cues, two negative controls, composed of pairs of identical chips (2.5YR vs. 2.5YR and 10YR vs. 10YR), were also used. It is important to emphasize that the chips used in the diagnostic tests have similar colors to mature fruits and leaves found in natural foraging situations [De Araujo et al., 2006; Dominy, 2004; Perini et al., 2009; Pessoa et al., 2005a; Prado et al., 2008; Savage et al., 1987; Terborgh, 1984]. Pairs of stimuli were presented to the animals in two series, each one containing three positive controls, a diagnostic test and a negative control.

An acrylic glass apparatus, similar to that described by Araújo et al. [2008], was used to assess the color discriminating abilities of the subjects. The apparatus was installed on the external side of the housing enclosure's' wire mash, and was composed of a portable shelf, a retractable shield and two cubes, all built in black acrylic. The Munsell chips were presented inside each cube, being exposed only through each cube's orifice (diameter 1.2 cm) (see Pessoa et al. [2005a] for a detailed scheme). The shield prevented animals from visualizing the stimuli between trials. We also used a D-65 lamp to constantly and homogeneously improve the illumination of the test apparatus (98 lux) that also received natural sunlight.

Procedure

The behavioral studies were conducted within the housing areas, between 10:00 and 15:00 hr. During the experiment, subjects were food deprived, but had access to water ad libitum. Brazil nut (*Bertholletia excelsa*, Lecythidaceae) was used as

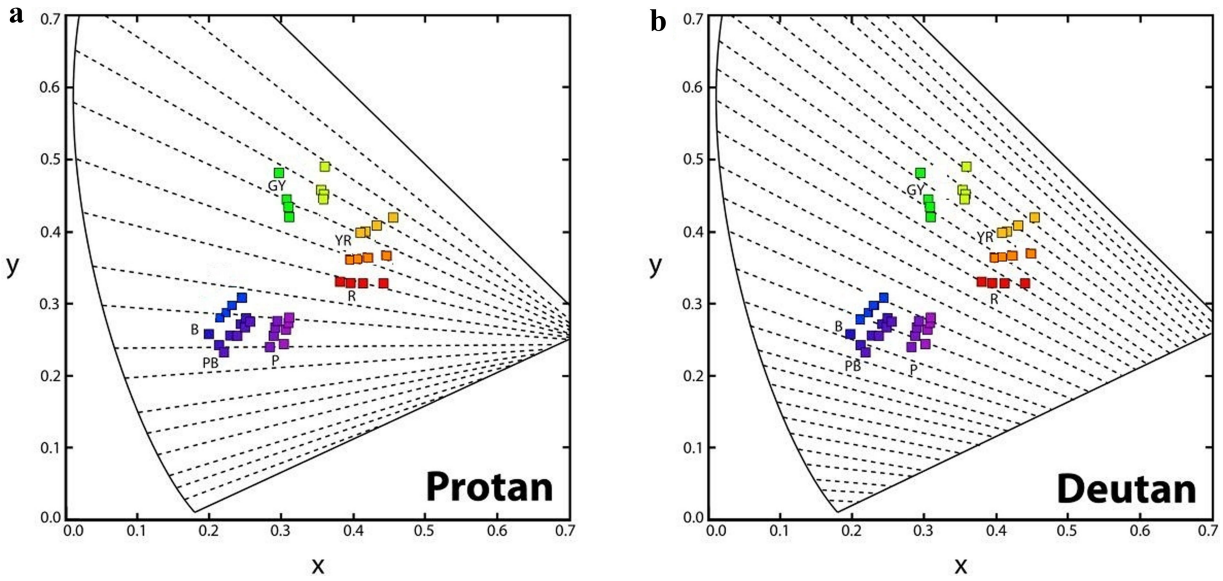

Fig. 1. Chromaticity of Munsell chips used on color discrimination tests in eight *Chiropotes utahickae*. *X* and *Y* axes indicate the *x* and *y* values in the CIE1931 chromaticity diagram, respectively. Dotted lines indicate confusion lines for human protans and deutans (stimuli that superimpose should not be discriminated).

reward. Initially, subjects underwent a period of behavioral modeling which was comprised by training phase 1 and 2 [Araújo et al., 2008; Gomes et al., 2002; Gutierrez et al., 2011]. Training phase 1 comprehended 11 sessions (50 min each), in which subjects were faced with only one cube with 2.5YR hue. They had to lift the cube in order to obtain the reward.

In training phase 2, subjects were isolated so that their behavior was not affected by conspecifics (Fig. S1 in the Supplementary Material). In this phase, a pair of cubes was presented to the subjects, 2.5YR (yellow-red) versus 5B (blue). The cube with orange hue was fixed as positive discriminative stimulus (SD+, the rewarded stimulus) and the one with blue hue was fixed as negative discriminative stimulus (SD−, never rewarded). Each subject's task was to choose one of the two cubes using the color cue. A correct response was represented by choosing cube 2.5YR and an incorrect response was represented by choosing cube 5B. The left or right position of the cubes was determined according to the Gellermann table of random numbers [Gellermann, 1933] in order to avoid position bias. Subjects varied in the number of sessions needed to reach a minimum of 80% of correct responses. Each session lasted 20 min/subject.

Finally, in the testing phase, the subjects were submitted to the positive and negative controls and to the diagnostic tests for color discrimination ability. In this phase, animals were presented to two experimental series, where each one contained (alternately) three pairs of positive control (easy discrimination), one pair of diagnostic test (difficult discrimination) and one pair of negative control (impossible discrimination) (Fig. 1). The first series had 2.5YR as the SD+, paired with following SD−: 5B, 2.5PB, and 7.5P (positive controls), 7.5GY (diagnostic test), and 2.5YR (negative control) (Fig. 2a). The second series comprised 10YR as SD+, paired with following SD−: 5R, 5PB, and 5P (positive controls), 5GY (diagnostic test), and 10YR (negative control) (Fig. 2b). Each session lasted 40 min and tested 1 pair of hue with 64 trials (that is, 4 variations in brightness of SD+ were paired with 4 variations in brightness of SD−, resulting in 16 pairs presented 4 times each).

Genetic Study

DNA extraction

DNA was extracted from blood samples of the 12 individuals using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. The polymerase chain reaction (PCR) was used to amplify and isolate the exons 3 and 5 of the X-linked opsin gene. Exons 3 and 5 were amplified using forward and reverse primers with the respective sequences: exon 3, 5′-GGATCACGGGTCTCTGGTC-3′/5′-CTGCTCCAACCAAAGATGG-3′; exon 5, 5′-GT GGCAAAGCAGCAGAAAG-3′/5′-CTGCCGGTTCAT AAAGACATAG-3′ [Mancuso et al., 2006]. PCR were adjusted to a final volume of 25 μl with the following reagents: 2.5 μl PCR buffer (10×), dNTPs (200 μM), $MgCl_2$ (1.5 mM), primer (0.2 μM), genomic DNA (10 ng), and Taq DNA polimerase (Invitrogen, 1 U). For the amplification reactions, an initial denaturation step of 2 min at 94°C was followed by 35 cycles of 94°C for 30 sec, 60°C for 45 sec, 72°C for 1 min, and final extension for 5 min at 72°C. The amplified

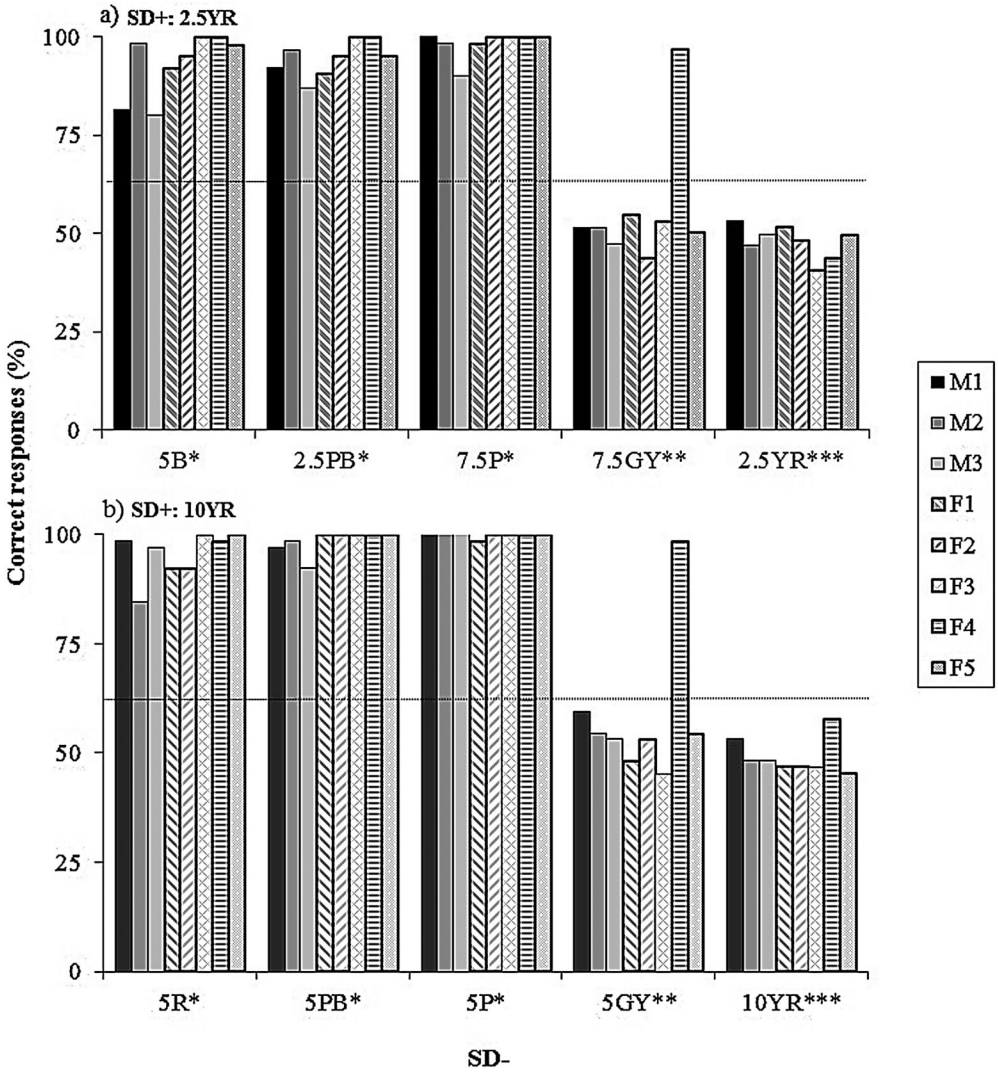

Fig. 2. Performance of eight *Chiropotes utahickae* on color discrimination tests: (**a**) 2.5YR (yellow-red) as positive discriminatory stimulus (SD+), and 5B (blue), 2.5PB (purple-blue), 7.5P (purple), 7.5GY (green-yellow), and 2.5YR (yellow-red) as negative discriminative stimuli (SD−); and (**b**) 10YR (yellow-red) as positive discriminatory stimulus (SD+), and 5R (red), 5PB (purple-blue), 5P (purple), 5GY (green-yellow), and 10YR (yellow-red) as negative discriminative stimuli (SD−). The horizontal line indicates the upper limit for randomness (63% correct responses) within the 95% confidence interval of discriminative performance. *Positive control, **diagnostic test, ***negative control.

products were visualized in agarose gel (1%) under UV light.

Gene Sequencing

Positive products were loaded in 1% agarose gel and subjected to 100 V for 30 min, then cut out to be purified using the GeneJET™ Gel Extraction Kit (Fermentas), according to the manufacturer's instructions. Purified products were used in a dideoxyterminal sequencing reaction with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems), and samples were then precipitated to run on an ABI 3130 automated sequencer (Applied Biosystems).

Heterozygous individuals were detected when two peaks appeared in a single nucleotide position on the sequencing chromatogram. In order to sort out alleles from heterozygotes, the purified fragments of the original PCRs were cloned using the pGEM-T Vector System I kit (Promega), following manufacturer's protocol. Ligated fragments were inserted on electrocompetent *Escherichia coli* DH5α, and then plated on agar-LB culture media with ampicilin and X-gal. Bacteria containing chimeric plasmids showed a bluish color and were selected to grow in Tartoff–Hobbs Broth with ampicilin. Chimeric plasmid isolation was carried out using lysis alkaline miniprep. Clones from each heterozygote were sequenced to identify the alleles.

Data Analysis

Behavioral tests

We used the binomial test to construct the 95% confidence intervals of the experimental design, based on the number of trials in the test [Savage et al., 1987]. In the case of 64 trials, the upper limit for correct responses was 40 correct trials or 63%. The performance of all individuals was compared with this confidence interval, and a performance above this limit was considered significant ($P < 0.05$).

Molecular genetics

Based on the amino acids found at sites 180, 277, and 285, we inferred the number of different kinds of M/L opsins expressed by each subject [Hiramatsu et al., 2005; Neitz et al., 1991]. In Neotropical primates, six amino acid combinations with their respective alleles have been found (the numbers represent the λ_{max} value of sensitivity for the cone opsins): P530 (Ala, Phe, and Ala), P538 (Ala, Tyr, and Ala), P545 (Ala, Phe, and Thr), P552 (Ser, Phe, and Thr), P553 (Ala, Tyr, and Thr), and P560 (Ser, Tyr, and Thr) [Hiramatsu et al., 2005, 2008]. However, in two ateline species the combinations Ser, Phe, and Thr and Ser, Tyr, and Thr correspond, respectively, to alleles P538 and P553 because mutation at sites 213 and 294 [Matsumoto et al., 2014].

RESULTS

Behavioral Tests

All subjects finished the behavioral training phase 2 with a performance above the upper limit of randomness ($P < 0.05$) for the pair 2.5YR versus 5B, indicating they have the capacity to discriminate colors. In the testing phase, a similar performance was obtained by seven subjects (four males and three females), in which their percentage of correct responses were above the upper limit of randomness in the positive controls ($P < 0.05$) and below the upper limit of randomness in the diagnostic tests (Fig. 2). For that reason, this behavior indicates that these seven *cuxiús* are dichromats. Another type of performance was shown only by Female 4, in which her percentage of correct responses was above the upper limit of randomness ($P < 0.05$) for both the positive controls and diagnostic tests. Such behavior points to a trichromatic color vision and this was the only trichromatic female found (Fig. 2). All subjects performed below the randomness level ($P > 0.05$) for the negative controls, indicating that they were incapable of resolving the discrimination without a color cue.

Molecular Genetics

We found alleles coding for three different M/L visual pigments in the *C. utahickae* (Table I): P530, P545, and P560. Female 4, the only one that showed a trichromatic performance in the behavioral experiments, was shown to be heterozygous, coding alleles P530/545. The remaining subjects were shown to be homozygous or hemizygous; two of them (Male 1 and Female 6) had allele P545, while nine of them (Males 2, 3, 4, 5, and 6, Females 1, 2, 3, and 5) had allele P560, which characterized them as dichromats.

DISCUSSION

Our genetic and behavioral results are in consonance and show that *C. utahickae* has polymorphic color vision; all males and most females were identified as dichromats, while one female was identified as a trichromat. Although dichromatic males and di- or trichromatic females are a common pattern among Neotropical primates [Jacobs, 2007; Mollon et al., 1984; Neitz et al., 1991], our results are

TABLE I. Description of the Amino Acids Found in Positions 180, 277, and 285 of Exons 3 and 5 for 12 *Chiropotes utahickae*, and Their Respective Genotypes and Phenotypes

	Exon 3	Exon 5			
Subject (microchip)	180	277	285	Genotype	Phenotype
Female 4 (039.549.805)	Ala	Phe	Ala	P530	Trichromat
	Ala	Phe	Thr	P545	
Female 6 (039.354.358)	Ala	Phe	Thr	P545	Dichromat
Male 1 (039.300.615)	Ala	Phe	Thr	P545	Dichromat
Male 2 (039.358.520)	Ser	Tyr	Thr	P560	Dichromat
Male 3 (039.527.527)	Ser	Tyr	Thr	P560	Dichromat
Male 4 (039.309.561)	Ser	Tyr	Thr	P560	Dichromat
Male 5 (039.273.285)	Ser	Tyr	Thr	P560	Dichromat
Male 6 (039.269.062)	Ser	Tyr	Thr	P560	Dichromat
Female 1 (039.548.868)	Ser	Tyr	Thr	P560	Dichromat
Female 2 (039.538.775)	Ser	Tyr	Thr	P560	Dichromat
Female 3 (039.292.017)	Ser	Tyr	Thr	P560	Dichromat
Female 5 (039.284.613)	Ser	Tyr	Thr	P560	Dichromat

the first to investigate the color vision of *Chiropotes* and to confirm the visual polymorphism of pitheciines [Boissinot et al., 1998] through both behavioral and molecular approaches.

We have identified the occurrence of three different alleles in the CENP colony of *C. utahickae*, P530, P545, and P560, based on the amino acid composition found in three sites of genes that encode M/L opsins. These alleles are a typical set found in Cebinae (squirrel monkeys and capuchins) [Hiramatsu et al., 2005], and also identified through molecular genetics in *Pithecia irrorata* [Boissinot et al., 1998]. However, we cannot rule out the possibility that additional studies with a larger sample of *Chiropotes* will not reveal additional M/L opsin alleles other than those found in the present study, as was the case for *Cebus* and *Saimiri*, with four alleles [Cropp et al., 2002; Soares et al., 2010], and for *Callicebus*, in which five alleles have been described so far [Jacobs & Deegan II, 2001]. Taking into account that *Chiropotes* share behavioral ecology characteristics with *Cacajao* [Norconk, 1996; Walker, 1996], we should expect a similarity of alleles between these two species; in fact, as previously highlighted, our results point out a consistency between spectral peaks of *cuxiús* and sakis.

Based on our behavioral data, we conclude that *cuxiús* have S cones, since they discriminated the pair 2.5YR versus 5B (Fig. 2a). This pair was out of the dichromats' confusion range (as illustrated by Fig. 1), since such stimuli are processed by the yellow-blue channel of opponency [Dominy & Lucas, 2001; Regan et al., 2001]. Other chromatic stimuli were also discriminated against by the same channel for processing; red, yellow-red, purple-blue, and purple, which constituted the positive control. The green-yellow stimuli (diagnostic test) were used in this study to evaluate the discrimination of the red-green channel, found only in trichromats [Dominy & Lucas, 2001; Regan et al., 2001]. We observed males and females *cuxiús* with a performance above randomness level in the positive control and below randomness level in the diagnostic tests, and in those *cuxiús* had only one allele P545 or P560; therefore, they were classified as color vision dichromats. In contrast, a female had performance above randomness level in both positive control and diagnostic tests, and had two alleles identified, P530 and P545, and was the only trichromat in this study. In addition, all *cuxiús* had a performance below randomness level in the negative controls, indicating that color cues were important and that they have not used non-chromatic cues to solve the tests. Although some studies have demonstrated that trichromats reveal a better chromatic discrimination when their phenotypes have longer intervals between the peak sensitivity of M/L photopigment alleles (P530-P560) [De Araujo et al., 2006; Melin et al., 2009; Osorio et al., 2004; Rowe & Jacobs, 2007], green-shifted phenotypes (dichromats P530 or trichromats P530-P545) also seem to be more advantageous than red-shifted phenotypes (dichromats P560 or trichromats P545–P560) [Melin et al., 2013, 2014]. However in natural populations, the P530 and P545 alleles, also found in *C. utahickae* by our study, are generally the rarest [Hiramatsu et al., 2005; Melin et al., 2013; Talebi et al., 2006], and allele P530 is absent in callitrichids [Osorio et al., 2004; Surridge et al., 2005]. In this study, we used chromatic stimuli with complex and broad spectral reflectance (Fig. 1) [Pessoa et al., 2003] in a way that a female with P530 and P545 alleles showed an above chance performance when presented to all tasks, with exception of the negative control.

The behavioral protocol using Munsell chips as a diagnostic approach for color vision was validated for the first time by genetic sequencing. Previous studies using this protocol had indicated its reliability and validity as a diagnostic test for evaluating the color vision of captive primates [Araújo et al., 2008; Gomes et al., 2002; Pessoa et al., 2003, 2005a,b,c; Prado et al., 2008], and other mammals, such as *Didelphis albiventris* [Gutierrez et al., 2011]. Here, we found only one female with a trichromatic behavioral phenotype (P530/P545 genotype), which makes it impossible to infer the relationship between color vision genotype and performance on behavioral tests. However, earlier studies, using the same behavioral methodology, were able to identify variation in the performance of supposedly trichromatic females [Pessoa et al., 2005c; Prado et al., 2008]. In addition, as two other trichromatic genotypes (P530/P560 and P545/P560) are also expected to occur in *C. utahickae*, we believe that future studies, expanding our sample size, should enable us to test for this relationship. On the other hand, our results indicate that in dichromatic individuals (P545 or P560) of *C. utahickae* there does not appear to be a relationship between performance and genotype, confirming what was found by Pessoa et al. [2005c]. Consequently, we can affirm that Munsell color chips, if applied with caution and paying special attention to brightness control, are an effective tool for, at least, diagnosing dichromats from trichromats.

Selection of ripe fruits that are conspicuous against the forest foliage has been one of the main hypotheses suggested and tested when examining the evolution of trichromatic vision in primates [Allen, 1879; Mollon, 1989; Regan et al., 2001]. In non-pitheciine primates, approximately 80% of exploited fruits have conspicuous chromaticity (violet, blue, yellow, orange, red, and brown) [Dominy, 2004]. Contrastingly, in pitheciines, the majority (54%) of exploited fruits are cryptic [Dominy, 2004], and probably the majority of them are unripe for consumption of seeds. The discovery of color vision polymorphism in *C. utahickae*, which has a diet

composed of 52% seeds (90% immature seeds) and 26% fruits (54% unripe fruits) [Santos et al., 2013], contradicts the classical view that trichromacy would result from the identification of ripe fruits. The high consumption of seeds and unripe fruits (traditionally considered cryptic) by *Chiropotes* suggests that dichromats could occur at higher frequencies in natural populations, because: (1) selective pressure for identification of conspicuous fruits should be lower; and (2) pressure for selecting dichromats could be higher if they were more advantageous in detecting cryptic targets as has been suggested by Mollon [1989]. However, we identified color vision polymorphism in *Chiropotes*, indicating that the trichromacy would also be in some way advantageous. On the other hand, at short-range distance, trichromatic phenotypes may be superior in green fruit detection, as showed in humans [Melin et al., 2013]. Therefore, it is important to carry out studies to identify the environmental characteristics and chromaticity of foraged fruits by natural populations. Furthermore, studies should investigate other selective pressures for trichromacy in pitheciines, such as choice of sexual partners, antipredatory behavior, and social structure in this taxonomic group, considered the most specialized among the platyrrhines [Kay et al., 2012; Norconk, 2007; Rosenberger, 1992].

To the best of our knowledge, no previous publication had investigated the color perception of *Chiropotes*. Our study is therefore the first to provide behavioral and genetic evidence of color vision polymorphism in *C. utahickae* and to evaluate a taxon that is specialized in consuming hard-husked fruits and immature seeds.

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Supporting Information

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