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RESEARCH ARTICLE

Color Vision Polymorphism in Wild Capuchins (*Cebus capucinus*) and Spider Monkeys (*Ateles geoffroyi*) in Costa Rica

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New World monkeys are unique in exhibiting a color vision polymorphism due to an allelic variation of the red-green visual pigment gene. This makes these monkeys excellent subjects for studying the adaptive evolution of the visual system from both molecular and ecological viewpoints. However, the allele frequencies of the pigments within a natural population have not been well investigated. As a first step toward understanding the relationship between vision and behavior, we conducted color vision typing by analyzing fecal DNA from two wild groups of white-faced capuchin monkeys (*Cebus capucinus*) and one group of black-handed spider monkeys (*Ateles geoffroyi*) inhabiting Santa Rosa National Park of Costa Rica. All color-typed monkeys were individually identified. In *C. capucinus* and *A. geoffroyi* we found three and two pigment types, respectively, and the spectral mechanism that created one of the two *Ateles* pigments was found to be novel. In one *Cebus* group and the *Ateles* group, all alleles were present, whereas in the other *Cebus* group only two alleles were found, with one allele predominating. This was likely due to the effect of close inbreeding, indicating that wild populations can exhibit a variety of allele compositions. This result also suggests that the color vision polymorphism can be easily distorted by natural factors, such as inbreeding, skewing the population structure. Am. J. Primatol. 67:447–461, 2005.

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INTRODUCTION

The color vision of New World monkeys (platyrrhine primates) is unique among animals in that it is highly polymorphic. Only a few species are known to be exceptions [Jacobs, 1998]. In New World monkeys, the males are dichromatic and the females are either dichromatic or trichromatic. This phenotypic variation results from an allelic polymorphism of the single-locus middle-to-long-wave-sensitive (M/LWS, or red-green) cone visual pigment gene residing on the X chromosome [Kawamura et al., 2001; Mollon et al., 1984]. Typically, three M/LWS pigment alleles are found in a species. By combinations of the M/LWS alleles, three different dichromacies and three different trichromacies are possible within the species. This unique feature has made New World monkeys an excellent subject for studies regarding both molecular genetics and the behavioral significance of color vision.

However, our knowledge about the color vision polymorphism relies mostly on data accumulated from various reports on wild and captive animals and preserved specimens [Cropp et al., 2002; Surridge & Mundy, 2002], and little is known about the pattern of color vision variation within natural populations. Recently, Surridge et al. [2002] developed a methodology to analyze visual pigment genes from fecal samples and used it to study the association between vision types and leadership of travel progression in wild tamarins in Peru [Smith et al., 2003a]. Cropp et al. [2002] studied three species of squirrel monkeys to assess gene frequencies in wild populations (*Saimiri oerstedii* in Costa Rica, *S. sciureus* in Suriname and Guyana, and *S. boliviensis* in Peru and Bolivia). However, that study was intended to reveal differences in gene frequencies between species of different geographical origins and under different dietary demands–not to reveal gene frequencies in individual social groups of monkeys. With the exception of those studies, no information on the gene frequency of M/LWS visual pigment alleles is currently available regarding natural populations of New World monkeys, and no studies have targeted vision-type compositions in other species in the wild.

To study color vision types using fecal DNA, and to understand vision–behavior relationships in wild monkeys, it is essential for the monkeys at the study site to be habituated to human observers and individually identified. It is also important to have ecological and sociological information regarding the study animals, and it is desirable to have two or more primate species of different ecology, phylogeny, and vision types present at the site for comparative purposes. Santa Rosa National Park in Area de Conservacion Guanacaste (ACG), Costa Rica, is an ideal study site where these conditions are satisfied [Chapman, 1990; Fedigan, 2003; Fedigan & Jack, 2001, 2004].

There are three sympatric primate species at Santa Rosa: white-faced capuchin monkeys (*Cebus capucinus*), black-handed spider monkeys (*Ateles geoffroyi*), and mantled howler monkeys (*Alouatta palliata*). The former two genera of monkeys are known to have a color vision polymorphism [Jacobs, 1998; Jacobs et al., 1996]. Capuchins and spider monkeys differ in diet (omnivores and frugivores, respectively), social structure (matrilineal in capuchins, and patrilineal and fission-fusion in spider monkeys) [Kinzey, 1997], and phylogeny (families Cebidae and Atelidae, respectively) [Schneider, 2000]. Capuchins are

reported to possess three M/LWS pigment alleles, whereas spider monkeys possess two [Jacobs & Deegan, 2001]. These conditions make capuchins and spider monkeys excellent study subjects. They complement callitrichine species (marmosets and tamarins), whose vision–behavior relationships have been most intensively investigated among New World monkeys [Caine, 2002; Caine & Mundy, 2000; Caine et al., 2003; Pessoa et al., 2003; Smith et al., 2003a,b].

The objective of this paper is to present data on M/LWS gene frequencies and color vision variation obtained by analyzing fecal DNA from wild groups of white-faced capuchins and black-handed spider monkeys inhabiting Santa Rosa National Park in Costa Rica. This study is the first step toward our planned research on the relationships among vision types, behaviors such as foraging and subgroup formation, and the spectral properties of visual objects and the ambient environment.

MATERIALS AND METHODS

Study Site and Subjects

Santa Rosa National Park is located in the tropical dry forest of northwest Costa Rica (10°50′N, 85°37′W) (Fig. 1). The climate of the area is divided roughly into two seasons: the rainy season from the middle of May to December, and the dry season from January to the beginning of May. The majority of the understory plants and nonriparian trees lose their leaves in the dry season, and the canopy of the forest rarely exceeds 30 m in height. The study subjects were two groups (LV and CP) of white-faced capuchin monkeys (*Cebus capucinus*) and one group of black-handed spider monkeys (*Ateles geoffroyi*). Each group consisted of 20–30 monkeys. The home ranges of the spider monkeys and the LV group of capuchins overlap, whereas the home range of the CP group is distinct from those of the other two (Fig. 1). All of the monkeys in these groups have been the subjects of long-term, continuous study and are individually recognized on the basis of

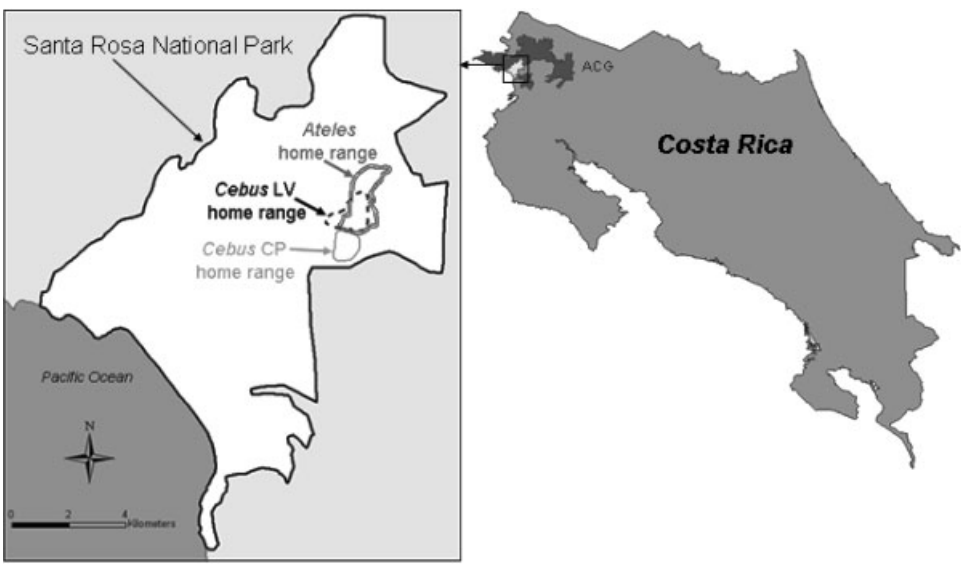

Fig. 1. A site map of the study field. Santa Rosa National Park is a part of the Area de Conservacion Guanacaste (ACG), Costa Rica. Home ranges of the spider monkeys (*Ateles*) and the groups LV and CP of the capuchins (*Cebus*) are indicated.

natural markings, such as pelage patterns and scars and distinctive facial markings.

Sample Collection

Samples of feces (about the size of a human fingertip) were collected using sterile cotton swabs and suspended in 5 ml of ASL lysis buffer (QIAamp DNA Stool Mini Kit; Qiagen, Crawley, UK), which was pre-aliquoted in sterile 15-ml screw-capped plastic vials. The samples were collected as soon as the monkeys defecated. Fecal samples were collected only when long-term observers were 100% certain of the identification of the monkey that had produced the sample. Collectors wore a mask and plastic gloves to minimize the chance of contamination by humans. The collected samples were stored at ambient temperature in labeled vials until the DNA was extracted. Some capuchin samples were stored in 100% ethanol or in silica beads immediately after collection. We obtained a total of 140 fecal samples from 44 capuchins (one to six samples per individual, average $= 3.2 \pm 1.4$) and 59 fecal samples from 22 spider monkeys (two to four samples per individual, average $= 2.7 \pm 0.7$). Skin samples were collected from three additional capuchins (in LV group) that had died of natural causes and were preserved in 100% ethanol. Sample collection and exportation to Japan or Canada were done under a permit from the Ministerio de Ambiente y Energia (MINAE) of Costa Rica. For the spider monkey samples (subjects of CITES Appendix W-I), an additional import permit was obtained from the Ministry of Economy, Trade and Industry (METI) of Japan.

DNA Extraction

We extracted genomic DNA from fecal samples using the QIAamp DNA Stool Mini Kit according to the manufacturer's instructions, with the following modifications: For feces stored in 100% ethanol, we evaporated the ethanol before extraction. Then we mixed 5 ml of ASL buffer and incubated the samples overnight at ambient temperature. For feces stored in silica beads, we added ASL buffer and incubated the samples as with the ethanol samples. For all samples, final elution of the DNA by buffer AE took 30 min (instead of 1 min, as in the original protocol). We recovered DNA in 200 μl of elution buffer and stored it at –20°C. Genomic DNA from the skin samples was prepared by the conventional phenol-chloroform extraction method [Sambrook & Russel, 2001]. As a precaution against contamination, we performed all extraction procedures from fecal samples at a clean bench in a separate room dedicated only to DNA extractions from feces and subsequently to the polymerase chain reaction (PCR) experiment.

Color Vision Typing (Background)

We previously demonstrated by site-directed mutagenesis that peak absorption spectra (λmax) of New World monkey M/LWS pigments were determined by amino acid composition at three sites (180, 277, and 285), and that sites 229 and 233, which were previously suspected to have spectral tuning effects [Shyue et al., 1998], in fact made little or no contribution to them [Hiramatsu et al., 2004]. Hence, we examined by PCR the nucleotide sequences of exon 3, encoding the amino acid site 180, and exon 5, encoding sites 277 and 285, for genotyping the M/LWS pigment genes [Neitz et al., 1991; Williams et al., 1992; Yokoyama & Radlwimmer, 1998, 1999, 2001].

According to the three-site amino acid compositions (see Fig. 2), alleles of the M/LWS pigments in New World monkeys are classified into five types: P530,

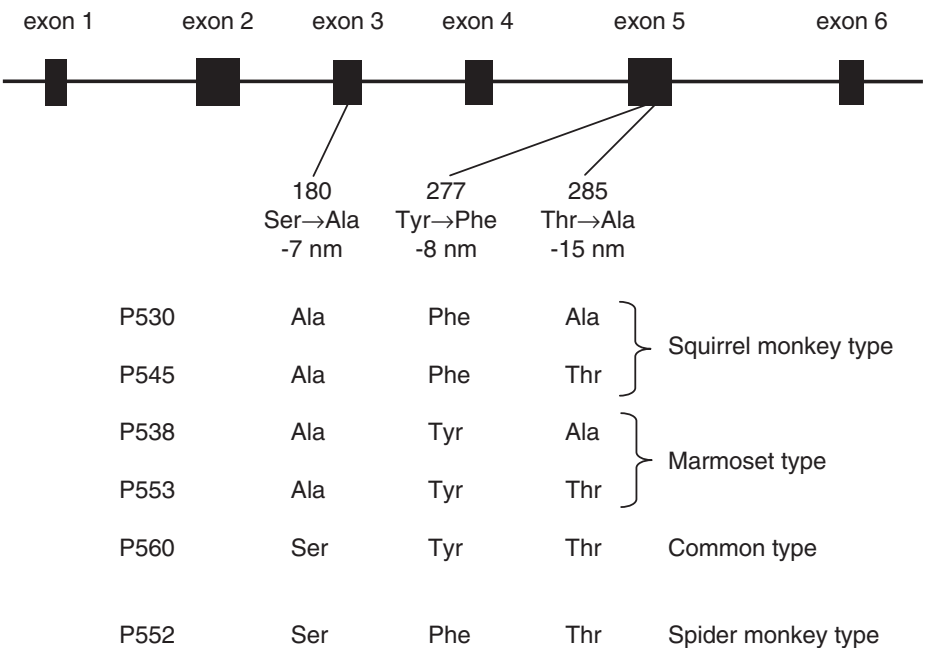

Fig. 2. Allelic types of M/LWS visual pigments in New World monkeys classified by amino acid compositions at three sites (180, 277, and 285). The locations of the three amino acid sites are schematically indicated in the exon-intron structure of the M/LWS visual pigment gene. The spectral effects of amino acid substitutions at these sites are indicated below the site numbers, and the pigment names are given to represent λmax values predicted from the "three-site rule" according to Yokoyama and Radlwimmer [2001].

P538, P545, P553, and P560 (the numbers represent λmax values expected from the amino acid compositions). P538 and P553 are seen in callitrichine species (designated here the marmoset type), P530 and P545 are found in non-callitrichines (squirrel monkey type), and P560 is common to both callitrichines and non-callitrichines (common type; see also Hiramatsu et al. [2004]). In spider monkeys (*Ateles*) and the closely related woolly monkeys (*Lagothrix*), two pigment types that are spectrally comparable to the squirrel monkey type P545 and the common type P560 have been observed electrophysiologically [Jacobs & Deegan, 2001]. As shown in the Results section, we found that the shorter-wave type of spider monkey pigment had a unique three-site combination, and thus designated it P552 (spider monkey type; Fig. 2).

Color Vision Typing (Procedures)

We designed PCR primer pairs inside exons 3 and 5 of the M/LWS pigment gene: exon 3, 5′-ggatcacgggtctctggtc-3′ (forward)/5′-ctgctccaaccaaagatggg-3′ (reverse); exon 5, 5′-gcaaagcagcagaaagagtc-3′ (forward)/5′-ctgccggttcataaagacat-3′ (reverse). We performed PCR in 50 μl using 1.5 units of high-fidelity Pyrobest polymerase (Takara, Tokyo, Japan) with 1 × Pyrobest buffer, 0.2 mM each of dNTPs, 0.6 μM each of the forward and reverse primers, and 5 μl of the DNA extract from the feces. We used pure water as the template for the negative control in every reaction. We carried out PCR for exon 3 at 98°C for 5 min followed by 40 cycles of 98°C for 45 sec, 56°C for 5 sec, and 72°C for 30 sec. For

exon 5, we carried out the reaction with the same cycle as in exon 3, but the annealing time at 56°C was changed to 30 sec. We purified the amplified DNA by agarose gel electrophoresis, and recovered the DNA fragments from the gel.

The purified DNA samples were directly sequenced in both strands using an automatic sequencer (model 3100; Applied Biosystems Japan, Tokyo, Japan) with a Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Japan, Tokyo, Japan). For exon 3, the forward sequencing primer (5′-tcctggga gaggtggctggtt-3′) was set inner the forward PCR primer, and the reverse sequencing primer was the same as the reverse PCR primer. For exon 5, the reverse sequencing primer (5′-ataatggggttgtagatagt-3′) was set inside the amplified region, and the forward sequencing primer was the same as the forward PCR primer. When two or three of the three critical amino acid sites (180, 277, and 285) were heterozygous, we assumed that their combination (i.e., haplotype) in each allele matched one of the known combinations.

When we examined the three-site composition for each sample, we also examined DNA sequences surrounding the sites and checked for contamination of other sequences (including human contamination), using as references the complete DNA sequences of the five M/LWS alleles determined for the capuchins and spider monkeys in this study (see below). When contamination was found despite the precautions taken, those samples were not included in further analyses. Only when we obtained DNA sequences of both exons 3 and 5 from a fecal sample did we accept the results from the sample as genotype data. In addition, only when we obtained identical genotyping results from two different fecal samples collected from the same individual (individual identity was established through visual examination of the defecating monkeys) did we consider that the genotype of the individual was determined. Furthermore, when a female was typed as homozygous by two different samples, we required that at least one sample contain no less than 200 pg of genomic DNA in the PCR, according to the genotyping criteria set in a microsatellite analysis of hair and fecal samples [Morin et al., 2001], to minimize the chance of missing one of the two alleles (i.e., allelic dropout) due to low concentration of genomic DNA. As for the three capuchins with only skin samples, both exons 3 and 5 were sequenced and the typing results from one sample were accepted as ''determined,'' because individual identities of the samples were certain and DNA recovery from the skin samples was comparable to that achieved with tissue samples, such as blood.

Quantification of Genomic DNA Amount

For females that were homozygous for the M/LWS pigment gene, we quantified the recovered genomic DNA from feces by the real-time PCR method based on the principle of the 5′ nuclease assay [Morin et al., 2001]. Using human genomic DNA of known concentration as a standard, one can quantify the amount of genomic DNA obtained from a test species by comparing the amplification of a marker gene that is highly conserved between the two species. The short-wavelength sensitive (SWS, or blue) visual pigment gene is a single-copy gene that is located on an autosome (chromosome 7 in humans) and is highly conserved among primates [Shimmin et al., 1998]; therefore, it is suitable for the marker.

Human placental DNA of known quantity (Sigma-Aldrich Japan, Tokyo, Japan) was diluted into a series of concentrations: 10,000 pg/μl, 1,000 pg/μl, 100 pg/μl, 10 pg/μl, and 0 pg/μl (containing buffer only). We carried out real-time PCR for the SWS gene using human DNA as the template (Smart Cycler System; Cepheid, Sunnyvale, CA). The amount of PCR product was monitored

through progression of PCR cycles by fluorescence intensity of a fluorophore FAM released by 5′-exonuclease activity of Taq DNA polymerase from the 5′-end of a oligonucleotide probe prehybridized to the SWS gene region to be amplified. The cycle number at which the second derived function of the fluorescence intensity gives the highest peak was defined as the threshold cycle that most effectively reflects the initial amount of the target DNA. Threshold cycle numbers were then plotted against the concentration series of the human DNA. By using the plot as a standard regression line (the values of the correlation coefficient were between –0.98 and –1.0), we evaluated the concentrations of the test DNA from their threshold cycles.

We amplified a 72-bp portion in exon 4 of the SWS gene by the primer pairs, 5′-acccagaaggctgaacgggaggt-3′ (forward) and 5′-acgtagcagacacagaaggat-3′ (reverse). The probe, 5′-agccgcatggtggttgtgatggta-3′, was synthesized with a 5′-FAM reporter dye and a 3′-TAMRA quencher dye (Takara, Shiga, Japan). The primer and probe sequences are those of the human SWS gene [Nathans et al., 1986] (GenBank accession no. U35874), and we confirmed that all of the sequences were completely conserved in our test species (*Cebus capucinus* and *Ateles geoffroyi* from Santa Rosa) by PCR using primers set outside the 72-bp region (data not shown). We carried out PCR in 25 μl containing 1 × buffer, 0.3 mM dNTPs, 1.5 mM $MgCl_2$, 0.3 μM each of the primers, 0.2 μM probe, 1 unit of Ex Taq R-PCR (Takara, Shiga, Japan), and 2.5 μl DNA extract from feces with initial incubation for 150 sec at 95°C followed by 50 cycles of 30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C, and 8 sec at 80°C (for fluorescence measurement). Five reactions were carried out for 10 pg/μl standard because of its relatively variable performance in amplification. For the 0 pg/μl standard, only one reaction was conducted. For all of the other reactions, including both the standard and the test, we conducted triplet PCR.

Determination of Full-Length Nucleotide Sequences of M/LWS Gene Alleles

To obtain the entire coding sequences of all the alleles found in this study, we PCR-amplified all exons of each allelic type from the fecal genomic DNA of three male capuchins with P530, P545, and P560, and two male spider monkeys with P552 and P560. The primer pairs used to amplify the exons were as follows: exon 1, 5′-ggacagggctttccatagcc-3′ and 5′-tggcctccatgggcctgtggc tcac-3′; exon 2, 5′-tctctcctctgcctcctgcccgcag-3′ and 5′-tgagcctgggccctgactggcttac-3′; exon 3, 5′-gtctgtctgctctcccctag-3′ and 5′-actccgtcttgcacccttac-3′; exon 4, 5′-actgg ctgccggcccttctctccag-3′ and 5′-agccaggagggaccggggtgctta-3′; exon 5, 5′-accgtccct gtctccctcag-3′ and 5′-ctgctgatggtgttgcttac-3′; and exon 6, 5′-gctcaatcactttctgtcct tccag-3′ and 5′-ggacgggtaggaggcagacc-3′. All primers were set outside the coding regions and designed according to previously sequenced marmoset or owl monkey M/LWS pigment genes [Kawamura et al., 2001, 2002]. We conducted the PCR at 98°C for 5 min followed by 40 cycles at 98°C for 45 sec, 56°C for 5–30 sec, and 72°C for 30 sec. The amplified DNA fragments were purified and directly sequenced in both strands using the PCR primers. We confirmed their nucleotide sequences in duplicate PCR experiments. These sequences have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession nos. AB193772 (*C. capucinus* P560), AB193778 (*C. capucinus* P545), AB193784 (*C. capucinus* P530), AB193790 (*A. geoffroyi* P560), and AB193796 (*A. geoffroyi* P552).

Reconstitution of the M/LWS Visual Pigments

We synthesized cDNA clones encoding amino acid sequences of the capuchin M/LWS opsin alleles by site-directed mutagenesis from opsin cDNA clones of squirrel monkey and common marmoset that were previously isolated [Hiramatsu et al., 2004; Kawamura et al., 2001]. We created a cDNA clone of the capuchin P530 by introducing mutations G-to-A at the coding-nucleotide site 673 (denoted G673A) and A835G to the squirrel monkey P530 cDNA, resulting in a valine-to-isoleucine substitution at residue 225 (denoted Val225Ile) and Ile279-Val, respectively. The capuchin P545 cDNA was created by introducing T193G, G331A, G484A, G673A, G823C, A835G, and G853A to the squirrel monkey P530, resulting in Phe65Val, Val111Ile, Val162Met, Val225Ile, Val275Leu, Ile279Val, and Ala285Thr. We created a cDNA clone of the capuchin P560 by introducing C181G, G193T, and G823A to the marmoset P560 cDNA, resulting in Leu61Val, Val65Phe, and Val275Met.

We recloned these cDNA clones into the pMT5 expression vector [Khorana et al., 1988], transfected the expression constructs into the cultured COS-1 cells (Riken Cell Bank, Tsukuba, Japan), reconstituted the photopigments by incubating the resulting proteins (opsins) with 11-*cis* retinal (Storm Eye Institute, Medical University of South Carolina, Charleston, SC), and purified the reconstituted pigments using the immobilized 1D4 antibody (Cell Culture Center, Minneapolis, MN) by following the method of Kawamura and Yokoyama [1998]. We recorded the absorption spectra of the reconstituted visual pigments from 250 to 750 nm at 0.5-nm intervals using the Hitachi U3010 dual beam spectrometer at 20°C for five times in the dark, and five more times after 3 min of light exposure, as described by Kawamura and Yokoyama [1998].

RESULTS

M/LWS Visual Pigment Alleles of Capuchins and Spider Monkeys at Santa Rosa

In white-faced capuchins at Santa Rosa, three M/LWS pigment alleles were found on the basis of amino acid compositions at three critical sites for spectral tuning: 180, 277, and 285 [Hiramatsu et al., 2004; Yokoyama & Radlwimmer, 1999]. As expected, one was the common type P560 and two were the squirrel monkey types P545 and P530 (see Fig. 2). The same three types have been identified in congeneric wedge-capped capuchins (*Cebus olivaceus*) [Shyue et al., 1998] (note that an alternative species name, *C. nigrivittatus*, is given in Shyue et al. [1998]). In the reconstructed phylogenetic tree (Fig. 3), these two species clustered by every allelic type, demonstrating that the collected DNA sequences were indeed those of the study animals and were not the result of contamination.

On the other hand, two alleles were found in black-handed spider monkeys at Santa Rosa. This observation of two alleles was consistent with previous electrophysiological observations [Jacobs & Deegan, 2001], and one allele was, as expected, the common type P560. However, the amino acid composition in the other allele was Ser, Phe, and Thr at sites 180, 277, and 285, respectively, and was novel among all vertebrate M/LWS pigments examined to date for the site composition [Yokoyama & Radlwimmer, 2001]. Its λmax was predicted to be 552 nm according to the three-site rule (Fig. 2) [Hiramatsu et al., 2004; Yokoyama & Radlwimmer, 1999]. In the phylogenetic tree (Fig. 3), the two alleles of the spider monkey were most closely related to the capuchin-marmoset clade, and formed a sister group with them separately from human genes. This is consistent

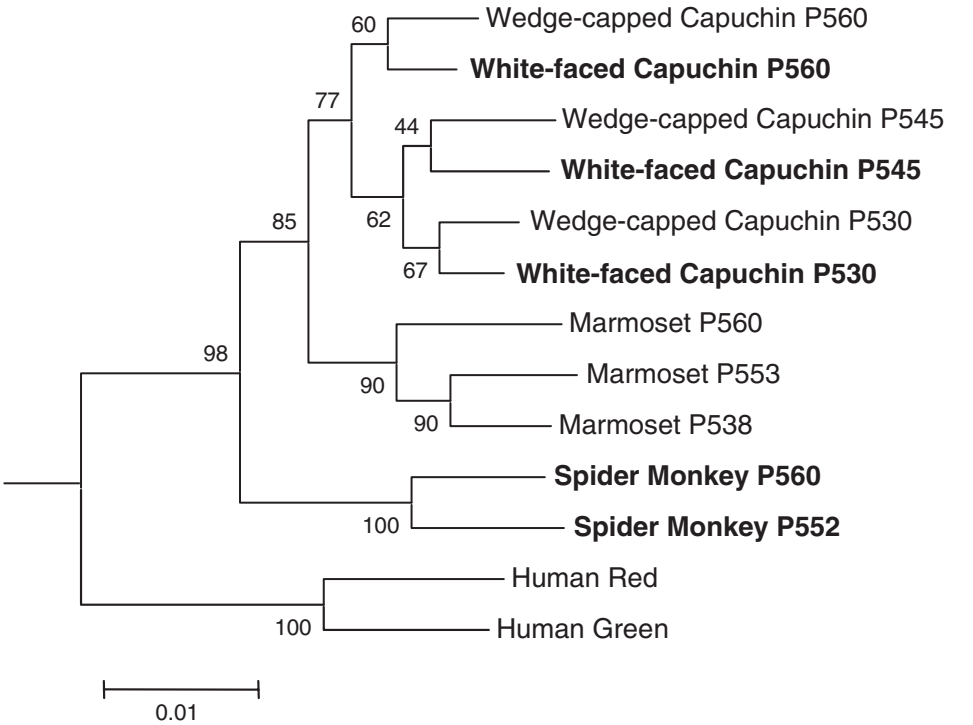

Fig. 3. A phylogenetic tree reconstructed from entire coding nucleotides of M/LWS visual pigment genes from selected New World monkeys and humans. The number of nucleotide substitutions per site (*d*) for two sequences was estimated by the method of Tamura and Nei [1993], and the phylogenetic tree was reconstructed by applying the the neighbor-joining method [Saitou & Nei, 1987] to the *d* values using the MEGA2 program version 2.1 [Kumar et al., 2001; Nei & Kumar, 2000]. The reliability of the tree topology was evaluated by bootstrap analysis with 1,000 replications, and the bootstrap probabilities are given to each node. The phylogenetic root was given by a prosimian (*Otolemur crassicaudatus*) M/LWS pigment gene [Kawamura & Kubotera, 2003] (GenBank AB112590). Genes sequenced in this study are highlighted with boldface letters. Scale bar, one nucleotide substitution per 100 sites. GenBank accession numbers: wedge-capped capuchin (*Cebus olivaceus*) P560, AF051618-23; wedge-capped capuchin P545, AF051624-9; wedge-capped capuchin P530, AF051630-5; marmoset (*Callithrix jacchus*) P560, AB046546; marmoset P553, AB046547; marmoset P538, AB046548; human red, M13300-5; human green, M13306, K03490-4.

with the accepted species phylogeny among them [Goodman et al., 1998; Schneider, 2000] and again is evidence against contamination.

To test the validity of the three-site prediction of λmax, the three allelic pigments of the capuchin were reconstituted in vitro. The absorption spectra of the reconstituted pigments were indeed concordant with the prediction from the three-site composition, with λmax of P530, P545, and P560 being 532 ± 1, 543 ± 1, and 561 ± 0.4 nm, respectively (Fig. 4). When the pigments were exposed to light, they showed an absorbance peak at around 380 nm that appeared as a negative peak in dark–light difference spectra (Fig. 4A–C insets), indicating that 11-*cis* retinal in the pigments was isomerized by light, all-*trans* retinal was released, and the reconstituted pigments were indeed photoreactive.

For females that we determined possessed only one allelic type in two different fecal samples, we assayed genomic DNA concentrations in the samples by the real-time PCR method, using the SWS visual pigment gene as a marker. In the majority of such monkeys, at least one sample contained over 200 pg of

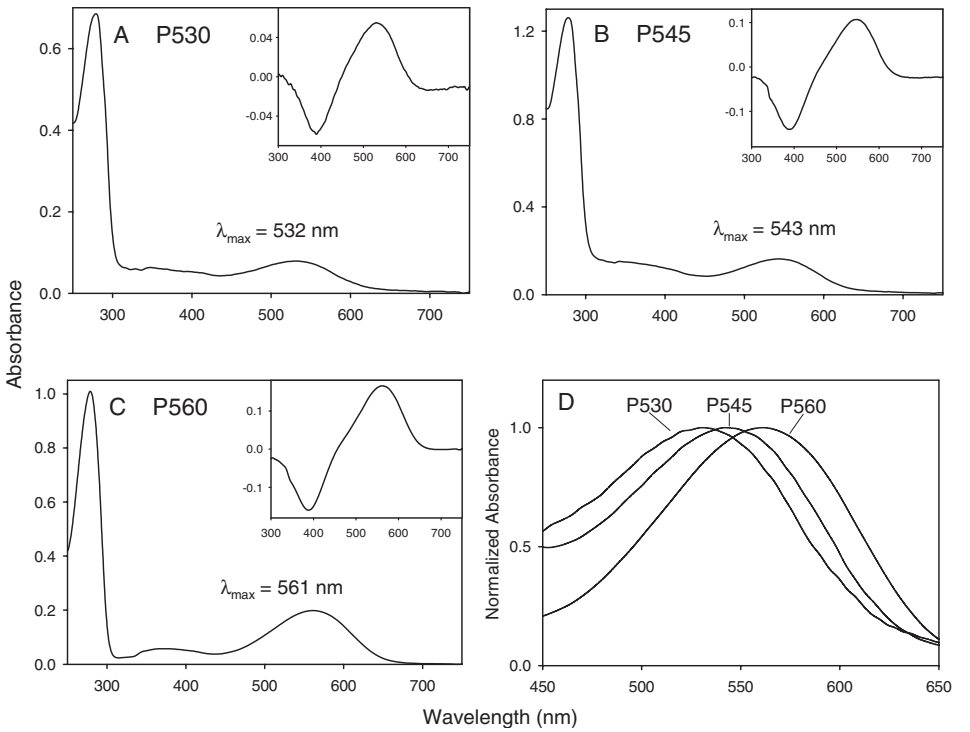

Fig. 4. Absorption spectra of P530 (**A**), P545 (**B**), and P560 (**C**) M/LWS visual pigments of white-faced capuchin reconstituted in vitro. Insets in **A–C**: dark–light difference absorption spectra. The λmax values were directly taken from the dark absorption spectra. **D:** Normalized absorbance of the three pigments presented together, with the peak height adjusted to one.

their genomic DNA in the 5-μl DNA extract (the volume used for one PCR experiment of color vision typing). Samples from three capuchin monkeys were all below the standard. For these monkeys, we adjusted the template DNA amount to 200 pg and repeated the PCR experiments. No additional sequence was detected in these samples, and we concluded that these females were indeed homozygous. It was noted that recovery of spider monkey DNA was generally stable and high, but that of the capuchins varied greatly from sample to sample. This variation does not appear to be associated with the preservation methods used for the samples, and probably reflects dietary differences between the two species (capuchins are omnivorous, whereas spider monkeys are mostly frugivorous). However, contrary to a note by Surridge et al. [2002], seedy feces were not necessarily poorer for DNA recovery than others, and at this point it is difficult for us to point out which features of the fecal samples are critical for DNA recovery.

Phenotypic Compositions and Allele Frequencies

In the LV group of capuchins, the color vision type of 19 out of 29 group members (66%) has been determined thus far (Table I). Of the individuals typed, five out of six females (83%) were trichromats, with all three possible combinations (P530/P545, P545/P560, and P530/P560) observed. As expected,

all of the males examined were dichromats. To date, no P530 dichromat has been identified in the group. The allele frequencies of P530, P545, and P560 were 12%, 28%, and 60%, respectively, in a total of 25 X chromosomes examined for the group (Table II). Although we still need to analyze the remaining 13 chromosomes and perform statistical tests for confirmation (see Table I), longer-wave alleles appeared to be more abundant than shorter-wave alleles (P530:P545:P560 was approximately 1:2:4). However, these results demonstrate that the three alleles can exist in a natural group of capuchin monkeys with phenotypic variations in both dichromats and trichromats.

In contrast, the CP capuchin group was found to consist of only dichromats, the majority of which were the P560 type (Table I). There was only one P530 dichromat in this group, a male that emigrated recently. The allele frequency of P560 was 96% in a total of 28 X chromosomes examined (Table II). In the CP group, 17 out of 20 group members (85%) were typed, and the color vision type of three females is as yet undetermined. Sequence results were obtained from one sample each from two of the three monkeys, and they were both P560. The third monkey is an infant that was recently born in the CP group. Therefore, the future

TABLE I. Observed Numbers of Individuals with Each Color Vision Phenotype in Capuchin and Spider Monkey Populations at Santa Rosa

	Capuchin group LV		Capuchin group CP		Spider monkey	
Phenotype	Female	Male	Female	Male	Female	Male
Dichromat						
P530	0	0	0	1	n/a	n/a
P545	0	3	0	0	n/a	n/a
P560	1	10	11	5	6	2
P552	n/a	n/a	n/a	n/a	3	1
Trichromat						
P530/P545	2	0	0	0	n/a	n/a
P545/P560	2	0	0	0	n/a	n/a
P530/P560	1	0	0	0	n/a	n/a
P552/P560	n/a	n/a	n/a	n/a	8	0
Total	6	13	11	6	17	3
(To be determined[a]	3	7	3	0	0	2)

[a]Including one male infant in LV and on female infant in CP whose samples have not been collected. Other individuals are the ones for which the second sample is to be examined.

TABLE II. Allele Frequencies of M/LWS Visual Pigments in Capuchin and Spider Monkey Populations at Santa Rosa

Allele type	Capuchin LV	Capuchin CP	Spider monkey
P530	0.12	0.04	n/a
P545	0.28	0	n/a
P560	0.60	0.96	0.59
P552	n/a	n/a	0.41
(X chromosome[a]	25	28	37)

[a]Number of X chromosomes examined.

completion of typing these individuals is unlikely to significantly change the allelic frequencies in this group.

In the spider monkey population, 20 out of 22 group members (91%) were typed, and two alleles (P552 and P560) were found (Table I). These alleles occurred at frequencies of 41% and 59%, respectively, in a total of 37 X chromosomes examined (Table II). However, as with the LV group of capuchins, there was a tendency for the longer-wave allele (P560) to be more abundant than the shorter-wave allele (P552). About half of the females typed (8/17) were trichromats (i.e., P552/P560) (Table I).

In all of our capuchin and spider monkey samples, the results of color vision typing were concordant with the kin relationships determined thus far through known maternal lines and partial microsatellite analyses (data not shown). Recombination of allele haplotypes has been reported in capuchins [Boissinot et al., 1998] and squirrel monkeys [Cropp et al., 2002]. For female trichromatic capuchins with P530/P560 and P545/P560, distinguishing the two alleles requires cloning of a DNA segment (~4 kb) encompassing the exons 3 and 5 for each allele from the individuals (see Fig. 2), which would not be feasible for fecal DNA. However, no recombinant allele was found in males, homozygous females, or heterozygous females with other allelic combinations in our study groups. This and the consistency of allele inheritance with the known kin relationships strongly suggest that no recombinant allele is present in the study groups examined to date.

DISCUSSION

We conducted color vision typing for two groups of capuchins (*Cebus capucinus*) and one group of spider monkeys (*Ateles geoffroyi*) inhabiting Santa Rosa National Park in Costa Rica by examining their M/LWS visual pigment genes, mostly in fecal samples. On the basis of amino acid compositions at three critical sites for spectral tuning (sites 180, 277, and 285) [Hiramatsu et al., 2004; Yokoyama & Radlwimmer, 1999], three allelic M/LWS pigment types (P530, P545, and P560) were found in capuchins, and two (P552 and P560) were found in spider monkeys. These numbers of alleles are concordant with the published information regarding allelic compositions in these two genera [Jacobs & Deegan, 2001, 2003]. However, the three-site composition of the spider monkey P552 was found to be a novel one (Fig. 2). The predicted λmax of the three capuchin pigments was confirmed in a reconstitution experiment for the pigments. We further showed that color vision polymorphism was indeed present in natural groups (of 20–30 members in both species), but that allelic compositions sometimes varied greatly among the groups.

In a study of spider monkeys (*Ateles geoffroyi*) in southwest Costa Rica, Riba-Hernandez et al. [2004] postulated the presence of squirrel monkey type alleles P530 and P545, as well as P560 in the spider monkey population, on the basis of an unpublished sequencing observation of M/LWS genes (their λmax values are given as 535, 550, and 562 nm, respectively, in Riba-Hernandez et al. [2004]). However, P552, which was found at our study site, was not reported in their study. The P552 allele may have arisen by a recombination of P545 and P560 resulting in the exchange of exon 3, or by a replacement of Tyr at site 277 to Phe in P560 (see Fig. 2). In future studies, spider monkey pigments should be reconstituted and measured for their absorption spectra to assess the applicability of the three-site rule to the spider monkey system. In comparison with Cebidae monkeys (Cebinae, Callitrichinae, and Aotinae), less is known about the color

vision of Atelidae, except for howler monkeys (*Alouatta*) which are nontypical New World monkeys with routine trichromatic color vision [Jacobs et al., 1996]. In an electrophysiological study, Jacobs and Deegan [2001] reported that woolly monkeys (*Lagothrix*), a closely related genus to spider monkeys, had a set of two M/LWS alleles similar to those of spider monkeys; however, their DNA sequences remain to be determined. Studies of M/LWS genes in different populations and congeneric species of spider monkeys and other atelid species, including woolly monkeys, would elucidate the origin and evolution of M/LWS alleles in Atelidae.

In the CP group of capuchins, the allele frequency of P560 was found to be close to 100%, and vision type was biased to P560 dichromats. From our long-term observations, we know that one male has occupied the alpha position for 11 years and has fathered 100% of the infants tested for paternity, including all of the present adult females [Jack & Fedigan, 2004, in press]. This is not typical for matrilineal monkeys, including our other capuchin study groups, and we suspect that the CP group has been genetically homogenized by the close inbreeding. In the LV group of capuchins, male dispersal is common and the alpha male is replaced every 4 years [Fedigan & Jack, 2004]. Although the case of the CP group may be fairly rare and influenced heavily by inbreeding, it alerts us that caution should be used in interpreting the gene frequency data of a natural population solely by natural selection, and that it is necessary to also consider its social structure.

Our planned comparison of behaviors across phenotypes, groups, and species in a natural environment will complement earlier studies on vision–behavior relationships in callitrichine species [Caine, 2002; Caine & Mundy, 2000; Caine et al., 2003; Pessoa et al., 2003; Smith et al., 2003a,b] and will help to illuminate the utility of color vision in wild New World monkeys. It is also essential to collect spectral data on visual objects under various irradiance conditions and to evaluate the observed vision–behavior associations in the light of models predicting differences among vision types in detecting visual objects with various spectral properties. Such models have been developed for primates in recent years [Sumner & Mollon, 2000a,b, 2003; Dominy & Lucas, 2001, 2004; Dominy et al., 2003; Lucas et al., 2003; Riba-Hernandez et al., 2004]. The collection of fecal DNA in this study also enabled us to conduct a population-genetics analysis of M/LWS allelic diversity in not only the three spectral tuning sites, but in other parts of coding sequences and introns as well. Together with results from behavioral observations, these findings will shed new light on the evolutionary forces, such as the nature of balancing selection (overdominance vs. frequency dependence), gene conversion, migration, inbreeding, and population size reduction/expansion, that act on alleles in natural populations.

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