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Allelic Variation in the Squirrel Monkey X-Linked Color Vision Gene: Biogeographical and Behavioral Correlates

Susan Cropp,[1,2] Sue Boinski,[3] Wen-Hsiung Li[1]

[1] Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, IL 60637, USA
[2] Department of Anatomy & Neurobiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA
[3] Department of Anthropology, 1112 Turlington Hall, P.O. Box 117305, University of Florida, Gainesville, FL 32611-7305, USA

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Abstract. Most Neotropical primate species possess a polymorphic X-linked and a monomorphic autosomal color vision gene. Consequently, populations are composed of both dichromatics and trichromatics. Most theories on the maintenance of this genetic system revolve around possible advantages for foraging ecology. To examine the issue from a different angle, we compared the numbers and relative frequencies of alleles at the X-linked locus among three species of *Saimiri* representing a wide range of geographical and behavioral variation in the genus. Exons 3, 4, and 5 of the X-linked opsin gene were sequenced for a large number of X chromosomes for all three species. Several synonymous mutations were detected in exons 4 and 5 for the originally reported alleles but only a single nonsynonymous change was detected. Two alleles were found that appeared to be the result of recombination events. The low occurrence of recombinant alleles and absence of mutations in the amino acids critical for spectral tuning indicates that stabilizing selection acts to maintain the combinations of critical sites specific to each allele. Allele frequencies were approximately the same for all *Saimiri* species, with a slight but significant difference between *S. boliviensis* and *S. oerstedii.* No apparent correlation exists between allele frequencies and behavioral or biogeographical differences between species, casting doubt on the speculation that the spectral sensitivities of the alleles have been maintained because they are specifically well-tuned to *Saimiri* visual ecology. Rather, the spectral tuning peaks might have been maintained because they are as widely spaced as possible within the limited range of middle-wave to longwave spectra useful to all primates. This arrangement creates a balance between maximizing the distance between spectral tuning peaks (allowing the color opponency of the visual system to distinguish between peaks) and maximizing the number of alleles within a limited range (yielding the greatest possible frequency of heterozygotes).

Key words: *Saimiri* — Opsin polymorphism — Dichromacy — Trichromacy

Introduction

All Neotropical primates surveyed to date, with the exception of howler monkeys (genus *Alouatta*), possess a single X-linked color photopigment gene (Jacobs et al. 1996; Boissinot et al. 1998; Hunt et al. 1998). The locus is known to possess three highly polymorphic alleles that code for photopigments with spectral sensitivity maxima around 535–543 nm (green opsin), 550–556 nm (yellow opsin), and 561–562 nm (red opsin) (Jacobs 1996a). Although the alleles differ in amino acid sequence at a number of sites, five residues are primarily responsible for their differences in spectral sensitivity. Substitutions at amino acid positions 180, 277, and 285 cause a shift of

Correspondence to: Susan Cropp, Department of Anatomy & Neurobiology, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA; *email:* cropp@pcg.wustl.edu

~5, 8, and 15 nm in spectral sensitivity, respectively, while those at positions 229 and 233 cause a shift of only 1–3 nm (Neitz et al. 1991; Jacobs et al. 1993; Shyue et al. 1998). Together with the autosomal color photopigment gene (blue opsin), this triallelic system confers trichromatic vision to females heterozygous at the X-linked locus. Males and homozygous females have dichromatic vision.

The amino acid variation at the X-linked locus is remarkably similar across the Neotropical taxa studied thus far, suggesting that the polymorphism originated in the ancestral lineage of New World primates. Although slight differences in spectral tuning of the middlewave (MW) and longwave (LW) pigments exist between species, they all use a common set of substitutions at three particular amino acid sites to affect the shift from MW to LW (Hunt et al. 1998). Within species, pairwise divergences in intron 4 sequences between alleles have been found to range between 2 and 4%, considerably higher than the average amount of divergence between two allelic sequences in humans (0.1%). However, despite the large differences in intronic sequences, it appears that frequent homogenization events have occurred between alleles. The preservation of the critical amino acids defining the three alleles in the face of repeated homogenizations suggests that balancing selection is playing a role in maintaining the specific character of each allele (Shyue et al. 1995).

The traditional view of the origin of primate color vision postulates that trichromacy evolved as an advantage for frugivory (Polyak 1957; Mollon 1991; Regan et al. 1998), because trichromacy facilitates the detection of colored fruits amongst dappled foliage (Mollon 1989; Osorio and Vorobyev 1996). Indeed, in a behavioral test of the advantages of trichromacy, Caine and Mundy (1999) demonstrated that trichromatic marmosets (*Callitrix geoffroyi*) were better able to detect orange-colored items than dichromats. Trichromacy could also allow a frugivorous primate to assess the ripeness of a particular fruit visually, a task which would otherwise be accomplished by time-consuming manual manipulation. An alternative theory is the evolution of trichromatic color vision as an aid to folivory (Lucas et al. 1997). In a study of four Old World trichromatic primate species, Dominy and Lucas (2001) found that many fruits chosen for consumption could be distinguished from background foliage using the yellow–blue color channel common to all dichromatic species. In contrast, the red–green channel was needed to detect the immature leaves commonly consumed by these primates. Similarly, Sumner and Mollon (2000a) demonstrated that primate trichromacy was not necessarily optimized for locating fruits but, rather, for locating among the foliage anything that was *not* a mature leaf.

Trichromatic vision is not a necessary condition for Neotropical primates to detect food items in their natural habitats (Sumner and Mollon 2000a). It has been shown that dichromatic individuals may be better able to detect color-camouflaged objects (Morgan et al. 1992), such as the cryptic arthropods and small vertebrates that are ingested by many species of Neotropical primates (DeVries 1987; Nickle and Heymann 1996; Enterovick et al. 1997). However, while not vital to foraging ability, trichromacy does facilitate the location of fruits and young leaves (Sumner and Mollon 2000a, b), possibly conferring a slight advantage for this task to those individuals possessing it. Therefore, a trichromatic individual would have a foraging advantage over a dichromatic one and overdominant selection should act to maintain polymorphism at the X-linked locus in a population. If polymorphism is maintained at the X-linked locus by heterozygote advantage, one might expect to find additional alleles at this locus, as this would increase the frequency of heterozygotes (Shyue et al. 1995). Also, habitat differences among monkeys from distinct biogeographical areas may be reflected in variation in the frequency and number of X-linked color photopigment alleles. However, to date, there have been no detailed studies of the genetic architecture of color vision among Neotropical primate populations from different biogeographical regions.

Squirrel monkeys are Neotropical primates of the genus *Saimiri* (Platyrrhini, Primates). Members of the genus are found primarily in tropical lowland rainforest throughout the Amazon basin, with a southern extreme of Paraguay and a northern extreme of Guyana, and a small, disjunct distribution of *S. oerstedii* in Central America (Fig. 1A) (Hershkovitz 1984; Thorington 1985). All *Saimiri* species are ecologically similar with respect to their habitat preferences, positional behavior, and food preferences (soft fruits, arthropods, and small vertebrates) (Terborgh 1983; Boinski 1987a, 1988, 1989; Janson and Boinski 1992). Nevertheless, significant biogeographical differences do exist across the range of the genus in the predominant plant families that bear the small, soft fruit that squirrel monkeys ingest (Gentry 1988; Terborgh and Andresen 1998). These taxonomic differences in available food resources appear to engender marked divergences in both social organization and food competition regimes among squirrel monkeys from distinct biogeographical regions (Boinski and Cropp 1999; Mitchell et al. 1991; Boinski 1999a, b). The invertebrate prey harvested by squirrel monkeys are distributed throughout the forest foliage at such low densities that aggressive defense or acquisition is rarely an economic strategy throughout the distribution of *Saimiri* (Boinski 1988; Mitchell 1990). On the other hand, fruit is typically available in patches of varying size and density across biogeographical regions, and the resultant differences in the defendability of these patches appear to underlie the marked differences in social relationships among troop members. Seasonal variation in fruit avail-

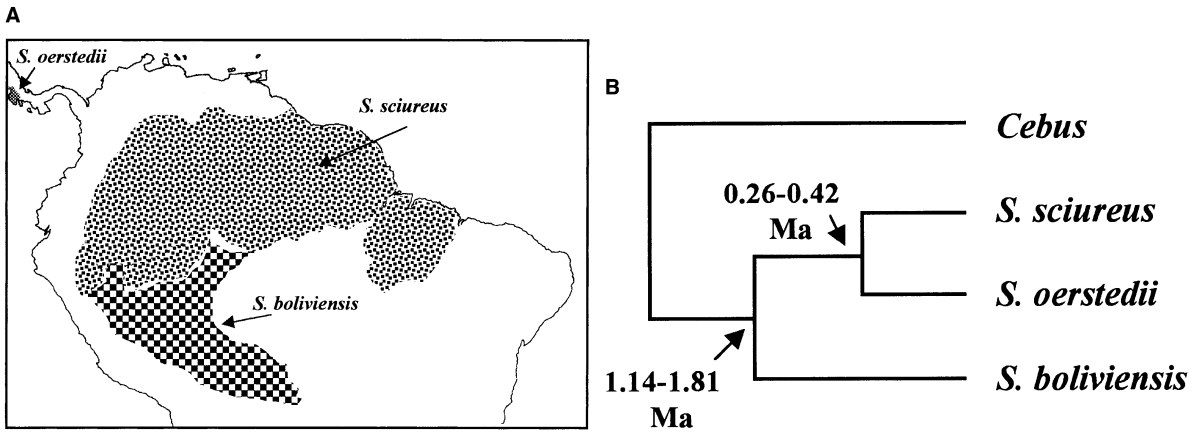

Fig. 1. **(A)** Geographic distribution (Hershkovitz 1984; Thorington 1985; Rylands et al. 1995) and **(B)** phylogenetic relationships and divergence times (Boinski and Cropp 1999; Cropp and Boinski 2000) for the three *Saimiri* species used in this study.

ability is equally marked between regions, with *Saimiri* in some regions having suitable fruit available throughout most of the year; in such areas, squirrel monkeys are much more frugivorous. In regions where fruit can be harvested only about half of the year, invertebrates predominate in the dietary intake of *Saimiri.*

Three species of squirrel monkeys, *S. boliviensis, S. oerstedii,* and *S. sciureus,* encompass much of the geographic and behavioral variation in the genus (Boinski and Cropp 1999). The Central American squirrel monkey, *S. oerstedii,* has an egalitarian social organization, with neither sex apparently dominating the other and negligible direct competition for food (Boinski 1987a, 1988). In this region, the plant families that bear the fruit preferred by squirrel monkeys typically grow in the form of shrubs or small trees, and only a few pieces of fruit per plant tend to ripen each day (Gentry 1988). As a result, food patches are small and widely dispersed. Female social bonds are weak. Males are integrated into the troop and usually remain in their natal troop, while females emigrate before their first mating season (Boinski and Mitchell 1994; Boinski and Cropp 1999). In Suriname, squirrel monkeys (*S. sciureus*) also exhibit weak female social bonds, but males are dominant to females, and direct food competition is frequent and intense (Boinski and Cropp 1999), with individuals vigorously defending access to food patches (Boinski 1999a). Due to the stringent seasonal availability of fruit resources for squirrel monkeys in Suriname, *S. sciureus* is more insectivorous than the other two species and subsists largely on invertebrates for periods extending as long as 6 to 7 months at a time (Boinski 1999a, unpublished data). Recent studies show that *S. sciureus* males emigrate from their natal troops, as does a low percentage of the females (Boinski, unpublished data). *Saimiri boliviensis* display yet another type of social organization, with females being dominant to males and forming kin-based matrilines that cooperate in the acquisition and defense of food (Mitchell 1990, 1994; Mitchell et al. 1991). Fruit resources are more consistently available throughout the annual cycle within the range of *S. boliviensis* and, therefore, compose a higher percentage of their dietary intake (Mitchell et al. 1991; Terborgh 1983). Consequently, trichromacy might confer a greater advantage to these squirrel monkeys than to their northern counterparts. In this species, males typically are excluded by females from the troop core to the periphery of the troop. Also in contrast to the other two species, *S. boliviensis* males are the only sex to emigrate, while all females remain with their natal troops (Mitchell 1994).

The phylogenetic relationships and divergence times between these three species have been reconstructed from molecular and fossil data (Fig. 1B) (Boinski and Cropp 1999; Cropp and Boinski 2000). Thus, *Saimiri* is a propitious study system for examining the evolution of color vision genes to elucidate the roles played by molecular-level processes (i.e., mutation, gene conversion, and recombination) and natural selection in causing and maintaining genetic diversification. Specifically, the goals of this study were to compare the number and relative frequencies of alleles at the X-linked color vision locus between squirrel monkeys from diverse biogeographical regions and to determine whether allele frequency differences (or lack thereof) correspond to differences in behavior and food resource biogeography among *Saimiri.*

Materials and Methods

Samples

Three species of squirrel monkeys were included in this analysis: *S. boliviensis* from Peru and Bolivia, *S. oerstedii* from Costa Rica, and *S. sciureus* from Suriname and Guyana. Samples of *S. boliviensis* were generously provided by Dr. Lawrence Williams (University of South Alabama, Mobile), Dr. Jean Dubach (Brookfield Zoo, Chicago, IL), and Dr. Pat Gullett (University of Miami Primate Center, Miami, FL). The remaining samples were collected by SB and her assistants. Genomic DNA was extracted from hair follicles using the tissue protocol

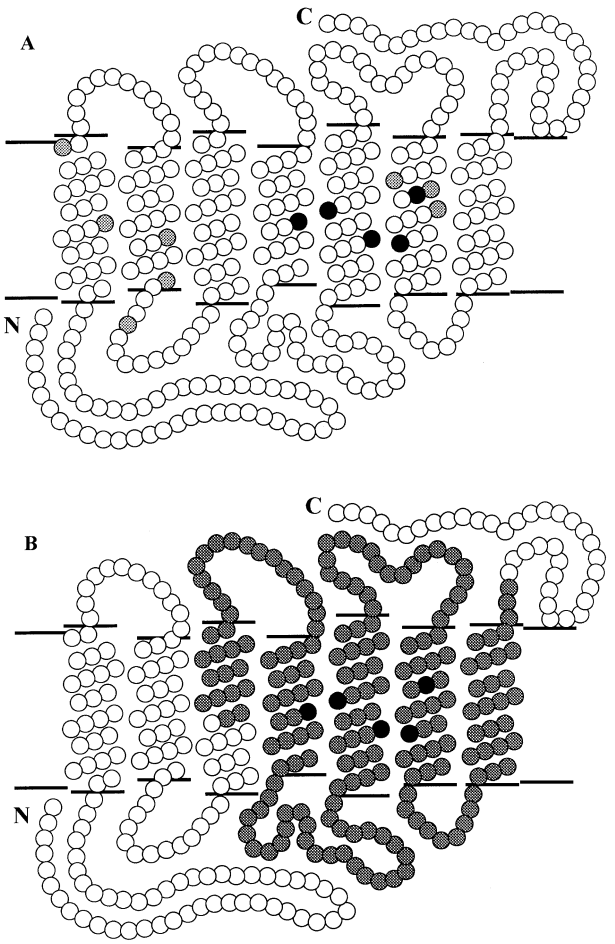

Fig. 2. Model of the squirrel monkey X-linked color photopigment gene. *Lines* represent the cell membrane and *circles* represent individual amino acids. N, N terminus; C, C terminus. **A** *Gray circles* indicate amino acid positions that differ among the three alleles for this locus. *Black circles* denote the critical amino acids responsible for spectral tuning differences among the three alleles. **B** *Gray circles* represent the amino acid positions sequenced in this study. These sites encompass exons 3, 4, and 5 of the gene. The critical amino acids are shown as *black circles.*

of the QIAamp DNA Mini kit (Qiagen Inc., Chatsworth, CA). Both subspecies of *S. boliviensis* were included in the analysis, as were both subspecies of *S. oerstedii.*

DNA Amplification and Sequencing

In squirrel monkeys, the three alleles for the X-linked color vision gene differ at a total of 13 amino acid positions, 5 of which have been found to be responsible for spectral tuning differences between alleles (Fig. 2A) (Shyue et al. 1998). Since these critical amino acid residues are encoded in exons 3, 4, and 5 of the gene, we amplified and sequenced those three exons (Fig. 2B), for a total of 93 X chromosomes from *S. boliviensis,* 70 X chromosomes from *S. oerstedii,* and 98 X chromosomes from *S. sciureus* (Table 1). Each exon was amplified and sequenced individually with primers designed to anneal to the intronic regions flanking the exons. The primers for exon 3 (SMCV1, 5′-GGAAGGAAGCAGTGATGTCGGAGGCT-3′; and SMCV3, 5′-CCTCAAGGTCACAGAGTCTGACCCT-3′) and exon 4 (SMCV5, 5′-GCCGGCCCTTCTCTCCAG-3′; and SMCV2, 5′-TGATTCAGGGGCAGAGAAGCTTAGGG-3′) were designed specifically for this study, while those for exon 5 (I5-5, 5′-CTGGG TCACCTGCCTCTTGC-3′; and I5-3, 5′-TCAGAGACATGATTC-CAGGTGG-3′) were designed by Shyue (1994). Double-stranded amplifications were performed for 30 cycles under a regime of 94°C for 35 s, 58–60°C for 35 s, and 70°C for 1 min.

Sequencing was accomplished using either a *fmol* cycle sequencing kit (Promega Corp., Madison, WI) and ^{32}P-radiolabeled dATP (NEN, Boston, MA) or the BigDye™ fluorescent labelling kit (PE Biosystems, Foster City, CA) and an ABI 377 automatic sequencer. Exons were sequenced in both directions to ensure accuracy. The sequences obtained in the present study were compared to those listed in GenBank (accession numbers AF051566-8, AF051572-4, and AF051578-80) and by Shyue et al. (1998) for the three alleles of the X-linked color vision gene for *Saimiri.*

Table 1. Sample sizes for the three *Saimiri* species used in this study

Species	Males	Females	Total no. of X chromosomes
S. boliviensis	15	39	93
S. oerstedii	24	23	70
S. sciureus	20	39	98

Results

Allele Sequences

The nucleotide sequences for exons 3, 4, and 5 are shown in Fig. 3. The sites that code for the critical amino acids responsible for spectral tuning are shown in boldface with boxes around them. The exon 3 sequences for all three alleles (Fig. 3A) are identical to previously published sequences (Shyue et al. 1998) with only one exception. According to the published sequences, the red and green alleles (GenBank Nos. AF051566 and AF051578, respectively) have an isoleucine at amino acid position 178 (ATC) and the yellow allele (GenBank No. AF051572) has a serine (AGC) (Shyue et al. 1998). However, none of the yellow alleles sequenced in this study (a total of 101 across three species) had the sequence AGC for amino acid position 178; all had the sequence ATC, coding for isoleucine. Another discrepancy between the published sequences and the data collected for this study is in exon 4 at amino acid position 197 (Fig. 3B). All three alleles have been reported to have a histidine at this position, with the codon CAT for the red and yellow alleles (GenBank Nos. AF051567 and AF051573, respectively) and the codon CAC for the green allele (GenBank No. AF051579). In the present study, the consensus sequence for the green allele at position 197 is CAT, with only four X chromosomes having the reported sequence of CAC.

Other than the discrepancy noted above for the yellow allele, no new mutations in exon 3 were discovered for

A. Exon 3

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Red             GG ATC ACG GGT CTC TGG TCC TTG GCC ATC ATC TCC TGG GAG AGG TGG CTG GTT GTC TGC AAA CCC TTT GGC AAC GTG AGA TTT GAT GCC
Yellow          .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ...
Green           .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ...
Amino Acid #              140                                   150                                   160

Red             AAG CTG GCC ATC GTG GGA GTT GCC TTC TCC TGG ATC TGG TCT GCT GTG TGG ACG GCC CCG CCC ATC TTT GGT TGG AGC AG
Yellow          ... ... ... ... ... ... ... .T. ... ... ... ... ... G.. ... ... ... ..A ... ..A ... ... ... ... ... ... ..
Green           ... ... ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... ..
Amino Acid #              170                                   180                                   190
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B. Exon 4

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Red             G TAC TGG CCC CAT GGC CTG AAG ACT TCC TGC GGC CCA GAC GTG TTC AGT GGC AGC TCG TAC CCC GGG GTG CAG TCT TAC ATG ATC GTC
 Red248T→G      . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Yellow          . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Yellow210C→T   . ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ...
Green           . ... ... ... ... ... ... ... ... ... ..T ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Green206T→C    . ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Green206T→C+197T→C . ... ... ... ..C ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Amino Acid #                          200                                   210                                   220

Red             CTC ATG GTC ACA TGC TGC TTC CTC CCA CTT GGC ATC ATT GTG CTC TGC TAC CTC CAA GTG TGG CTG GCC ATC CGA GCT
 Red248T→G      ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
Yellow          ... ... ... ... ... ... A.. ... ... ..C A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
 Yellow210C→T   ... ... ... ... ... ... A.. ... ... ..C A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
Green           ... ... ... ... ... ... A.. ... ... ..C A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
 Green206T→C    ... ... ... ... ... ... A.. ... ... ..C A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
 Green206T→C+197T→C ... ... ... ... ... ... A.. ... ... ..C A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..G
Amino Acid #                          230                                   240
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C. Exon 5

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Red                GTG GCA AAG CAG CAG AAA GAG TCC GAA TCC ACC CAG AAG GCA GAG AAG GAA GTG ACG CGC ATG GTG GTG GTG ATG ATC ATG GCG TAT TGC
 Red279V→I         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Yellow             ..C ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... C.. A.A .TC ...
 Yellow321C→T      ..C ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... C.. A.A .TC ...
Green              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.. A.A .TC ...
 Green276A→G       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.. A.. .TC ...
Amino Acid #            250                                 260                                 270
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Red                GTC TGC TGG GGA CCC TAC ACC TTC TTT GCA TGC TTT GCT GCT GCC AAC CCT GGC TAC GCC TTC CAC CCT CTG ATG GCT GCC CTG CCA GCC
 Red279V→I         A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Yellow             A.. ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Yellow321C→T      A.. ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Green              A.. ... ... ... ... ... G.. ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Green276A→G       A.. ... ... ... ... ... G.. ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Amino Acid #            280                                 290                                 300
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Red                TAC TTT GCC AAA AGT GCC ACT ATC TAC AAC CCC ATT ATC TAT GTC TTT ATG AAC CGG CAG
 Red279V→I         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Yellow             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Yellow321C→T      ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ...
Green              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
 Green276A→G       ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Amino Acid #            310                                 320
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Fig. 3. Nucleotide sequences for the red, yellow, and green alleles and their respective mutations for **(A)** exon 3, **(B)** exon 4, and **(C)** exon 5 of the X-linked color photopigment gene in *Saimiri.* The consensus sequence for the red allele is used as a reference. *Periods* for the other alleles indicate that the sequence is identical to that of the red allele. Amino acid residue numbers relative to the entire gene are shown below the nucleotide sequences. The codons for the critical amino acids for spectral differences are shown in *boldface* with *boxes* around them.

any of the three alleles (Fig. 3A). There were, however, mutations for all three alleles in exons 4 and 5. In exon 4 (Fig. 3B), we discovered a mutation in the last base of the exon (amino acid 248), from a T to a G in the red allele (denoted Red$^{248T\rightarrow G}$). A mutation in exon 4 of the yellow allele (Yellow$^{210C\rightarrow T}$) occurred in the third base position of amino acid 210. This mutation always occurred in the background of another mutation in the yellow allele in exon 5 (described below). A yellow allele containing both the exon 4 and the exon 5 mutations is denoted Yellow$^{210C\rightarrow T+321C\rightarrow T}$. Two mutations were found in exon 4 of the green allele: Green$^{206T\rightarrow C}$ and Green$^{197T\rightarrow C+206T\rightarrow C}$. Both of these new mutants involved a change from a T to a C in the third base position of amino acid 206. Mutant Green$^{197T\rightarrow C+206T\rightarrow C}$ has the additional mutation of a T to a C in the third base of codon 197. All of these mutations in exon 4 are synonymous changes. A mutation for the green allele (Green$^{276A\rightarrow G}$) in exon 5 from A to G occurred in the third position of codon 276, adjacent to one of the critical sites for spectral tuning (Fig. 3C). In *S. sciureus,* the Green$^{276A\rightarrow G}$ mutation always occurred in a green allele that also had the Green$^{206T\rightarrow C}$ mutation. Both mutations together in the same allele are denoted Green$^{206T\rightarrow C+276A\rightarrow G}$. For the yellow allele, a mutation (Yellow$^{321C\rightarrow T}$) from a C to a T was discovered in the third base position of amino acid 321. Mutants Green$^{206T\rightarrow C}$ and Yellow$^{321C\rightarrow T}$ are both synonymous substitutions. The only nonsynonymous change found was for the red allele in exon 5 in the first base position of codon 279 (Red$^{279V\rightarrow I}$), which is located in the second transmembrane domain of the gene. This mutation results in an amino acid change from valine to isoleucine. All of the red alleles containing this mutation also contained a mutation in exon 4 (Red$^{248T\rightarrow G}$); therefore, this mutant allele is designated Red$^{248T\rightarrow G+279V\rightarrow I}$.

Of the seven mutations described above, four were located in either the cytoplasmic region or the extracellular region, while three were located in transmembrane domains (Fig. 4). Of the three found in transmembrane regions, two (Red$^{279V\rightarrow I}$ and Green$^{276A\rightarrow G}$) involved mutations in codons that were known to vary between alleles.

One *S. boliviensis* female possessed an allele that appeared to be the result of a recombination event between a red and a green allele; the nucleotide sequences of exons 3 and 4 were consistent with those of a green allele, while the sequence of exon 5 was consistent with that of a red allele. Based on the spectral tuning effects of the critical amino acids in such a combination (Shyue et al. 1998), this recombinant allele would have a putative spectral sensitivity maximum of 558 nm, which is between the spectral maximum of the red (562 nm) and that of the yellow (550 nm) alleles. Therefore, we call this recombinant allele the *orange* opsin. One *S. sciureus* female also possessed a recombinant allele. In this instance, exons 3 and 5 had the nucleotide sequence of a green allele, and exon 4 the sequence of a red allele. However, in this case, such a combination of critical amino acid sites results in a calculated spectral sensitivity maximum of 534 nm, which is likely not functionally different from the spectral maximum of the original green allele (535 nm).

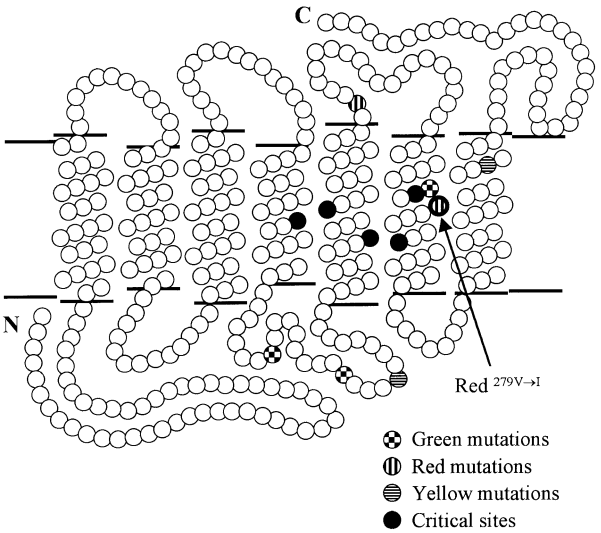

Fig. 4. This model of the squirrel monkey color vision gene shows the locations of the various mutations (as indicated by *patterned circles*) found for all three alleles in relation to the critical amino acid positions (*black circles*). All mutations are synonymous substitutions with the exception of Red$^{279V\rightarrow I}$, which is labeled.

Allele Frequencies

The frequencies of the original and mutant alleles for each species are listed in Table 2. Since none of the observed point mutations resulted in a functional difference for a particular allele, mutations were tallied along with their respective alleles. Therefore, the frequency of the red allele = Red + Red$^{248T\rightarrow G}$ + Red$^{248T\rightarrow G+279V\rightarrow I}$, etc. Within species, the frequencies of the three alleles are approximately equal for *S. boliviensis* and *S. sciureus* ($\chi^2 = 5.59$, $p > 0.10$, 2 df, and $\chi^2 = 0.97$, $p > 0.10$, 2 df, respectively) but not for *S. oerstedii* ($\chi^2 = 7.23$, $p < 0.05$, 2 df). The allele frequencies of *S. boliviensis* and *S. sciureus* are not significantly different from each other ($\chi^2 = 2.11$, $p > 0.10$, 2 df), nor are the allele frequencies of *S. oerstedii* and *S. sciureus* ($\chi^2 = 4.66$, $p > 0.10$, 2 df). There is a significant difference between the allele frequencies of *S. oerstedii* and *S. boliviensis* ($\chi^2 = 7.90$, $p < 0.05$, 2 df).

The frequencies of the various mutations vary considerably between the species (Table 2). In Fig. 5, the occurrence of mutations for each allele has been overlaid on the species phylogeny from Fig. 1B. For *S. bolivien-*

Table 2. Observed allele frequencies of the X-linked color vision gene for the three species of *Saimiri*[a]

	S. boliviensis		*S. oerstedii*		*S. sciureus*	
	Number	% total	Number	% total	Number	% total
Red	27	29	0	0	0	0
$Red^{248T\rightarrow G}$	0	0	28	41	25	26
$Red^{248T\rightarrow G+279V\rightarrow I}$	0	0	1	1	7	7
Total Red		29		42		33
Yellow	30	32	25	37	36	37
$Yellow^{210C\rightarrow T+321C\rightarrow T}$	1	1	0	0	0	0
$Yellow^{321C\rightarrow T}$	10	11	0	0	0	0
Total Yellow		44		37		37
Green	15	16	0	0	0	0
$Green^{206T\rightarrow C}$	0	0	10	15	13	13
$Green^{206T\rightarrow C+197T\rightarrow C}$	0	0	4	6	0	0
$Green^{276A\rightarrow G}$	9	10	0	0	0	0
$Green^{206T\rightarrow C+276A\rightarrow G}$	0	0	0	0	16	16
Total Green		26		21		29
Recombinant	1 (orange)	1	0 (—)	0	1 (green)	1

[a] Designations of the mutant alleles are explained in the text.

sis, no mutations were found in any exon for the red allele, while the mutation in exon 4 for the red allele ($Red^{248T\rightarrow G}$) was fixed for *S. sciureus* and *S. oerstedii.* The nonsynonymous mutation in the red allele (valine to isoleucine at codon 279) was found in both *S. sciureus* and *S. oerstedii* and always in conjunction with the $Red^{248T\rightarrow G}$ mutation. The occurrence of the same two mutations of the red allele ($Red^{248T\rightarrow G}$ and $Red^{248T\rightarrow G+279V\rightarrow I}$) in these two species suggests a polymorphism in their common ancestor. The green allele mutation in exon 4, $Green^{206T\rightarrow C}$, was also fixed in the populations for *S. sciureus* and *S. oerstedii* and not found in *S. boliviensis.* An additional mutation of the $Green^{206T\rightarrow C}$ allele was found in *S. oerstedii,* $Green^{206T\rightarrow C+197T\rightarrow C}$. The occurrence of a green allele mutation at amino acid 276 for some *S. boliviensis* individuals ($Green^{276A\rightarrow G}$) and in a background of the $Green^{206T\rightarrow C}$ mutation for some *S. sciureus* ($Green^{206T\rightarrow C+276A\rightarrow G}$) suggests that the mutation from A to G in the third base position of codon 276 in the green allele may have arisen twice. Mutations of the yellow allele ($Yellow^{321C\rightarrow T}$ and $Yellow^{210C\rightarrow T+321C\rightarrow T}$) occurred at low frequencies and were found only in *S. boliviensis.*

Discussion

In the absence of a chance duplication of the X-linked locus endowing constant trichromacy to all individuals (as in the case of humans, Old World primates, and howler monkeys), polymorphism at the X-linked locus in Neotropical primates enables at least some individuals in

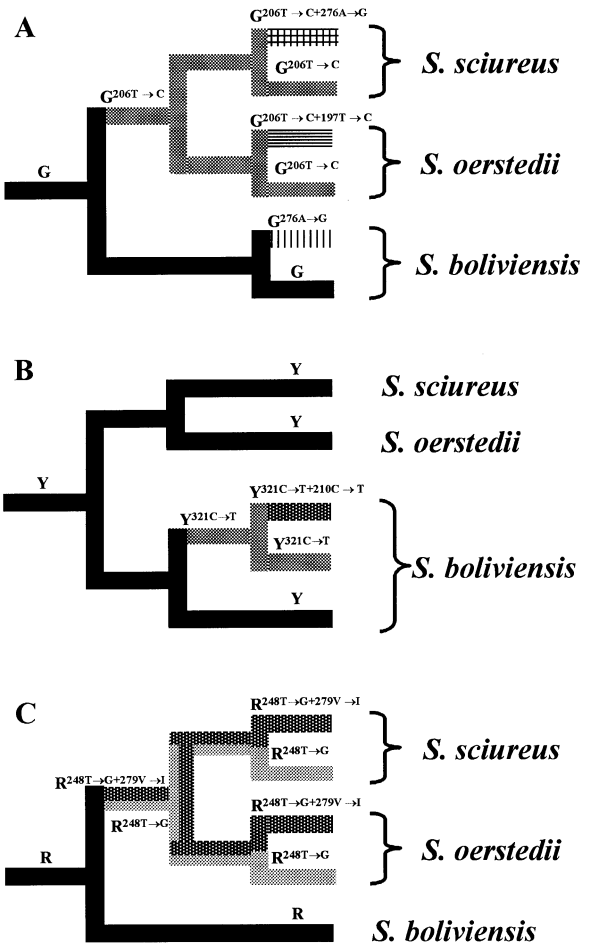

Fig. 5 The occurrence of allelic mutations in the X-linked color vision gene superimposed on the phylogeny of *Saimiri* for **(A)** the green allele, **(B)** the yellow allele, and **(C)** the red allele.

a population to be trichromatic. Trichromatic vision has been postulated to confer advantages in foraging efficiency to such individuals and the three alleles that have been previously identified at the X-linked locus are thought to be maintained because of heterozygote advantage (Mollon et al. 1984; Jacobs and Neitz 1987; Bowmaker et al. 1987; Shyue et al. 1995). In this situation, we might expect to find more than three alleles at the X-linked locus, as this would increase the frequency of heterozygotes in a population. Assuming Mendelian inheritance, random mating, no selection, and equal allele frequencies, a gene with two equally frequent alleles would result in half of the females in the population being heterozygous, but a locus with three equally frequent alleles could yield a population composed of two-thirds heterozygous females. Increasing the number of alleles to four would increase the frequency of heterozygotes even more, to three-quarters of the females. However, with the exception of the one recombinant (*orange*) allele, no new unique alleles were identified at the X-linked color photopigment locus. Why are there no more than three alleles at this locus?

One obvious explanation is that only three stable, functional allelic possibilities exist. Selection could be acting to maintain those combinations of critical sites responsible for functional differences among alleles, while gene conversion is acting to homogenize the remaining sites at the level of the nucleotide sequence. Gene conversion has been demonstrated to occur in the X-linked color vision gene of humans, Old World and New World primates (Balding et al. 1992; Deeb et al. 1994; Boissinot et al. 1998), while selection is operating for the retention of the distinct functional differences of exons 3, 4, and 5 of the red and green opsin genes (Shyue et al. 1994; Zhou and Li 1996). Clearly, in squirrel monkeys, mutations have occurred in all three alleles, yet all but one ($Red^{279V\rightarrow I}$) are synonymous changes. Most of the mutations in the three alleles were found only in certain species, but none of the mutations appeared to affect the spectral tuning of the alleles and differences in the frequencies of the silent mutations between species are more likely due to drift rather than selection. The $Red^{279V\rightarrow I}$ allele may have survived in the population because it resulted in the substitution of one amino acid (isoleucine) with a charge similar to that of the original concensus amino acid (valine) and therefore is not functionally different from the other forms of the red allele. However, a number of studies have demonstrated that amino acid changes at just a few sites can cause subtle shifts in spectral tuning (Neitz et el. 1991; Asenjo et al. 1994; Shyue et al. 1998). Additionally, mutagenesis experiments (Merbs and Nathans 1993; Sun et al. 1997) have demonstrated that the addition of hydroxyl groups can create peak shifts in either the red or the blue direction, depending upon the location of the addition. Even though they occurred at a low frequency in the populations, two alleles with exon combinations that appeared to be the result of recombination events were found in this study. Although one of those alleles appears to be functionally a green allele, there is still a slight (1-nm) shift from the spectral absorbance peak of the originally reported green allele. Furthermore, the recombinant *orange* allele has an estimated spectral sensitivity maximum between that of the red and that of the yellow alleles, making it unlikely that only three allelic prospects exist.

Alternatively, the spectral sensitivity maxima of the red, yellow, and green alleles may be the most useful for squirrel monkeys and selection is acting to maintain them in the population. Our finding of nearly equal allele frequencies within two of the species, *S. boliviensis* and *S. sciureus,* certainly suggests three equally advantageous, functional alleles, and even though the allele frequencies for *S. oerstedii* are slightly different from those of the other two species, all three species do possess alleles with the same three spectral tuning frequencies. The unequal allele frequencies for *S. oerstedii* could be attributable to an inadequate sample size or a recent population bottleneck. *Saimiri oerstedii* are severely endangered due to habitat destruction and other human activities (Rylands et al. 1995; Boinski et al. 1998). However, squirrel monkeys are not unique in the spectral tuning of their color vision. In fact, all of the diurnal New World primates possessing a single X-linked color photopigment gene exhibit the same three alleles with approximately the same spectral sensitivities for each respective allele (Jacobs 1996a; Boissinot et al. 1998). Therefore, it is difficult to believe that the same three spectral sensitivity maxima could be especially useful to all New World primates given the wide range of platyrrhine dietary regimes (Emmons and Feer 1997). Even within a single genus, *Saimiri,* the dietary composition varies widely between species, from *S. boliviensis,* inhabiting forests with year-round fruit availability (Terborgh 1983; Mitchell et al. 1991), to *S. sciureus,* which has only seasonal access to reliable fruit resources and must rely heavily on insects and arthropods for 6 to 7 months of the year (Boinski 1999a; unpublished data). The plant taxa comprising the background foliage against which squirrel monkeys must detect fruit, as well as the size, shape, and color of the fruit, are also markedly different across the range of this genus (Gentry 1988; Hammond and Brown 1995; Terborgh and Andresen 1998; ter Steege et al. 2000). However, this study failed to reveal any significant differences in allele frequencies that could be correlated with food resource availability between squirrel monkeys from different biogeographical regions.

A number of studies have demonstrated that the absorbance spectra of photopigments are not always correlated with the visual ecology of the organisms possessing them. In a comparison of the reflectance spectra of fruits

and the color space appropriate to catarrhines, Sumner and Mollon (2000b) found that the maximum absorption spectra of the primate MW and LW photopigments were not optimal for discerning fruit ripeness. Similarly, a review of the mechanisms of color vision in insects possessing UV–blue–green trichromacy revealed that some insects with distinctly different ecologies possessed nearly identical sets of color receptors and receptor types could only occasionally be interpreted as adaptive for specific environments (Briscoe and Chittka 2001).

Although a correlation between trichromacy and increased foraging efficiency for red/yellow food items has been demonstrated in captive marmosets (Caine and Mundy 2000), predicting whether such an advantage exists under natural conditions is problematic without field observations. In *S. boliviensis,* females form kin-based matrilineal groups that cooperate in the defense of food resources (Mitchell 1990, 1994; Mitchell et al. 1991) and, therefore, any trait, such as trichromatic vision, that would give one group a foraging advantage over another would likely be maintained in the population. However, within their home ranges, squirrel monkeys are well acquainted with the locations of fruit-bearing trees, and in Peru, *S. boliviensis* may also be guided by sympatric capuchins (Terborgh 1983; Boinski 1987a, 2000). Such spatial knowledge of fruit tree locations could preclude the need to search for colored fruit against dappled foliage, further reducing the advantage conferred by trichromacy. Alternatively, trichromats may have a slight advantage over dichromats in being able to visually inspect fruit for ripeness at close range rather than handling and tasting the fruits, which is time-consuming and decreases foraging efficiency (Boinski 1987a). However, Caine and Mundy (2000) found that trichromats had an advantage over dichromats in detecting colored food items only at a distance; at detection distances of less than 0.5 m, no significant difference was detected between them. In a species such as *S. sciureus,* where food competition among individuals is fierce and trichromats must compete for fruit resources in an area where cryptic food sources are more reliably available (Boinski and Cropp 1999; Boinski 1999a), the advantages of trichromacy for foraging efficiency are not obvious. Since dichromatic individuals may be better able to distinguish color-camouflaged objects (Morgan et al. 1992), and the insect prey of *S. sciureus* is more likely to be cryptically colored, in this species, a dichromat might even have a slight advantage over a trichromat. In *S. oerstedii,* for which food resources of all types are sparsely distributed (Gentry 1988) and agonistic competition over food resources among individuals is virtually nonexistent (Boinski 1987a, 1988), the foraging advantages of either type of color vision system are hard to envision. However, not only do all three species possess the same alleles at the X-linked locus, but the alleles are at approximately equal frequencies across the species, with no apparent correlation with the specific food resource ecology of the various species.

An alternative explanation for the existence of only three alleles at the X-linked locus involves constraints on the neurological system processing input signals from different classes of cone cells. The platyrrhine green (MW) and red (LW) photopigments have spectral absorbance maxima in the same approximate range as the equivalent catarrhine photopigments, about 530 nm and 560 nm, respectively (Deeb et al. 1994; Shyue et al. 1995). In a study of the efficacy of catarrhine trichromacy, Sumner and Mollon (2001a) concluded that the spectral absorbance maxima of the MW and LW pigments are constrained by the properties of the foliage background against which primates must detect targets. They found that possessing photopigments with sensitivity peaks at 531 and 561 nm minimized the chromatic noise of mature foliage while still allowing catarrhines to detect fruit and edible leaves, even though these photopigments are not especially tuned to the chromaticities of the food items. Nagle and Osorio (1993) have also shown that the chromatic noise of mature leaves is minimized by the catarrhine red/green color subsystem. Platyrrhines must search for food amidst the noise of foliage as well, which is likely limiting the spectral sensitivities of their MW and LW pigments to the same range as for catarrhines. Within this limited range, platyrrhines have acquired a third photopigment with a spectral sensitivity maximum generally halfway between those of the other two (Shyue et al. 1995). This additional allele increases the potential proportion of heterozygous females in the population from one-half in a two allele system to two-thirds in this three allele system. However, Sumner and Mollon (2000a) have also demonstrated that the magnitude of the signal and quantum noise limit how similar catarrhine MW and LW pigments can be. They propose that the chromaticities of the background foliage, not the fruit or leaf food targets, constrain the MW and LW spectral sensitivity maxima. We propose that a similar type of constraint exists for the separation of the spectral sensitivity maxima of the three alleles of the platyrrhine X-linked photopigment locus. In other words, a minimal distance between spectral sensitivity peaks for any two pairs of different alleles is necessary for a heterozygote to possess trichromatic vision. The neural subsystem which detects color opponency in the MW to LW range (Mollon 1991) may not be able to distinguish signals from two alleles that are too closely matched in spectral sensitivity, due, in part, to background signal interference. Selection could be acting to maintain this minimal distance between the spectral sensitivities of the alleles within the limited MW to LW range, and not necessarily the specific absorbance spectra of the alleles themselves. Therefore, three alleles at the X-linked color photopigment locus would strike the balance between maximizing the distance between their spectral tuning

peaks (and thereby allowing the color opponency system to distinguish between peaks) and maximizing the number of alleles to allow for the greatest possible proportion of heterozygotes (and therefore trichromats) in the population.

Clearly, the genetic architecture of color vision in Neotropical primates is being maintained across a wide variety of behavioral and dietary niches. Trichromacy seems to be useful to primates regardless of their lifestyle (Osorio and Vorobyev 1996). However, increased foraging efficiency may not be the selective force maintaining the genetic structure of primate color vision. Other aspects of primate ecology, such as antipredator and social behaviors, need to be considered along with chance evolutionary events and potential genetic and neurological constraints (Briscoe and Chittka 2001) before a complete picture of the forces that have shaped the evolution of Neotropical primate color vision can be elucidated. Without detailed analyses quantifying fitness differences between individuals possessing different genotypes, adaptationist arguments of the advantages of trichromacy over dichromacy should be taken with caution.

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