

Genetic Basis of Polymorphism in the Color Vision of Platyrrhine Monkeys

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It was earlier proposed that the polymorphism of color vision observed in some neotropical monkeys could be accounted for by assuming that these animals have only a single photopigment gene locus on the X-chromosome. Three kinds of evidence have been added to existing data sets in an effort to evaluate the adequacy of the single locus model: (1) photopigment complements of squirrel monkeys (*Saimiri sciureus*) have been determined using electroretinogram flicker photometry; (2) photopigment pedigrees have been established for several families of squirrel monkey; (3) X-chromosome pigment genes obtained from six dichromatic monkeys (three squirrel monkeys; three tamarins—*Saguinus fuscicollis*) have been examined to search for sequence polymorphisms at those gene loci believed crucial for spectral tuning. All of these results are in accord with the idea that some species of platyrrhine primate have only a single type of photopigment gene on the X-chromosome.

Platyrrhine monkeys *Saimiri sciureus* *Saguinus fuscicollis* X-chromosome Photopigment genes Color vision

INTRODUCTION

The genetic basis of human color vision variation has been much studied (reviewed by Kalmus, 1965; Pokorny, Smith, Verriest & Pinckers, 1979; Piantanida, 1991). Although it was early established that red–green color vision defects are inherited as X-chromosome linked traits, the exact nature of the genetic mechanism has been a subject of controversy. From study of pedigrees and psychophysical investigations one common idea that emerged is that there are two X-linked loci that encode the opsins for middle (MWS) and long-wavelength sensitive (LWS) cones. Variations in color vision were then postulated to arise by the replacement of these genes by alleles that specified variant opsins leading to shifts in the spectra of the cone photopigments. Recent molecular genetic results show this model is incorrect (Nathans, Thomas & Hogness, 1986). Many individuals having normal color vision have more than two X-chromosome photopigment genes, and defective color vision is correlated (albeit, so far somewhat weakly) with the presence of variant arrays of normal pigment genes and fusion genes, the latter apparently produced by unequal recombinations of normal pigment genes (Nathans, Piantanida, Eddy, Shows & Hogness, 1986).

In contrast to the complex picture that now seems to characterize the genetics of human red–green color vision, it has been proposed that the color vision variation among some genera of platyrrhine monkey might have a simpler genetic explanation. An example is the squirrel monkey (*Saimiri sciureus*). Like humans, these monkeys show significant variations in red–green color vision. However, unlike humans, (a) the rate of color vision variation is very high, (b) at least six distinct phenotypes of red–green color vision can be distinguished, and (c) males are exclusively dichromatic whereas female monkeys may have either trichromatic or dichromatic color vision (Jacobs, 1984). Based on microspectrophotometric (MSP) measurements of cone pigment variations in ten squirrel monkeys (Mollon, Bowmaker & Jacobs, 1984) and behavioral measurements of color vision in thirty-six animals (Jacobs & Neitz, 1985), it was proposed that, unlike the human, the squirrel monkey might have only a single X-linked photopigment locus. The presence in the population of three alleles coding for three different MWS/LWS photopigments would then account for the features of color vision variation noted above.

To attempt to evaluate this idea, cone pigment variation was examined in a sample consisting of a total of seventy-eight squirrel monkeys (Jacobs & Neitz, 1987a). The results provided support for the single locus model in that all the males examined were dichromatic as would be required, the frequency of female trichromacy was close to that predicted by the model based on the assumption of three equipotent alleles in the population, and cone pigment pedigrees obtained for nine

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families were all in accord with the requirements of this model. Because the presence of a single X-chromosome pigment locus in platyrrhines like the squirrel monkey may well have significant implications for understanding how color vision evolved among the primates (Mollon, 1989; Jacobs, 1991), we have been motivated to examine this issue further by compiling an expanded sample of monkeys whose cone pigment complement have been established, and by making a direct molecular genetic examination of the X-chromosome pigment genes in representatives drawn from two genera of platyrrhine monkey.

METHODS

Subjects

Electrophysiological procedures were used to infer the photopigment complements for six squirrel monkeys (*Saimiri sciureus*). These individuals constituted the membership of two families, each family consisting of three animals. Photopigment gene analyses were carried out on six male monkeys, three squirrel monkeys and three saddle-backed tamarins (*Saguinus fuscicollis*). These six animals served as individual representatives of all the dichromatic phenotypes found in these two genera.

Electrophysiology

The procedures used to determine photopigment complements with electroretinogram (ERG) flicker photometry have been detailed elsewhere (Neitz & Jacobs, 1984; Jacobs & Neitz, 1987a). In brief, the ERG was differentially recorded from the eyes of anesthetized animals using bipolar contact-lens electrodes. The anesthesia was induced by i.m. injection of 15 mg of ketamine hydrochloride mixed with 0.15 mg of acepromazine maleate followed by i.p. injection of 10 mg/kg of sodium pentobarbital. The stimuli were 53 deg circular stimulus fields presented in Maxwellian view. To measure spectral sensitivity the intensity of a rapidly flickering monochromatic light was adjusted to produce a response that matched in amplitude the response elicited by an interleaved flickering reference light. The test and reference light were equal in duration; they were separated by a dark interval also of the same duration. The total pulse rate from the photometer was 62.5 Hz. Repetition of this procedure for test wavelengths between 450 and 650 nm (in 10 nm steps) yields a curve that accurately characterizes the absorption spectra of the MWS and LWS pigments present in the retina of the subject (Neitz & Jacobs, 1984; Jacobs & Neitz, 1987a). To determine if the animal had one or more than one MWS/LWS cone pigment (i.e. whether it would be predicted to have dichromatic or trichromatic color vision) we used a chromatic adaptation procedure (Jacobs & Neitz, 1987a). In this case, an ERG flicker photometric equation was made between 540 and 630 nm lights. This equation was repeatedly determined, first when the eye was neutrally adapted and then again when there was concurrent chromatic adaptation of either 540 or

630 nm. The two adaptation lights were adjusted in intensity so that each elevated threshold by 0.5 log unit. A systematic shift in the equation obtained with the two conditions of chromatic adaptation was taken to indicate the presence of more than one MWS/LWS cone pigment. The magnitude of the shift has been found to serve as one diagnostic of the trichromatic pigment complement—those individuals having MWS/LWS pigment pairs separated by <15 nm have systematically smaller adaptation effects than do those individual monkeys having a pigment pair separated by 25–30 nm (Jacobs & Neitz, 1987a).

Molecular genetics

Two separate genomic DNA samples were obtained from each monkey and carried through the procedure described below to amplify and sequence fragments of the X-linked pigment genes. The polymerase chain reaction (PCR) was used to amplify each of four exons of the MWS/LWS pigment genes. The primer sequences derive from the human X-linked pigment gene sequences reported by Nathans *et al.* (1986). Among the human X-linked pigment genes for which sequences have been reported, those corresponding to the primers are invariant. The primers used to amplify exon 2 were: 5'GCCCCCTTCGAAGGCCCGA and 5'CACACAGGGAGACGGTGT. The primers used to amplify exon 3 were 5'GGATCACAGGTCTCTGGT and 5'CTGCTCCAACCAAGATG. Exon 4 was amplified with primers 5'GTACTGGCCCCACGGCCTG and 5'CGCTCGGATGGCCAGCCA. Exon 5 was amplified with primers 5'GTGGCAAAGCAGCAGAAA and 5'CTGCCGGTTCATAAAGAC. Amplification reactions were carried out in a Perkin Elmer Cetus thermal cycler. Reactions were incubated at 94°C for 5 min, then subjected to 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec. The reactions were incubated at 72°C for 10 min. Amplified DNA fragments were purified by electrophoresis through a 5% polyacrylamide gel and ligated into a plasmid vector (Bluescript, Strategene). Two to four clones were isolated for each of the exons, and the nucleotide sequences of both strands of each clone were determined (Sanger, Nicklen & Colson, 1977).

For exons 3 and 5 from each monkey, aliquots of genomic DNA were also used in asymmetric PCR to generate single-stranded DNA corresponding to each of the DNA strands (Gyllenstein, 1989). The nucleotide sequence was then determined from each of the single strands. For each monkey this latter procedure was carried out with two separate samples of genomic DNA. This second analysis was done to obtain additional confirmation of the DNA sequence for exons 3 and 5.

The primers used in PCR (described above) correspond to the nucleotide sequences encoding the first and last six amino acids of each exon examined. This molecular genetic approach thus provides the nucleotide sequences for exons 2–5 of the monkey X-linked visual pigment genes with the exception of those corresponding to the regions used to prime the amplification reactions.

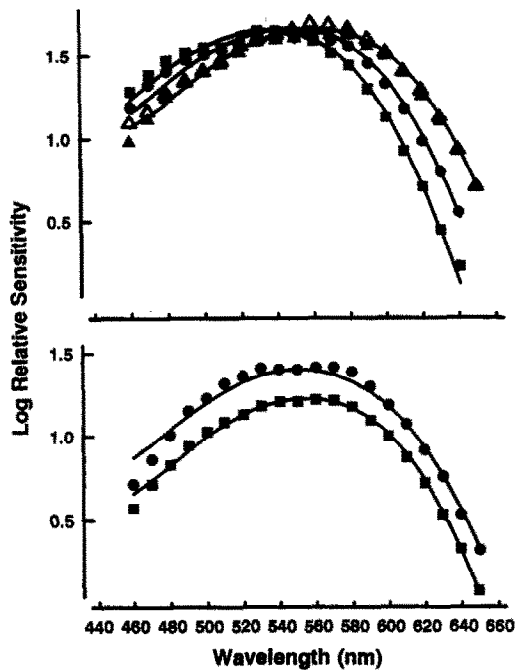


FIGURE 1. ERG spectral sensitivity functions for six squirrel monkeys. Each function was obtained under test conditions strongly favoring the detection of light by MWS/LWS cones. Top panel: functions for four dichromatic male monkeys. These animals were diagnosed as dichromatic because they showed no evidence for differential chromatic adaptation over the 540/630 nm portion of the spectrum. This group contains representatives having each of the three classes of MWS/LWS cone pigment found in this species. The continuous lines drawn through the data points are best-fit cone pigment nomograms. The peak sensitivity values for these functions (from left to right) are 533, 544, and 559 nm. There are two representatives of the latter phenotype (open and solid triangles) and one each (squares and circles) of the other two phenotypes. Bottom panel: ERG spectral sensitivity functions obtained from two trichromatic squirrel monkeys. The trichromacy of these animals was established through chromatic adaptation tests. The sensitivity data points were best fitted by pairwise summing of pigment nomograms representing the three squirrel monkey MWS/LWS cone pigments. For the animal at the top, the best combination was 534 (37%) + 561 (63%) (chromatic adaptation index = 0.11 log unit); for the other animal the best fit combination was 550 (70%) + 561 (30%) (chromatic adaptation index = 0.06 log unit). The functions for these two animals are arbitrarily displaced on the sensitivity axis for ease of viewing.

RESULTS

Photopigment complements and pedigrees

All squirrel monkeys share in common a short wavelength sensitive cone having peak sensitivity of about 430 nm (Bowmaker, Jacobs & Mollon, 1987). The polymorphism of squirrel monkey color vision arises from individual variation in the presence of three classes of cone pigment peaking in the middle to long wavelengths. Our current best estimates of the average peak values of these three pigments are 534, 550 and 561 nm. These numbers differ slightly from those given in earlier publications reflecting a change in the method used to estimate the peak value. The current estimates were obtained by shifting absorption curves (Ebrey & Honig, 1977) along a log wavenumber axis (Baylor, Nunn & Schnapf, 1987) to get the best fit to the lens-corrected ERG sensitivity data. The new technique

is believed to be more accurate in that it yields generally better fits between the absorption curves and the sensitivity values.

As noted above, the single-locus hypothesis requires that all males have dichromatic color vision while female monkeys may be either dichromatic or trichromatic. The pigment complements of the six additional squirrel monkeys follows this prediction (Fig. 1). Each of the four male monkeys was found to have only a single type of MWS/LWS pigment (one each from the 534 and 550 phenotype and two from the 561 phenotype). Both of the females studied were trichromatic. One had a chromatic adaptation effect (i.e. equation shift) of 0.11 log unit; for the other the shift was 0.06 log unit. As noted in the Methods section, this implies that the former has the 534/561 pigment complement. From the size of the adaptation effect the second monkey could have either the 550/561 or the 534/550 pigment combination. A comparison was made of the best fits obtained from these two possible combinations. The goodness of fit (the least mean difference squared between the data set and the best fitting combination) was 3.1×10^{-3} log unit for the 534/550 combination and 6.0×10^{-4} log unit for the 550/561 combination. This indicates (Fig. 1) that this monkey has the 550/561 pigment combination.

The cone pigment phenotypes of the two squirrel monkey families, as determined by ERG flicker photometry, are shown in Fig. 2. In that figure they have been added (as families 7 and 8) to the earlier compilation of pedigrees (Jacobs & Neitz, 1987a). In each of the eleven cases the pigment complement of the offspring agrees with the prediction that its inheritance in the squirrel monkey results from three allelic versions of a single X-chromosome photopigment gene. In particular, note that all of the male offspring can be seen to have inherited a photopigment found in the mother's complement and father/son transmission is excluded in all known cases.

Following the initial examination of the inheritance of photopigments in squirrel monkeys we were able to examine photopigment complements in members of three other platyrrhine genera—two Cebids (*Cebus apella* and *Callicebus moloch*) and a Callitrichid (*Saguinus fuscicollis*) (Jacobs & Neitz, 1987b; Jacobs, Neitz & Crognale, 1987). Although the set of MWS/LWS pigments differ for Cebid and Callitrichid species (in the Callitrichid the new fitting procedures yield average peak values of 543, 556 and 562 nm), in each case there is evidence for a photopigment polymorphism similar in character to that described for the squirrel monkey, i.e. each species appears to have the three classes of middle to long wavelength cone pigments with individual animals having any one or any pair of these three. The number of animals examined from these species was somewhat restricted, but it also seemed likely that the same single locus mechanism proposed for the squirrel monkey might account for the polymorphism of cone photopigments in these other platyrrhine monkeys. On the presumption that this idea may be correct, we have compiled the summed representation of trichromacy and

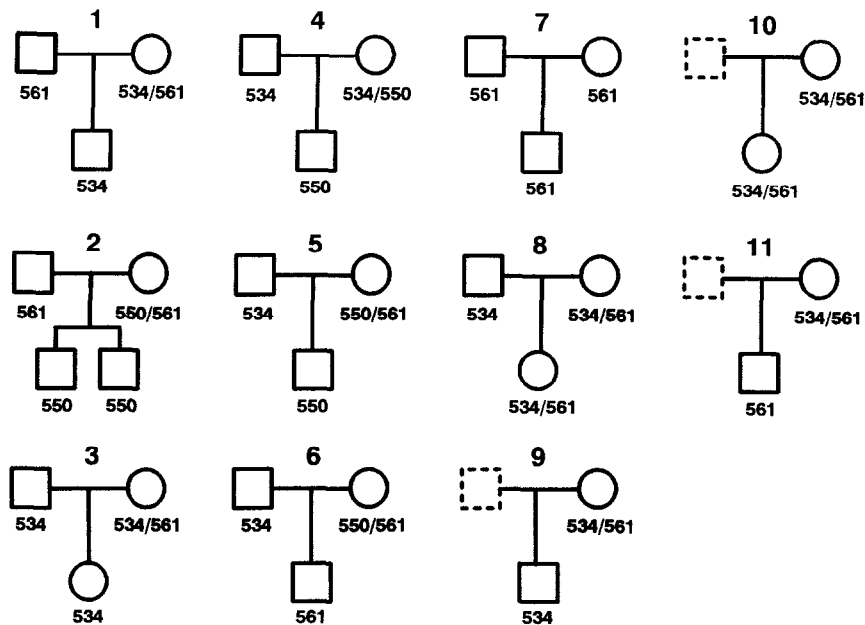


FIGURE 2. Cone pigment pedigrees for eleven families of squirrel monkey. The cone complement of each animal was determined with ERG flicker photometry. The numbers specify the average peak sensitivity values for each of the three cone pigment types. The identity of the male parent in families 9, 10 and 11 is not known.

TABLE 1. Representation of trichromacy and dichromacy among 109 platyrrhine monkeys

Sex	Dichromatic	Trichromatic
Female	18	34
Male	57	0

dichromacy in all of these species (Table 1). For a total of 109 monkeys, the result corresponds favorably with expectation: of the 57 male monkeys examined, none were found to have other than dichromatic color vision; of 52 female monkeys similarly tested, 34 (70%) were trichromats. The latter value is very close to the 67% representation that would be predicted by the single locus model based on the assumption that the three pigment alleles are equifrequent in these monkey populations.

Absence of gene polymorphism

The genes encoding the opsins of MWS/LWS photopigments consist of six exons. Of these, exons two to five specify the membrane spanning regions of the opsin (Nathans *et al.*, 1986). On the assumption that only these exons are likely to be linked to spectral tuning properties of the encoded pigment, we recently determined the nucleotide sequences for these four exons for each of eight primate pigment genes that specify MWS/LWS pigments (Neitz, Neitz & Jacobs, 1991). Included in this group were genes from three dichromatic squirrel monkeys and genes from three tamarins also classified as dichromatic. Comparisons of the deduced amino acid sequences of these eight pigments suggested that only three amino acid substitutions might be required to spectrally tune photopigments over the approx. 30 nm peak range encompassed by MWS/LWS pigments. These substitutions appear to act in a linearly additive

fashion, i.e. the spectral shift accomplished by two such changes is the sum of the shifts produced by either of the changes alone.

The outcome of the present genetic analysis was unambiguous: the genes examined for the six dichromatic monkeys showed no within-animal variations indicative of more than one type of X-chromosome pigment gene. This was true both for sequence comparisons among the two to four clones examined for exons 2–5 for each of the monkeys and for the direct sequences of amplified DNA obtained for exons 3 and 5 from each monkey.

We here illustrate the results of the genetic analysis by focusing on those three sequence locations assumed by the spectral tuning model to be crucial for peak shifting the produced photopigment (Neitz *et al.*, 1991). These exon sequence differences among the dichromatic phenotypes are summarized in Table 2.

In the DNA sequence analysis there appears to be only one gene sequence in each animal. This point is illustrated in Fig. 3 by the autoradiograms of the nucleotide sequence from two of the monkeys. Direct sequence analysis of all of the X-linked pigment genes in an individual is shown in each autoradiogram. If an individual had two different X-linked pigment genes, then any nucleotide differences between them would be

TABLE 2. Exon sequence differences between dichromatic platyrrhine monkeys that may be responsible for the spectral tuning of MWS/LWS photopigments

Exon	Nucleotide position	Squirrel monkey			Tamarin monkey		
		534	550	561	543	556	562
3	1032	G	G	T	G	G	T
5	1325	C	C	T	C	C	C
5	1347	A	G	A	G	A	A

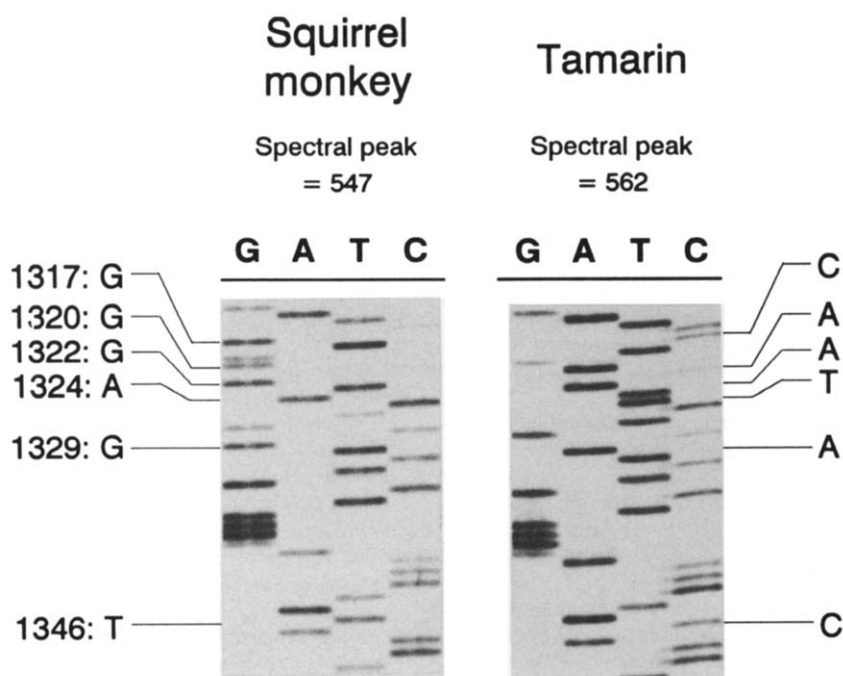


FIGURE 3. Autoradiograms of nucleotide sequence gels for X-linked pigment genes from two South American monkeys. The sequences extend from nucleotide 1346 to 1312 (numbering system from Nathans *et al.*, 1986). The numbers indicate nucleotides that differ between the two animals; for example, nucleotide position 1317 is G in the squirrel monkey X-linked pigment, C in the tamarin pigment. The occasional faint bands occurring in a second lane are the normal background always seen in these sequence ladders. None of these faint bands were reliable in replications of the sequence analysis, and they thus do not represent two genes in individual animals.

evident as two bands at one position in the autoradiogram. The positions that are the most likely candidates to have two bands are those that are polymorphic across animals. Thus, if two genes are present in an animal, these highly polymorphic regions are the ones likely to reveal this fact. The sequences shown in Fig. 3 span the most polymorphic region of the X-linked pigment genes of the squirrel monkey and the tamarin. Ten of the thirty-eight nucleotide positions shown vary across the six animals we examined, and two of these variant positions are responsible for encoding two of the three amino acid differences thought implicated in spectral tuning (Neitz *et al.*, 1991). No two of the six tamarin and squirrel monkey sequences were identical in this region. At each of these variant positions there appears to be only a single nucleotide present in each sequence.

DISCUSSION

Observations of cone photopigment and color vision variations among squirrel monkeys first suggested the idea that, unlike humans, these primates might have only a single X-chromosome pigment locus (Mollon *et al.*, 1984; Jacobs & Neitz, 1985). We have marshaled three kinds of evidence to evaluate this view: the statistics of pigment variation in a sizeable sample of squirrel monkeys (in particular, the absence of male trichromacy and the relative representations in the population of female trichromacy and dichromacy), the inheritance of cone pigment complements in eleven cases, and a search for sequence polymorphisms in the X-chromosome pigment genes. In none of these results does one see anything to

call the single-locus model into question. To the contrary, taken as a package, these results strongly support the view that squirrel monkeys have only a single photopigment gene locus on the X-chromosome.

Whereas the evidence for the single-locus model seems very compelling for the squirrel monkey, a Cebid species, and would appear also to account for color vision in the saddle-backed tamarin, a representative Callitrichid monkey, it would be inappropriate to blindly assume that all platyrrhines have this same arrangement. Part of the reason for caution comes from the simple fact that there are many platyrrhine species for which there is no information, either about photopigment genes, photopigments or color vision. Beyond that, on the basis of a correlative study of MSP and behavioral results, Tovee, Mollon and Bowmaker (1990, 1992) have expressed some doubt that the single locus X-chromosome model can completely account for the photopigment polymorphism seen in another platyrrhine, the common marmoset (*Callithrix jacchus*). Keeping in mind these reservations, it seems clear that the single locus model correctly describes the genetic basis of color vision polymorphism in some (if not all) of the platyrrhines. Thus the contention that catarrhine and platyrrhine primates show clear differences in the genetic mechanisms underlying their color vision would appear to be well founded.

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