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Cone pigment variations in four genera of new world monkeys

Gerald H. Jacobs [a,*], Jess F. Deegan II [b]

[a] Neuroscience Research Institute and Department of Psychology, University of California, Santa Barbara, CA 93106, USA
[b] Department of Psychology, California State University, Bakersfield CA 93311, USA

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Abstract

Previous research revealed significant individual variations in opsin genes and cone photopigments in several species of platyrrhine (New World) monkeys and showed that these in turn can yield significant variations in color vision. To extend the understanding of the nature of color vision in New World monkeys, electroretinogram flicker photometry was used to obtain spectral sensitivity measurements from representatives of four platyrrhine genera (*Cebus*, *Leontopithecus*, *Saguinus*, *Pithecia*). Animals from each genus were found to be polymorphic for middle to long-wavelength (M/L) sensitive cones. The presence of a short-wavelength sensitive photopigment was established as well so these animals conform to the earlier pattern in predicting that all male monkeys are dichromats while, depending on their opsin gene array, individual females can be either dichromatic or trichromatic. Across subjects a total of five different M/L cone pigments were inferred with a subset of three of these present in each species.

Keywords: New world monkeys; Cone photopigments; Color vision; *Cebus*; *Leontopithecus*; *Saguinus*; *Pithecia*

1. Introduction

Anthropoid primates have followed two distinct routes to trichromatic color vision. Catarrhines (Old World monkeys, apes, humans) have two (or more) X-chromosome opsin genes specifying the pigments in middle (M) and long (L) wavelength sensitive cones which, in conjunction with an autosomal gene producing a short (S) wavelength sensitive photopigment, provide the photopigment basis for trichromatic color vision (Jacobs, 1996; Nathans, Thomas, & Hogness, 1986). By contrast, most platyrrhine monkeys have only a single X-chromosome opsin gene with allelic versions of this gene present in different animals (Jacobs, 1998). Heterozygous females get alternative versions of the M/L opsin genes on their two X-chromosomes, thus producing separate populations of M and L cones and allowing trichromacy. All other individuals of these species, including every male, have only a single type of M/L cone and are dichromatic. There are two exceptions to this picture. The owl monkey *Aotus*, the only nocturnal anthropoid species, has neither polymorphism of X-linked pigment genes nor a viable population of S-cones and thus lacks a conventional color vision capacity (Jacobs, Deegan, Neitz, Crognale, & Neitz, 1993; Jacobs, Neitz, & Neitz, 1996). Howler monkeys (*Alouatta*) have an opsin gene/photopigment/color vision arrangement similar to that of the catarrhines and they thus also differ from their polymorphic cousins (Dulai, von Dornum, Mollon, & Hunt, 1999; Jacobs, Neitz, Deegan, & Neitz, 1996; Kainz, Neitz, & Neitz, 1998).

The basic picture of gene, photopigment and color vision variations in New World monkeys outlined above was first established in squirrel monkeys (*Saimiri*) (Jacobs, 1984; Jacobs & Neitz, 1987a; Mollon, Bowmaker, & Jacobs, 1984), animals from the subfamily Cebinae (Harada et al., 1995), and thereafter also in two species from the subfamily Callitrichinae—saddle-backed tamarins (*Saguinus fuscicollis*) and common marmosets (*Callithrix jacchus*) (Jacobs, Neitz, & Crognale, 1987; Tovée, Bowmaker, & Mollon, 1992; Travis, Bowmaker, & Mollon, 1988). The Cebid and Callitrichid representatives from a single family (Cebidae) were found to differ somewhat in the spectral sensitivities of their M and L cone types, but were otherwise in accord with the arrangement sketched above. In particular, each of these species had three M/L opsin gene alleles thus allowing as many as six separate color vision phenotypes in the

* Corresponding author. Tel.: +1-805-893-2446; fax: +1-805-893-2005.
E-mail address: jacobs@psych.ucsb.edu (G.H. Jacobs).

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population. We have since used a noninvasive electrophysiological procedure, electroretinogram (ERG) flicker photometry, to extend these measurements to additional New World monkeys and summarize here observations made on animals from four genera (*Cebus*, *Leontopithecus*, *Saguinus*, *Pithecia*). The results expand support for the view that polymorphism is the dominant theme of platyrrhine color vision.

2. Methods

2.1. Subjects

Recordings were obtained from 56 platyrrhine monkeys distributed as follows: 15 Cebus monkeys (9 *Cebus apella*, 6 *Cebus capucinus*); 10 Golden Lion tamarins (*Leontopithecus rosalia rosalia*); 27 Cottontop tamarins (*Saguinus oedipus*); 2 Golden-handed tamarins (*Saguinus midis*); 2 white-faced Saki monkeys (*Pithecia pithecia*).

2.2. Apparatus and procedures

Details of our use of ERG flicker photometry to measure spectral sensitivity in sedated monkeys have appeared in several previous publications (Jacobs & Deegan II, 2001; Jacobs & Neitz, 1987a; Jacobs, Neitz, & Krogh, 1996). In the work reported here recordings were made at a number of different animal facilities and consequently monkeys were anesthetized to differing veterinary standards. In the majority of cases this was accomplished by intramuscular injections of ketamine hydrochloride (20 mg/kg), often given in conjunction with one of the following agents: acepromazine maleate (0.2 mg/kg), butorphenol (0.03 mg/kg) or diazepam (1.0 mg/kg). A few of the animals were initially sedated with ketamine, then intubated and maintained on an inhalant mixture of oxygen and isoflurane (1.5%–3%). There was no evidence of variations in the quality of recording obtained with different anesthetic regimes. In every case the cornea was topically anesthetized with proparacaine hydrochloride (0.5%), the pupil was dilated through application of a mydriatic (either 0.04% atropine sulfate, 10% phenylephedrine hydrochloride, or 1% cyclopentalate hydrochloride), and ERGs were differentially recorded from a bipolar contact-lens electrode. The system used to process ERG signals has been described (Jacobs et al., 1996).

Stimuli from each of three light sources were optically superimposed and presented in Maxwellian view (59°). The three constituted a test light originating from a monochromator (half-energy passband = 10 nm), a reference light (achromatic, 2450 K; retinal illuminance of 3.3 log td), and an adaptation light that was passed through interference filters (Optical Thin Films, 10 nm half bandwidth) for one of the experiments. Recordings were made under photopic levels of illumination (range at different test sites of 150–400 lx).

Photometric equations were completed by adjusting the position of a neutral-density wedge and thus changing the intensity of a test light until it had the same effectiveness in producing an ERG as did the reference light. These two lights were presented as an interleaved train of square wave pulses and equations were based on the average of the fundamental responses to the last 50 cycles of a total of 70 stimulus cycles. The setting on a density wedge in the test beam pathway was recorded at the point of equation (to a precision of 0.01 log units). Each equation was made at least twice during the recording session and these individual values were subsequently averaged to obtain a final photometric equation.

For each monkey we measured a complete spectral sensitivity function at a pulse rate of 31.25 Hz. Equations were made at 10 nm steps, usually over a range from 450 to 650 nm. Subsequently, a test for response univariance was conducted to determine whether the retina of each subject contained more than a single type of M/L cone. To do this, a photometric equation was made for a 540 nm test light and a 630 nm reference light as the eye was alternately adapted to 540 and 630 nm light. The intensities of the adaptation lights were set so that each elevated the threshold of a 550 nm test light by 0.5 log units. A systematic change in the equation under the two adaptation conditions is taken as a failure of response univariance (Jacobs & Neitz, 1987a). Finally, for a subset of the monkeys we attempted to obtain objective evidence for the presence of S-cones. To accomplish this, spectral sensitivity was measured under test conditions favorable for detecting contributions from the S-cones including, (1) a slower stimulus pulse rate (12.5 Hz), (2) a short-wavelength (460 nm) reference light, and (3) concurrent chromatic adaptation consisting of exposing the eye to long-wavelength light (produced by using a long-pass filter having 50% transmission at 585 nm). Under these conditions spectral sensitivity measurements were made at 10 nm steps, usually from 430 to 520 nm.

2.3. Analysis of spectral sensitivity data

Since one goal of this research was to draw inferences about monkey cone photopigments, spectral sensitivity functions were fit to standard photopigment absorption functions and combinations of these functions. When we started this line of work some 15 years ago there were few such functions available. At that time a set of polynomial based functions was found to be the best for characterizing data from the ERG measurements (Dawis, 1981). Principally for reasons of consistency, we have continued to use these same templates on a variety of spectral data obtained in the intervening years. In

recent years several alternative standardized photopigment absorption functions have been proposed (Baylor, Nunn, & Schnapf, 1987; Carroll, McMahon, Neitz, & Neitz, 2000; Govardovskii, Fyhrquist, Reuter, Kuzmin, & Donner, 2000; Lamb, 1995; Palacios, Goldsmith, & Bernard, 1996; Stavenga, Smits, & Hoenders, 1993). We have now compared the fits obtained to the monkey spectral sensitivity data from application of these various templates and comment on those comparisons below.

3. Results

3.1. Variation in number of M/L cone types

The ERG test involving a comparison of photometric equations between 540 and 630 nm lights obtained under two conditions of chromatic adaptation has been shown to provide a sensitive and reliable assay to establish whether a retina contains one or more classes of M/L cone (Jacobs & Neitz, 1987a). The results from this test are summarized in the form of a frequency histogram in Fig. 1 that plots the size of the difference in the equation values obtained under red and green adaptation for all of the subjects. For the majority of these monkeys (40/56) there was no consistent difference (defined as <0.02 log unit) in the equation obtained for the two test conditions (mean equation shift = −0.002 log units; SD = 0.0075; $n = 16$). These monkeys would be judged to have only a single type of M/L cone and, consequently, the retinal basis for dichromatic color vision. The remainder of subjects each showed a systematic change in the equation values (entries to the right of the vertical dashed line in Fig. 1). These animals all require relatively more 540 nm light to achieve a photometric equation when the eye was concurrently adapted to 540 nm and the reverse when the adaptation was changed to 630 nm. Such monkeys must have two classes of independently adaptable M/L cones and are therefore potential trichromats. Dichromatic and trichromatic individuals were detected in four species of monkey. Only two animals were tested from the fifth species (*S. midis*) and they were both dichromatic.

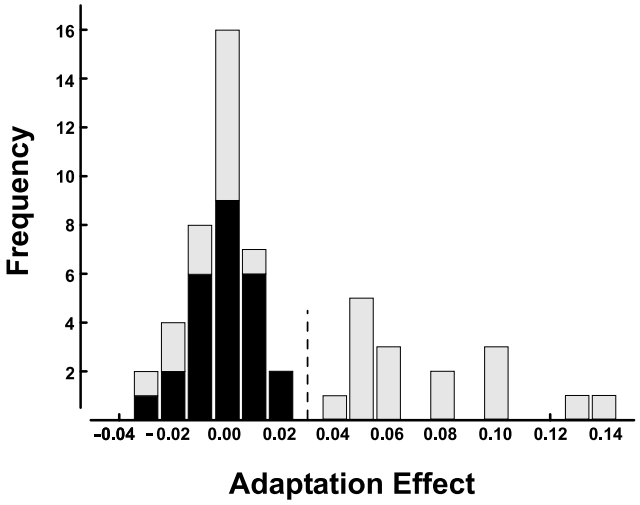

Fig. 1. Results from a test of response univariance run on 56 platyrrhine monkeys. The adaptation effect is the difference (in log units) between the photometric equations made for 540 and 630 nm lights under conditions of red and green adaptation. Results obtained from male monkeys are indicated by the solid shading; those for females are shown in gray. The absence of an effect (values of 0.02 or less) indicates the presence of a single type of M/L cone pigment and predicts dichromatic color vision. Values to the right of the dashed line indicate the presence of more than one M/L cone pigment in the retina and predict trichromacy.

The single X-chromosome opsin gene characteristic of most platyrrhine monkeys means males are obligatory dichromats (Jacobs, 1998). That was also consistently true for the animals of this sample. The results for males and females are keyed separately in Fig. 1 from which it can be seen that all of the 27 males tested were dichromatic, while 16 of 29 females (55%) had more than one M/L pigment and are predicted to have trichromatic color vision.

3.2. Cebus monkeys

Eleven of 15 Cebus monkeys showed no significant differential responses to the two conditions of chromatic adaptation. The spectral sensitivity functions obtained from these animals are shown in Fig. 2A. There are no published measurements of absorption by ocular media in Cebus monkeys. In the absence of such measurements the sensitivity values of Fig. 2A (solid circles) have been corrected by using lens absorption measurements made on the squirrel monkey, another species from the family Cebinae (unpublished measurements). The spectral sensitivity curves for these animals fall into three groups. To characterize these functions we averaged them and then best fit the results using the photopigment absorption functions recently provided by Govardovskii and colleagues (Govardovskii et al., 2000). The wavelengths of the peaks of the best fit function (λ_{max}) are noted in Fig. 2A. There was relatively small variation in the spectral positioning of the curves of the animals within each of the three groups.

The retinas of the remaining four Cebus monkeys each had two types of M/L cone. The averaged spectral sensitivity functions obtained from these animals are in Fig. 2B. Both the consistent size of the adaptation effect for these animals and the shapes of the functions suggest that each had same types of cone pigment, those represented by the shortest and the longest of the three classes found in the dichromats (Fig. 2A). Accordingly, these functions were best fit (continuous lines) by linear sums of two photopigment absorption functions having

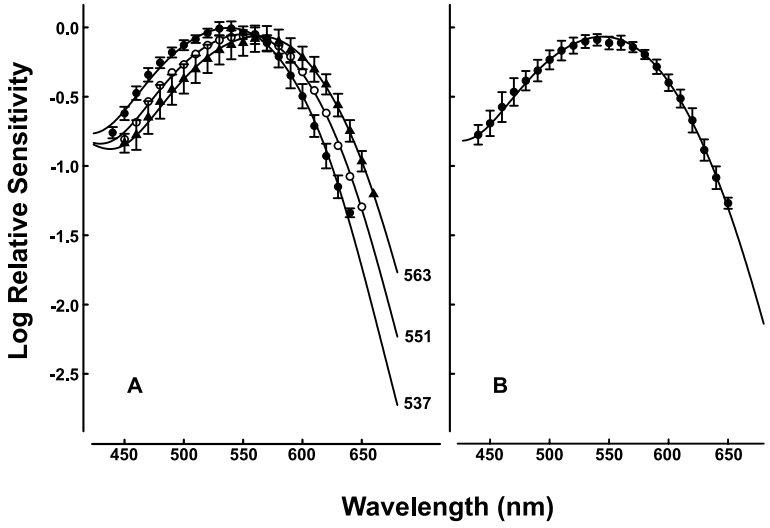

Fig. 2. ERG spectral sensitivity functions recorded from Cebus monkeys. (A) The plotted points are averaged sensitivity values obtained for three types of dichromatic monkeys (three males; eight females). Here and in subsequent figures the error bars are ±1 SD. The continuous curves are best fitting photopigment absorption spectra obtained as described in the text and normalized to have the same average peak sensitivity. The peaks of the best fit curves are noted on each function. (B) Averaged spectral sensitivity curves obtained from four female Cebus monkeys having two types of M/L photopigment. The best fit curve was obtained by linear summation of two photopigment absorption spectra having peak values of 537 and 563 nm.

respective peak values of 538 and 563 nm. This strategy yielded a good account of the spectral sensitivity functions with the relative proportions of 538 (65.6%) + 563 (34.4%).

3.3. Cottontop tamarins

From the results of the chromatic adaptation experiment (Fig. 1), 21 members of this group were judged to have only a single type of M/L cone pigment. These fell into three spectral positions. The majority of these animals (15/21) have an M/L pigment that peaks in the region from 543 to 548 nm. The averaged sensitivity function for these animals is shown in Fig. 3A. As for Cebus monkeys, there are no measurements of preretinal absorption in Cottontop tamarins and so we assumed that lens absorption measurements made for another Callitrichid monkey, the common marmoset, would be most appropriate (Tovée et al., 1992). Analogous M/L spectral sensitivity functions for the

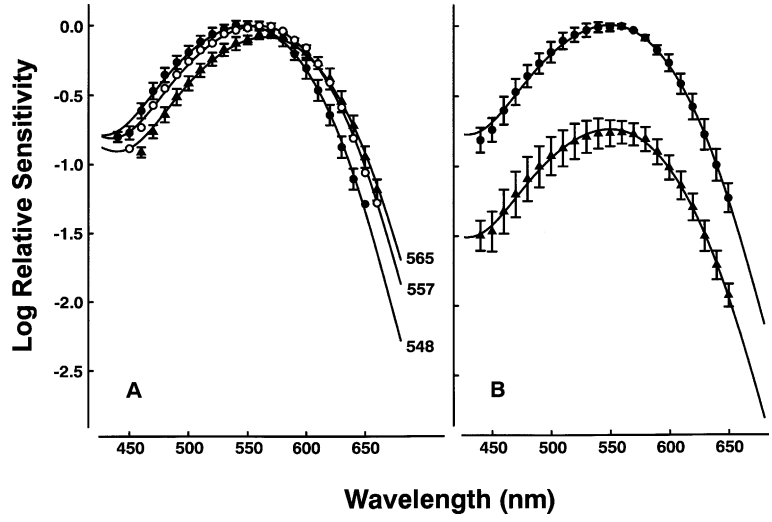

Fig. 3. ERG spectral sensitivity functions recorded from Cottontop tamarins. (A) Averaged values for three types of dichromatic animals (16 males; 5 females). The peaks of the best fit curves are as indicated. (B) Averaged functions for six female monkeys found to have two types of M/L photopigment. The data from the three animals at the top were fitted by summing curves having peak values of 548 nm (66%) and 557 nm (34%); the data for the three animals below were similarly fitted by summing pigment curves having peaks of 548 nm (74%) and 565 nm (26%).

remainder of the dichromatic Cottontop tamarins also appear in Fig. 3A. Five of these animals have a pigment whose peak is around 565 nm, while a single animal had a pigment with peak of about 557 nm.

Spectral sensitivity curves obtained from six trichromatic Cottontop tamarins are shown in Fig. 3B. As before, they have been best fitted using linear summations of photopigment absorption curves for two M/L pigments. The template curves employed in the fits were drawn from the three dichromatic spectral sensitivity functions. The selection of the pair of curves used for each animal was based on the size of the chromatic adaptation effect and the position of the peak of the spectral sensitivity curve. Four of the six animals were also members of families whose spectra were measured so the choice of pigments for these animals was also supported by examination of the pigment pedigrees (see below).

3.4. Golden-handed tamarins

Spectral sensitivity curves for the two dichromatic males of this species are shown in Fig. 4. The retinas of these animals contained distinctly different M/L cone pigments—one having a peak value of about 542 nm, the other with peak of about 557 nm.

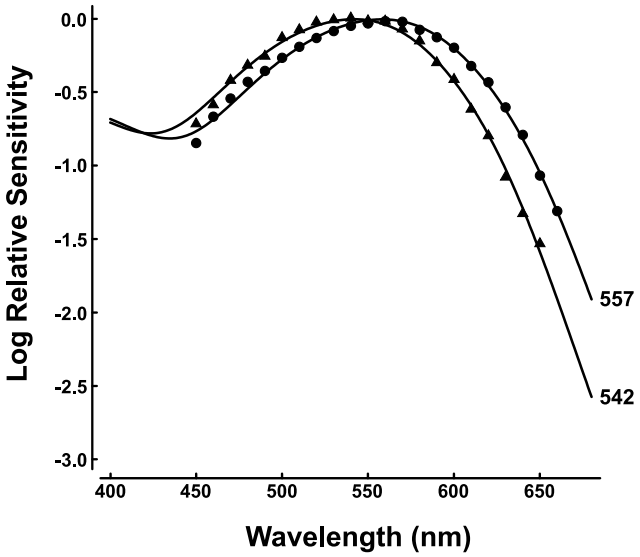

Fig. 4. ERG spectral sensitivity functions for two dichromatic male Golden-handed tamarins. The peaks of the best fit functions are indicated.

3.5. Golden lion tamarins

Two different M/L cone pigments were detected in the five animals found to be dichromatic. The averaged spectral sensitivity curves obtained from them are in Fig. 5A. Of these monkeys, four had an M/L pigment with a peak of around 546 nm while the spectral sensitivity of the remaining animal predicts a photopigment with a peak of about 555 nm. Spectral sensitivity curves for the five trichromatic Golden Lion tamarins are shown in Fig. 5B. The data from these animals have been best fit using linear summations of photopigment absorption functions having λ_{max} values appropriate for the respective two types of dichromatic monkey, i.e., 546 and 555 nm.

3.6. White-faced Saki

The male Saki monkey had a single type of M/L pigment (Fig. 6, top) that was best fit by an absorption function having λ_{max} of 565 nm. The female was trichromatic. She had a relatively large chromatic adaptation effect and that fact, in conjunction with the shape

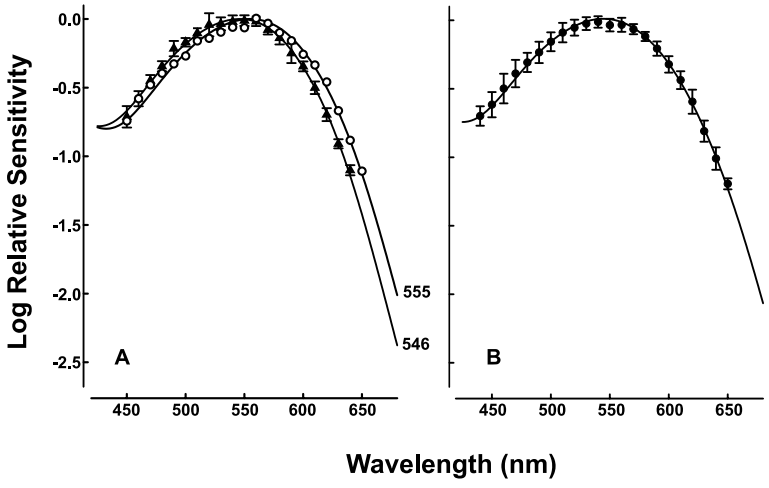

Fig. 5. ERG spectral sensitivity functions for Golden Lion tamarins. (A) Best fit functions obtained from two types of dichromats (a total of five males). (B) Spectral sensitivity curves obtained from five female Golden Lion tamarins each having two types of M/L pigment. The curve is the best fit linear summation of pigment templates having peak values of 546 nm (28%) and 555 nm (72%).

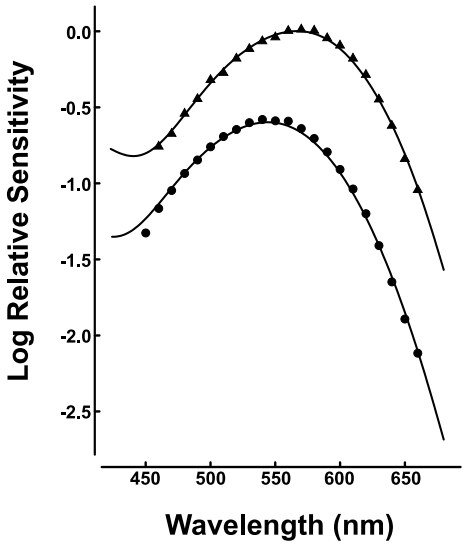

Fig. 6. ERG spectral sensitivity functions for white-faced Saki monkeys. The male monkey whose data are shown at the top had only a single M/L pigment ($\lambda_{max} = 565$ nm). The data from the female shown below had two M/L pigments and the curve fitted to those data is a linear summation of template curves having peak values of 537 nm (65%) and 563 nm (35%). See text for further details.

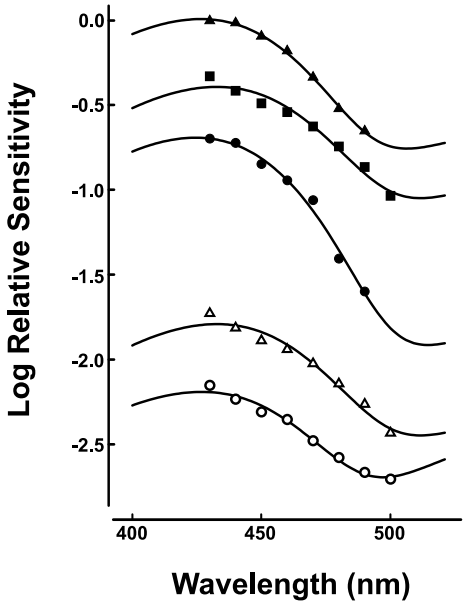

Fig. 7. ERG spectral sensitivity functions obtained from three Cebus monkeys (top, solid symbols) and two Cottontop tamarins (below, open symbols) under test conditions conducive for detecting contributions from S-cones. These spectra were derived as described in the text, by determining the best fitting combinations of S and M/L pigments. The identity of the M/L pigment had been previously determined for each animal (Figs. 2 and 3) and that value was fixed in the subsequent search procedure while the peak of the second pigment was determined by searching for the spectral location of an S-pigment that yielded the best summative fit. Other than for separation into two groupings, the five functions are arbitrarily positioned on the sensitivity axis.

of the spectral sensitivity function, suggests that her retina contains a pair of M/L pigments that are spectrally well separated. In the absence of direct measurements of the other M/L pigments in this species, her spectral sensitivity function (Fig. 6, bottom) was fit using templates that had peak values appropriate for the averages of the longest and shortest M/L pigments inferred for the Cebus monkeys (537 and 563 nm). This choice of pigment would also be in accord with inferences drawn from the structure of opsin genes found in a closely related species, the bald-faced Saki, *Pithecia irrorata* (Boissinot et al., 1998).

3.7. Presence of S-cones

Signals originating from activation of S-cones can be readily detected in flicker ERGs recorded using a short-wavelength reference light and concurrent long-wavelength adaptation. We tested a total of seven animals (three Cebus monkeys, three Cottontop tamarins, one Golden Lion tamarin) to verify that, as seemed likely, the retinas of these monkeys contained viable populations of S-cones. In each case S-cone contribution appeared as an elevation of sensitivity in the short-wavelengths relative to longer wavelengths. Indeed, under these test conditions sensitivity to middle and long-wavelengths was sufficiently depressed that it was typically impossible to make spectral sensitivity measurements for test lights much longer than about 500 nm. Spectral sensitivity functions obtained using this procedure for five of the subjects (three Cebus monkeys, two Cottontop tamarins) are in Fig. 7. For each, the spectral data points were fitted using linearly summed combinations of M/L and S-cone templates. To do this, the position of the M/L pigment was taken as specified by the direct measurements made as outlined above, and a search procedure was then used to find the spectral position of a standard S-cone mechanism that in combination with the M/L cone provided the best overall fit to the spectral sensitivity function. For the three Cebus monkeys the computed positions of the λ_{max} of the S-cone mechanism were 425, 426 and 427 nm; the corresponding S-cone locations for the Cottontop tamarins were 428 and 432 nm.

3.8. Photopigment pedigrees

Among the monkeys tested were 14 members of three families (two composed of Cottontop tamarins, one of Golden Lion tamarins). The M/L photopigment complements of these animals are summarized in the form of pedigrees in Fig. 8. In each case the M/L pigments are in accord with the hypothesis that they reflect a single-site polymorphism of X-chromosome opsin genes. Family 2 presents a strong test of the idea. The parents of that

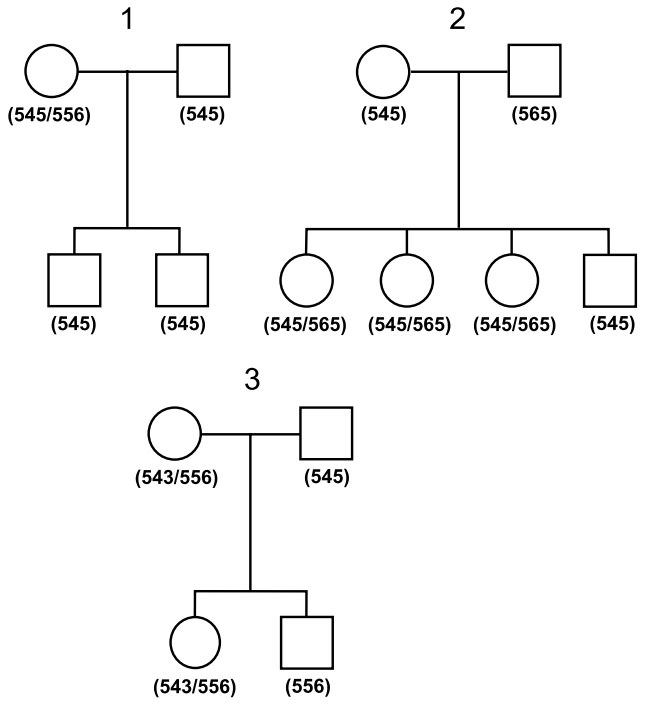

Fig. 8. M/L photopigment pedigrees for three families of platyrrhine monkey assessed from ERG spectral sensitivity measurements. Families 1 and 2 were Cottontop tamarins; family 3 were Golden Lion tamarins. In each case the spectral peak of the M/L pigment is indicated with a single value for a dichromatic animal and two values for potential trichromats.

family are both dichromatic, but they have different M/L pigments thus requiring that all female offspring are trichromats. There were three daughters in this family and, indeed, each was trichromatic. The model would also predict that any male offspring have the same pigment as the dichromatic mother and that was true for the single son of this pairing. The pigments found in other two families are also in accord with the same single-locus interpretation, although neither provides as compelling a test as does family 2.

3.9. Comparison of template fits

As noted above, at least seven different templates for fitting cone absorption spectra have been proposed. We examined the fits generated from each of the seven for four different sets of photopigment data that were selected to span the spectral range occupied by platyrrhine M/L cone pigments. These data sets were for the three separate M/L pigments measured from Cebus monkeys (a total of 19 animals, 11 from the current experiment and 8 from earlier measurements—Jacobs & Neitz, 1987b) and for one of the three M/L pigments of the Cottontop tamarins (peak of c. 548 nm; $n = 15$). Using a least-squares procedure each of the functions was individually best fit with each of the seven templates to a spectral positional accuracy of 0.1 nm. In making these fits, (a) all of the test wavelengths 460 nm and longer were used, (b) each of the functions was corrected for lens absorption as described above, and (c) a photopigment density of 0.15 was arbitrarily assumed. With one exception there was only small variation in the spectral positions obtained for fits using the various templates. That exception was the first of the proposed templates (Dawis, 1981). For all four of the M/L pigment positions, the λ_{max} values obtained using that template yielded a fit that was on average 1.5–2.3 nm shorter than those for the other six templates. These other six templates yielded λ_{max} estimates that covered a total range averaging only 0.93 nm across all four of the M/L pigment locations. For example, the mean λ_{max} value obtained for a photopigment estimated for 11 Cebus monkeys for the Dawis template was 560.9 nm while the mean value for the other six templates fit to the same data yields an estimated peak of 562.8 nm (SD = 0.19). With regard to measurements of the goodness of fit, the Dawis template also gave consistently poorer fits than those obtained for the other six templates. The metric aside, there were no consistent differences in the relative positioning (i.e., shorter versus longer) of the peak values of the spectra nor were there any consistent differences in the goodness of fit estimates for the various templates. This outcome is perhaps not surprising since all of these more recent templates share in common many assumptions that went into their derivation. Finally, it should be noted that although six of the seven templates examined provide virtually indiscriminable accounts of platyrrhine M/L cone photopigments, these templates do differ significantly in the assumptions made about the shape of the spectral absorption curves at spectral wavelengths shorter than those examined here (460 nm) and those differences can be matters of concern for specific analysis of short-wavelength sensitivity.

4. Discussion

Two of the types of monkey examined here (Cebus and Cottontop tamarins) have been subjects of other experiments involving measurements of photopigments and/or color vision. Early behavioral experiments on Cebus monkeys raised the possibility of individual variations in color vision (Gunter, Feigenson, & Blakeslee, 1965) and measurements of photopigments or opsin gene identifications in Cebus monkeys both supported that conclusion and additionally provided evidence that these animals are polymorphic at a single X-chromosome opsin gene site (Bowmaker & Mollon, 1980; Hunt et al., 1998; Jacobs & Neitz, 1987b; Lee et al., 2000). On the other hand, behavioral experiments conducted with Cottontop tamarins (Savage, Dronzek,

& Snowden, 1987) and Cebus monkeys (Pessoa, Tavares, Aguiar, Gomes, & Tomaz, 1997) suggest other interpretations. Both of these latter investigations examined the abilities of monkeys to discriminate various pairings of Munsell color papers and each found that male monkeys succeeded at particular color discriminations that would seem impossible for a dichromat (e.g., red versus green papers). Such experiments, however, probably are not definitive for the purpose of classifying color vision since human dichromats easily discriminate pairs of colored papers that were suggested as crucial for demonstrating trichromatic color vision in male Cebus monkeys (Gomes, Pessoa, Thomas, & Pessoa, 2002; Jacobs, 1999). Accordingly, it seems unlikely that these behavioral studies provide exceptions to the conclusions drawn here and elsewhere about color vision variations in New World monkeys.

As noted above, early research done on monkeys from the family Cebidae indicated that, aside from the nocturnal Aotus monkey, each of the species tested was polymorphic. However, the spectral positions of the M/L pigments differed for animals drawn from two subfamilies of this group. That conclusion is strengthened by the measurements reported here. To summarize these data we derived estimates of the average spectral locations of M/L photopigments for monkeys of this family as obtained from ERG measurements of the present work ($n = 54$) and for those examined in earlier experiments in this laboratory. Spectral sensitivity functions from a total of 104 dichromatic monkeys were best fitted using the spectral absorption curves of Govardovskii et al. (2000). To minimize the influence of any preretinal filters these functions were truncated so that only wavelengths longer than 500 nm were considered (Sharpe et al., 1998), and an optical density value of 0.15 was assumed. Fig. 9 is a frequency histogram of the spectral peaks derived for all the animals of this sample and it shows, in support of earlier work involving behavioral, electrophysiological, genetic and microspectrophotometric measurements (summarized in Jacobs, 1998), that the M/L pigments of these platyrrhines cluster in five discrete spectral locations. It is also clear from Fig. 9 that these spectral locations differ for species of the two subfamilies. The two species from Cebinae share three pigment positions having average peak values of about 535, 549, and 562 nm respectively. The four species from Callitricinae have analogous positions of about 544, 556, and 563. The latter pigment was not detected in the monkeys from two of these species, but the samples are small and the presence of this third pigment in them seems probable. Measurements made of the single M/L pigment found in Aotus are included in Fig. 9. The Aotus M/L pigment fall in the middle group and is indistinguishable from one of the pigments characteristic of the Callitrichids. These spectral data are further summarized in Table 1. An ANOVA run on

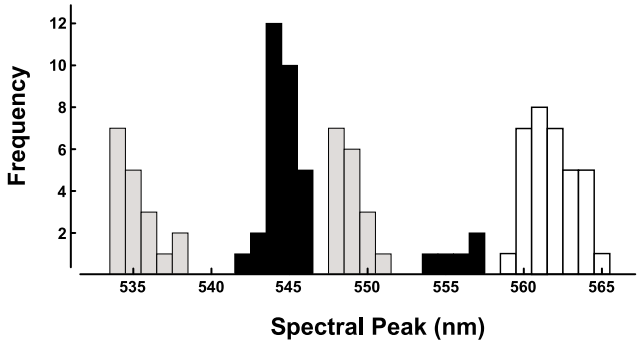

Fig. 9. Frequency histogram of the spectral peaks for M/L cone pigments measured in dichromatic platyrrhine monkeys of the family Cebidae. The method used to derive these peaks is explained in the text. Monkeys whose data are indicated in black are exclusively from the subfamily Cebinae while those shown in gray are exclusively from the subfamilies Callictrichinae and Aotinae. Note that there is no overlap in subfamily representation among these four spectral clusters. The unshaded bars include results obtained from animals drawn from more than one of the subfamilies.

the data of Table 1 verified that there are significant differences in spectral peaks estimated for the various positions ($F(4, 99) = 869.75$, $p < 0.001$) while post-hoc comparisons (Scheffé test) yield highly significant differences for all pairwise comparisons made between spectrally adjacent categories ($p < 0.001$). Although these results suggest quite strongly that the M/L pigments of monkeys from the family Cebidae occupy only five different spectral positions, we do note that the variability in the peak estimates is too great to resolve the possible presence of any potential polymorphic variations that might produce very small (1–2 nm) spectral shifts like those found in humans (Neitz & Neitz, 2000).

The number of animals available for testing from some of the species was restricted, but even so the results indicate that each of the species that constituted the targets of this investigation are polymorphic at a single X-chromosome opsin gene site and, accordingly, would be predicted to show the striking individual variations in color vision of the kind earlier documented for other platyrrhine monkeys (Jacobs, 1998). With these earlier results it now seems evident that monkeys drawn from at least nine of the 16 genera constituting *Platyrrhini* have M/L opsin gene polymorphisms. Of the remaining seven, we noted above that apparently no such polymorphisms occur in two genera (*Alouatta* and *Aotus*). Still remaining to be examined are representatives from five genera (*Bracytelles*, *Chiroptes*, *Cacajao*, *Callimico*, *Cebuella*). By virtue of their relationships of these animals to others already studied it seems probable that they too have M/L photopigment polymorphisms, although the example recently provided from studies of *Alouatta* suggests some reason for caution. In summary, although the picture is not yet completed, polymor-

Table 1
ERG estimates of spectral peak locations (in nm) of M/L pigments obtained from dichromatic platyrrhine monkeys of the family Cebidae

Species	Position 1	Position 2	Position 3	Position 4	Position 5
Cebinae					
Squirrel monkeys	534.77 (0.92)[a] ($n = 14$)		548.43 (1.98) ($n = 13$)		561.18 (1.46) ($n = 17$)
Cebus monkeys	535.63 (2.41) ($n = 4$)		548.83 (0.68) ($n = 4$)		562.77 (1.51) ($n = 11$)
Callitrichinae					
Cotton top tamarins		545.64 (1.79) ($n = 15$)		556.71 ($n = 1$)	563.3 (3.07) ($n = 5$)
Golden lion tamarins		544.5 (0.9) ($n = 4$)		554.4 ($n = 1$)	
Golden-handed tamarins		544.7 ($n = 1$)		557.0 ($n = 1$)	
Saddle back tamarins		543.15 (0.9) ($n = 4$)		555.35 (0.49) ($n = 2$)	563.5 ($n = 1$)
Aotinae					
Owl monkeys		544.65 (0.98) ($n = 6$)			
Mean λ_{max} (n)	534.96 (18)	544.93 (30)	548.52 (17)	555.76 (5)	562.07 (34)
SD	1.35	1.62	1.74	1.10	1.93

[a] Mean (SD).

phism of M/L cone opsin genes and photopigments is quite clearly a predominant feature impacting color vision in New World monkeys.

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