

The Relationship Between Cone Pigments and Behavioural Sensitivity in a New World Monkey (*Callithrix jacchus jacchus*)

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Microspectrophotometric measurements of visual pigments and behavioural measurements of spectral sensitivity are reported for individual marmosets from 3 family groups. The sex differences and polymorphism that characterise the long-wave cone pigments in this species are well reflected by variations in the behavioural sensitivities. With one exception, the pattern of inheritance is compatible with a genetic model in which the long-wave pigment is specified by a single polymorphic locus on the X-chromosome. Measurements are also reported for the spectral absorbance of the marmoset lens, and these are used to reconstruct short-wave behavioural sensitivity from the microspectrophotometric measurements of the short-wave cones.

Color vision *Callithrix jacchus* Visual pigments Cones Polymorphism Sex differences Lens

INTRODUCTION

The marmoset, like several other species of New World monkey, exhibits a polymorphism of cone photopigments (Travis, Bowmaker & Mollon, 1988), and so offers an attractive opportunity to correlate physiological variations with behavioural variations in spectral sensitivity. In this paper we report three types of measurement on the same individual animals: behavioural measurements of increment thresholds, measurements of the absorbance of the eye lens, and microspectrophotometry (MSP) of individual photoreceptors.

Previous microspectrophotometric measurements suggest that all members of the species share the same short-wave cone pigment; but in the long-wave spectral region at least three different pigments occur, with peak sensitivities (λ_{max}) at 543, 556 and 563 nm (Travis *et al.*, 1988; Tovée, Mollon & Bowmaker, 1990). Male marmosets can possess any one of these long-wave pigments, whereas females can possess either one or two of the three. This polymorphism is similar, but not identical, to that found in the squirrel monkey, *Saimiri sciureus* (e.g. Mollon, Bowmaker & Jacobs, 1984; Jacobs & Neitz, 1987a). To account for the latter case, Mollon *et al.* (1984) proposed that there is only a single genetic locus for a cone pigment in the long-wave region, that the locus is on the X-chromosome, and that there are three

alleles for the locus. The gene for the short-wave cone pigment is assumed to be autosomal, as in the human case (Kalmus, 1955).

Crucial to the theory is the random inactivation of one X-chromosome in individual cells of female mammals (Lyon, 1962): any long-wave cone cell will express only a single pigment gene. So if a female is heterozygous at this locus, possessing different pigment alleles on her two X-chromosomes, the two alleles will be expressed in different receptors. Such a monkey will be trichromatic, provided her nervous system is flexible enough to learn to compare the signals from her two subsets of long-wave cones. A female homozygous for the gene will possess only one pigment in the long-wave region, and so will be dichromatic. Males, being hemizygous at this locus, will always be dichromatic.

In the case of squirrel monkeys, this theory is consistent with an electroretinographic study by Jacobs and Neitz (1987b) on 25 members of 9 families. But it is not yet known whether an analogous model will hold for other New World monkeys. The microspectrophotometric study of 21 marmosets by Travis *et al.* (1988) suggested certain inconsistencies. Firstly, only two types of trichromatic female were convincingly found, whereas the theory calls for three. Secondly, one member of a pair of female twins appeared to exhibit only the 556 nm pigment whereas its co-twin exhibited only the 563 nm pigment: the theory forbids two forms of female dichromat from the same parents, since, whichever of their mother's genes they inherit, they must inherit the same pigment gene from their father. In the present study, to

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allow additional tests of the single-locus X-chromosome theory, we have drawn the subjects from 3 family groups.

In order to relate microspectrophotometric measurements to variations in actual visual sensitivity, we tested monkeys behaviourally on an increment-threshold spectral-sensitivity task similar to that used by Sperling and Harwerth (1971) for Old World monkeys and by Jacobs, Neitz and Crognale (1987) for New World monkeys. The use of a bright white field and of large, long-lasting, test flashes favours detection by opponent mechanisms (King-Smith & Carden, 1976; Mollon, 1982). A trichromatic animal should have a spectral sensitivity with a clear notch (Sloan's notch) in the long-wave region, indicating an opponent interaction of two cone channels. A dichromat should lack the notch and exhibit only a single peak in this region.

METHODS

Subjects

Microspectrophotometric data were obtained from 14 marmosets (*Callithrix jacchus jacchus*), drawn from 3 families (for pedigrees, see Fig. 2). In addition, behavioural data were obtained from 8 of the animals.

(i) Behavioural testing

Apparatus. A 12 V, 50 W tungsten-halogen bulb provided the light source for three test beams, each containing infra-red and ultra-violet filters (f1), a grating monochromator (M1-M3; Applied Photophysics, model 7300, spectral bandpass: 11.6 nm), and an additional gelatin blocking filter (f2) appropriate to the wavelength in use (Fig. 1). After passing through a circular neutral-density wedge (W1-W3), mounted on a computer-controlled stepping motor, each beam was collimated before being directed through a slot in an integrating sphere (I.S.) to project on to a frosted screen. The beam diameters were regulated by apertures at positions a1 and a2. Shutters were placed at S.

The background field was provided by a fibre-optic source (L2) containing a 12 V, 50 W tungsten-halogen lamp. This source was connected by a solid, transparent perspex rod (r) to an integrating sphere, 250 mm in dia. One section of the sphere was removed, allowing it to fit against the frosted screen. When a white field was required, the tungsten light was left unfiltered and the luminance of the field (measured with an SEI photometer) was $108 \text{ cd} \cdot \text{m}^{-2}$. To obtain coloured fields, a filter was placed in a holder between the end of the perspex rod and the integrating sphere.

In the case of the blue adapting field, a modification was made to favour the isolation of non-opponent visual mechanisms (Mollon, 1982): a sectored disc rotating at 1 Hz was introduced into the paths of the test beams so as to give two 10 msec pulses of light every second when one of the test shutters was concurrently raised.

Procedure. The animal's task was a three-alternative forced choice. A 10 mm monochromatic disc was pro-

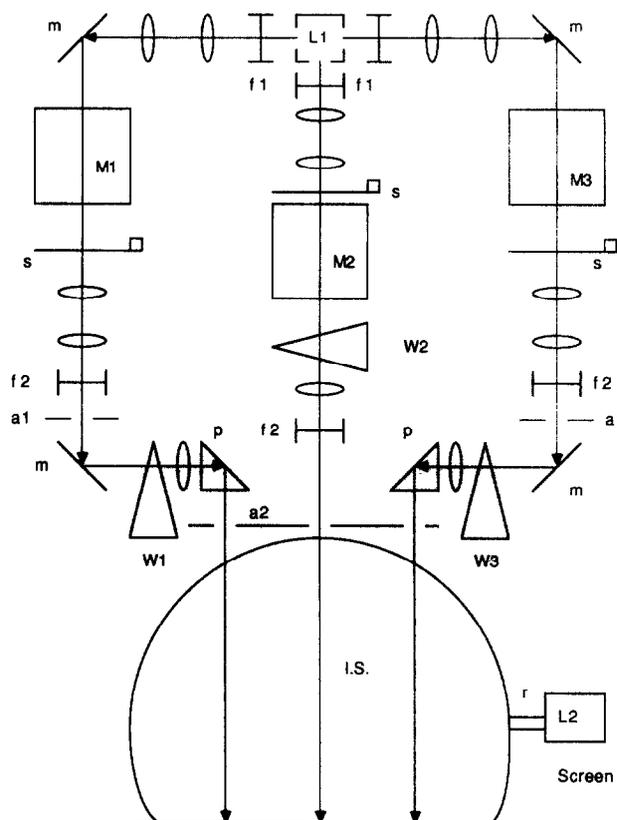


FIGURE 1. Diagram (not to scale) of the optical system used in the behavioural experiments. See text for details. (L1, light source; f1, heat absorbing glass and UV-absorbing gelatin filters; f2, Wratten gelatin blocking filters; m, mirrors; M1-M3, monochromators; W1-W3, neutral density wedges; a1-a2, apertures; p, prisms; I.S., integrating sphere; r, perspex rod; L2, fibre-optic light source.)

jected on to a 200 mm dia, homogenous white field in one of three positions, 25 mm apart. Within the viewing chamber, the monkey was unrestrained, but its minimum distance from the frosted screen was 50 mm. The stimulus remained present for 12 sec or until the monkey responded, whichever was the shorter. To secure a reward of banana milk-shake, the monkey had to touch the position on the screen at which the test flash had been projected. A correct response was followed by a tone, which indicated that reinforcement could be obtained by licking a spout. The animal's performance determined the stimulus intensities used in successive blocks of four trials, according to the BUDTIF procedure of Campbell and Lasky (1968). The changes in stimulus radiance were governed by the rule $S_2 = S_1/2n$, where S_1 was the initial step size (0.3 log units) and S_2 was the step size at that particular point in the test run (Findlay, 1978). n was initially zero; its value increased by 1 if the wedge reversed direction after a given block or remained in the same position; it decreased by 1 (to a limit of zero) if the wedge moved in the same direction. When the value of n was 3 (corresponding to a step size of 0.05 log units), the monkey was judged to have reached threshold.

For a given measurement, three staircases were run and the average value was taken as the threshold for that day. Each point in the threshold curve is based on three

such averages. Wavelengths were tested in random order.

Before a given day's set of threshold measurements, the maximal radiance of each beam at the test wavelength was measured at the screen by mounting a PIN10 silicon photodiode at each in turn of the three possible positions of the target. By compensatory adjustments of the wedges, the computer program ensured that the three beams were matched in output during the threshold measurements.

In addition to the achromatic field, two chromatic fields were used: a $4.5 \text{ cd} \cdot \text{m}^{-2}$ blue field (Schott blue glass filter, BG 23), and a $3.5 \text{ cd} \cdot \text{m}^{-2}$ yellow field (Wratten 16 gelatin). For the chromatic field measurements, the diameter of the test stimulus was 6 mm. For the blue-field measurements, in order to favour stimulus detection by non-opponent mechanisms, the test stimulus was presented as a train of brief pulses (see above). The train of pulses lasted for 12 sec or until the monkey responded, whichever was the shorter.

(ii) *Microspectrophotometry and lens measurements*

The monkeys were killed by an i.p. injection of nembutal, after being anaesthetised with an intramuscular injection of ketamine hydrochloride. Enucleation of the eyes was performed in dim red light. The lens from each eye was removed and its absorbance was measured by the method of Douglas and McGuigan (1989). Retinal tissue was prepared for microspectrophotometry as described by Mollon *et al.* (1984). Samples were taken from the foveal region, and measurements began roughly 30 min after the monkey's death. The spectral absorbances of the outer segments of individual receptors were measured with a dual-beam Liebman microspectrophotometer (Liebman & Entine, 1964; Knowles & Dartnall, 1977). If a structure had its maximal absorbance in the short-wave region, it was measured three times, and then bleached for 5 min; if it proved photosensitive, three post-bleach measurements were made.

The λ_{max} for each MSP record was estimated by fitting a template curve to the absorbance data. The template used was Dartnall's standard spectrum for frog rhodopsin (Knowles & Dartnall, 1977). The template was computed for $\lambda_{\text{max}} = 502 \text{ nm}$ and was then re-expressed on an abscissal scale of log frequency, since Mansfield (1985) has shown that the absorbance curves of visual pigments have almost the same shape if expressed on such an abscissa. In the study of Travis *et al.* (1988), an abscissal scale of $\lambda^{1/4}$ was used. The effect of changing the fitting procedure is to shift the λ_{max} of long-wave cones by about 2 nm towards shorter wavelengths. For discussion, see Bowmaker, Astell, Hunt and Mollon (1991).

The procedure for fitting the template to the microspectrophotometric data, and the criteria for accepting records, were the same as those used previously (Travis *et al.*, 1988). Owing to the limited data, no selection criteria were used for short-wave cones, but two estimates of λ_{max} were obtained, the first from the right-hand

limb of the average of the three pre-bleach records and the second (λ_{maxb}) from the right-hand limb of the curve representing the difference between the pre- and post-bleach spectra.

(iii) *Comparison of microspectrophotometric and behavioural data*

In order to compare MSP data with behavioural sensitivities, the measured absorbance spectra were converted to log absorbance (Knowles & Dartnall, 1977), assuming that the outer segments of cones had a length of $20 \mu\text{m}$ and a specific density of 0.015 per micron (i.e. an axial absorbance of 0.3). A correction was also made for lens absorbance. However, no correction was made for absorption by the macular pigment, because the monkeys were not constrained in their fixation and the part of the retina that detected the test flash in a given presentation was not known.

In the case of the two female monkeys A3 and B3, where two long-wave pigments are present, we fitted the blue-field behavioural sensitivities by summing the two

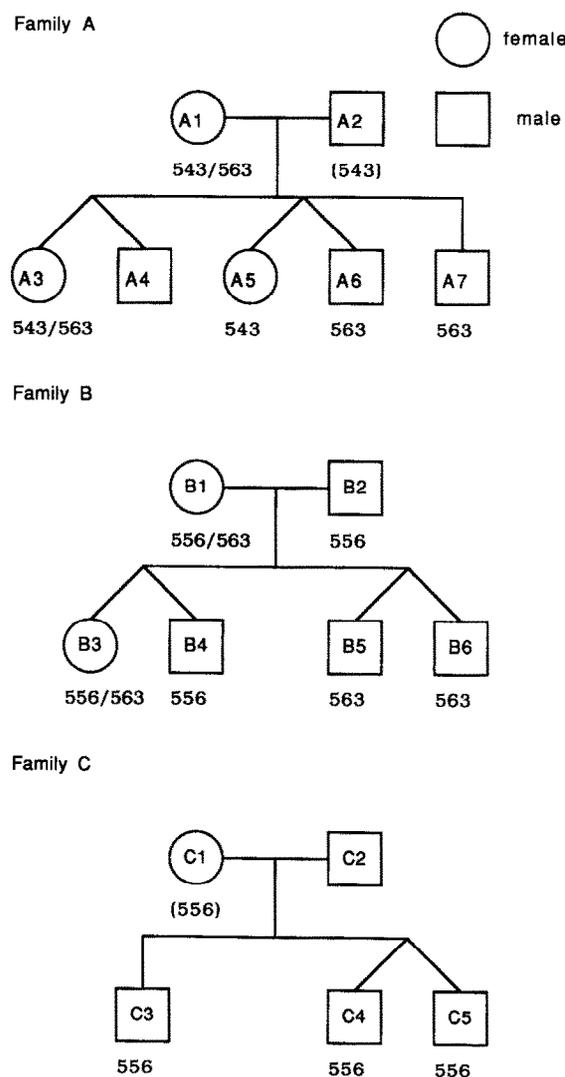


FIGURE 2. Pedigrees for the 3 families of marmosets. The numbers shown below individual animals represent their nominal phenotypes and are based on microspectrophotometric estimates of the λ_{max} values for their long-wave pigments; values shown in brackets are inferred.

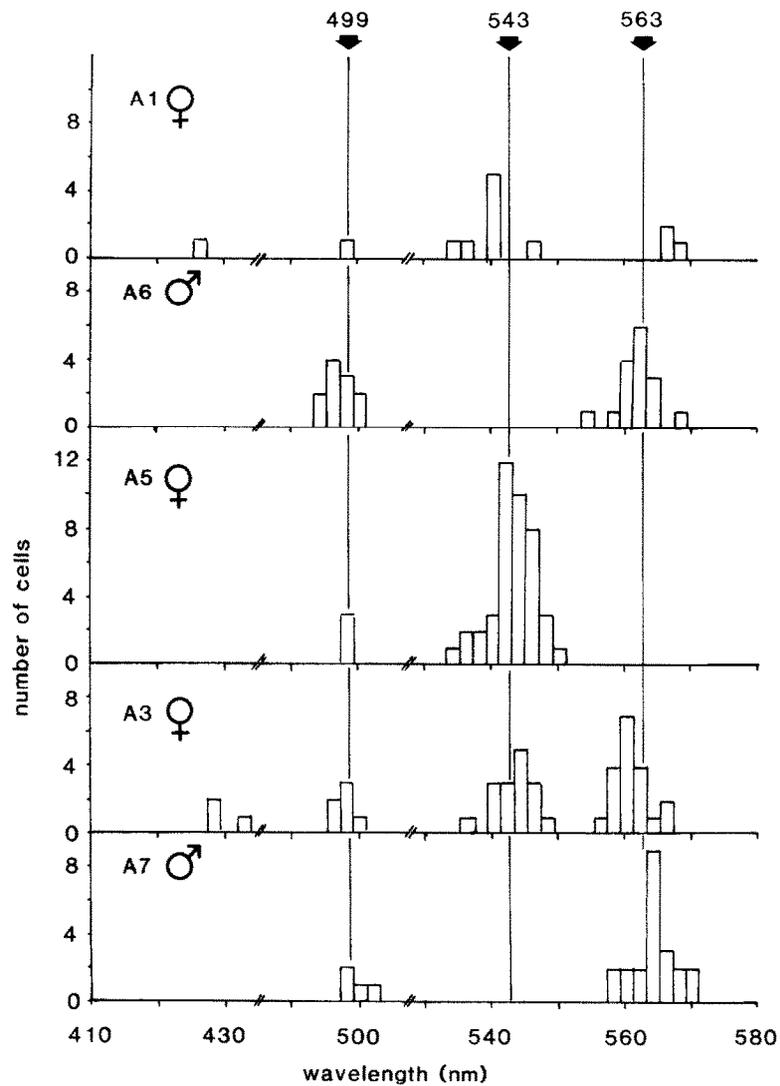


FIGURE 3. Distribution of values of peak sensitivity for individual receptor cells from the members of Family A. The bin size is 2 nm.

MSP absorbance curves with equal weighting, since the behavioural conditions were ones chosen to minimise detection by chromatically opponent mechanisms. But in the case of the white-field sensitivities for these animals,

where the conditions favoured an opponent mode of detection, we modelled the behavioural data with subtractive functions similar to those used by Sperling and Harwerth (1971).

TABLE 1. The table shows the average λ_{\max} values for the rods and long-wave cones of each animal determined by MSP. Each value is the average of the λ_{\max} values for all the records of that class of photoreceptor for a given animal

	Rods			543 nm			556 nm			563 nm		
	<i>n</i>	λ_{\max}	SD									
A1 (f)	1	499.0		11	540.2	3.6				3	566.3	1.0
A3 (f)	6	500.4	1.0	16	543.4	2.9				20	563.1	2.7
A5 (f)	11	498.3	0.4	42	543.6	3.2						
A6 (m)	11	497.5	2.1							16	562.0	2.7
A7 (m)	4	499.6	1.2							21	563.4	3.3
B1 (f)							9	555.8	2.0	13	562.8	1.4
B2 (m)	5	498.6	2.6				24	556.0	4.0			
B3 (f)	16	499.1	1.2				19	553.8	3.7	23	564.2	2.5
B4 (m)	1	498.9					22	556.7	3.0			
B5 (m)	4	499.8	0.5							27	564.2	3.4
B6 (m)	3	500.8	1.7							27	560.6	4.7
C3 (m)	3	496.1	2.3				28	555.7	3.3			
C4 (m)	5	498.5	2.1				37	556.2	3.7			
C5 (m)	3	501.1	1.3				8	556.7	3.1			

RESULTS

Long-wave cones

Figure 2 summarises the pedigrees for the 3 families. Shown against individual animals are the nominal phenotypes to which we assign them on the basis of the detailed evidence given below.

Family A. MSP data were obtained from the mother and four offspring in this family. The distributions of individual records are shown for each animal in Fig. 3, and mean values for rods and long-wave cones are listed in Table 1. Application of Kruskal's test (Giacomelli, Wiener, Kruskal, Poweranz & Loud, 1971) showed that the cone records obtained from the mother (A1) had a significantly bimodal distribution [dip intensity (d.i.) = 4.76, $P < 0.01$]: one group of cones had an average λ_{max} of 541 nm, and the other an average of 566 nm. The records from the cones of the daughter, A3, also had a significantly bimodal distribution (d.i. = 256.91, $P < 0.01$), with a λ_{max} of 544 nm for one group and 564 nm for the other. The data from the third female in this family, A5, had a distribution that was not significantly non-normal by Geary's test (D'Agostino, 1970; $a = 0.76$, $P > 0.10$), and had an average λ_{max} at 543 nm. The two sons, A6 and A7, exhibit long-wave cones with average λ_{max} values near 563 nm. The distributions of their cone records did not differ significantly from normality ($a = 0.74$, $P > 0.10$; $a = 0.736$, $P > 0.05$) or from each other (t -test, $t = 0.44$, d.f. = 33, $P > 0.10$); but they were significantly different from the distribution for the dichromatic female, A5 (one-way randomised ANOVA test, $F = 331.11$, d.f. = 2.74, $P < 0.01$).

Behavioural data were obtained from three of the offspring in this family, A3, A5 and A6. The white-field data for the female, A3, show clear dips at 480 and 580 nm and peaks at 450, 510–520 and 610 nm (Fig. 4). The white-field conditions favour opponent interactions, and this result is consistent with there having been two classes of cone in the long-wave region. The long-wave lobe of the behavioural sensitivity (S) has been fitted (solid line) by $S = 0.9L - 1.0M$, where L and M are the sensitivities of the microspectrophotometrically measured P564 and P544 pigments, after correction; and the middle-wave lobe has been fitted by $S = 1.3M - 1.0L$ (see Methods). Under blue-field conditions, which favour a non-opponent mode of detection, A3's behavioural data exhibit a single broad peak, near 560 nm. This peak was broader than the peaks obtained from A3's siblings under the same conditions. The MSP curve fitted in Fig. 4 to A3's blue-field sensitivities is the log of the sum of the absorbance curves for her two types of long-wave pigment. This fit depends on the assumptions that, firstly, the blue field adapts the two classes of cone to approximately the same extent, and secondly, random inactivation of the X-chromosomes produce equal numbers of the two types of cone.

Under white-field conditions, the threshold sensitivity function from the female A5 shows peaks at 450 and 540 nm and a dip at 500 nm (Fig. 4). This suggests that

there was only a single cone class in the long-wave region in this animal, a conclusion supported by the blue-field data, which also show a single peak in the long-wave region, of the same shape, and at the same position as under white-field conditions. If there were two or more

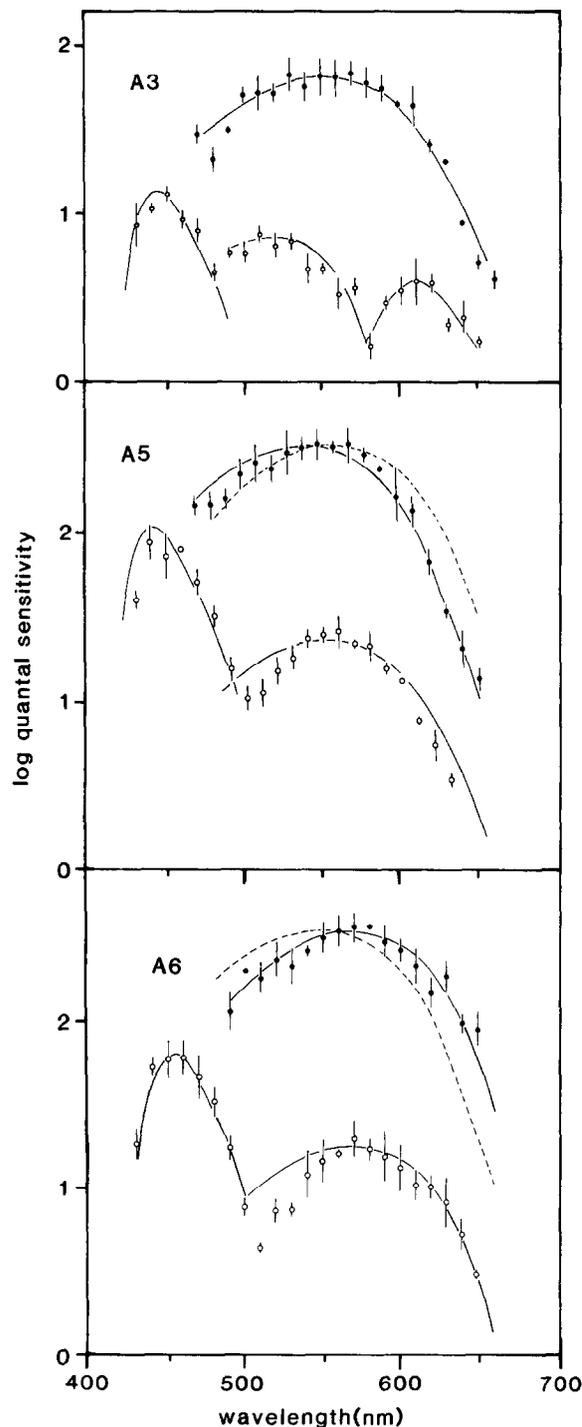


FIGURE 4. Spectral sensitivities obtained behaviourally for 3 members of Family A. Open circles represent sensitivities (reciprocals of log thresholds expressed in quantal units) on white fields; and solid circles represent sensitivities on blue fields. Note that the notch near 580 nm in the white-field data for A3 is not seen in her blue-field data or in the white-field data for the other two animals. The solid lines represent the sensitivities predicted from microspectrophotometric (MSP) measurements for the corresponding animal; in the case of A5 we also show for comparison (dashed line) MSP sensitivity for A6, and in the case of A6, we show (dashed line) the MSP sensitivity of A5.

cone classes in the long-wave region, the different background fields should have induced different patterns of adaptation in these cones, and that ought to have altered the shape and position of the peak in the spectrum. The average MSP absorbance curve for the cones from this animal is fitted to the behavioural data in Fig. 4.

The behavioural data from A6 suggest that it, too, was a dichromat. Under both white- and blue-field conditions there was a single peak in the long-wave region at around 560 nm. The MSP absorbance curve obtained from A6's cones is fitted to the blue-field data in Fig. 4, and the fit is consistent with A6's having been a 563 nm dichromat. To illustrate the difference between A6 and his dichromatic sister, A5, we show as a dashed line in the bottom panel of Fig. 4, the blue-field sensitivity that would be reconstructed from A5's MSP results; and in the case of A5 (middle-panel) we similarly show as a dashed line the analogous sensitivity for A6.

Family B. MSP data were obtained from a breeding pair and four of their offspring (Fig. 5 and Table 1). The distribution of the cone records from the mother, B1, was significantly non-normal ($\alpha = 0.86$, $P < 0.05$) and significantly bimodal (d.i. = 3.59, $P < 0.05$). One group

of cones had an average λ_{\max} of 557 nm and the other an average of 564 nm. The daughter, B3, had a similar distribution of cone records, which was also significantly non-normal ($\alpha = 1.21$, $P < 0.01$) and significantly bimodal (d.i. = 0.79, $P < 0.01$). One group of cones had a λ_{\max} at around 557 nm and the other a λ_{\max} at about 564 nm.

The distribution of the cone records from the father (B2) was not significantly non-normal ($\alpha = 0.78$, $P > 0.10$), and had a λ_{\max} at 556 nm. The male offspring, B4, had a similar distribution of cone records (t -test: $t = 0.76$, d.f. = 42, $P > 0.10$), which were also not significantly non-normal ($\alpha = 0.79$, $P > 0.10$), and had an average λ_{\max} at 556 nm. The distribution of the records from the male B5 was also not significantly non-normal ($\alpha = 0.82$, $P > 0.10$), but these records had an average λ_{\max} at 563 nm. The distribution was significantly different from those of B2 and B4 (t -test: $t = 7.66$, d.f. = 47, $P < 0.005$). The distribution of the cone records from the male B6 was not significantly non-normal ($\alpha = 0.78$, $P > 0.10$). However, the average λ_{\max} of the cones was 560 nm and the distribution was significantly different from that of B5 (t -test: $t = 3.19$, d.f. = 52, $P < 0.005$)

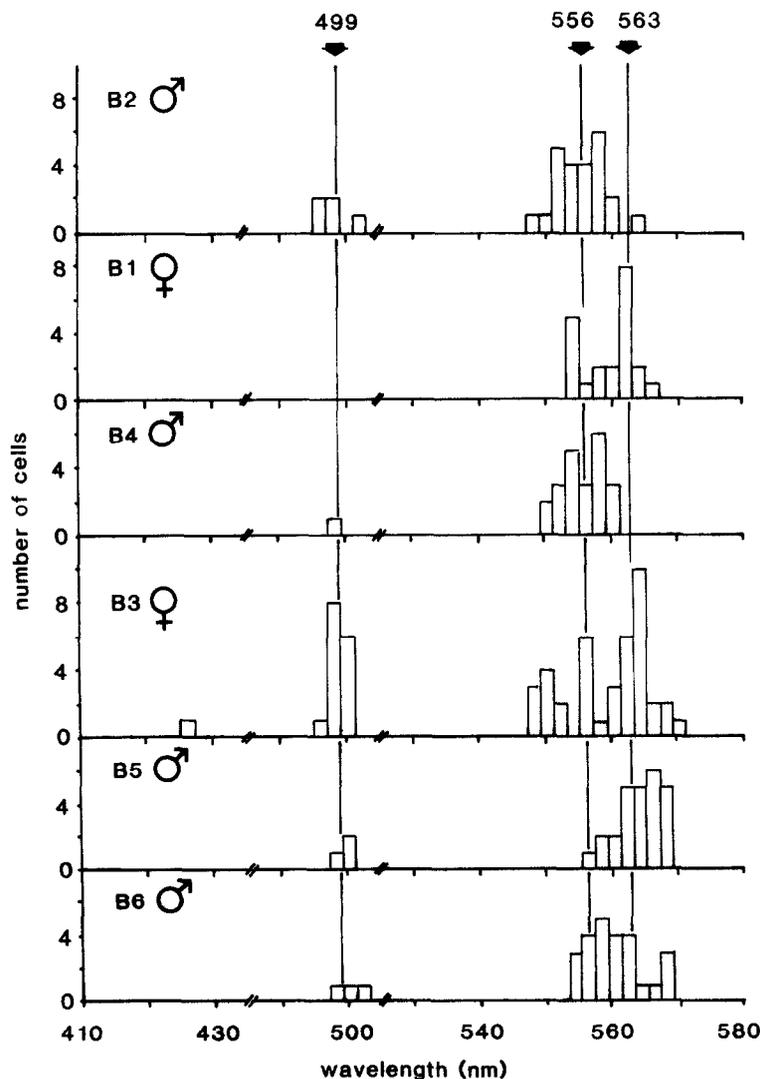


FIGURE 5. Distribution of values of peak sensitivity for individual receptor cells from the members of Family B. The bin size is 2 nm.

and from those of B2 and B4 ($t = 3.19$, d.f. = 47, $P < 0.005$).

Behavioural data were obtained from B2, B3 and B4. Under white-field conditions, the threshold sensitivity function for the female, B3, shows peaks at 450, 550 and 610 nm, and dips at 500 and 580–590 nm (Fig. 6). The long-wave lobe of her behavioural sensitivity has been fitted by $S = 1.0L - 1.0M$ and the middle-wave lobe by $S = 1.3M - 1.0L$. Under blue-field conditions there is only a single peak in the long-wave region, which is not broader than that of dichromatic animals under the same conditions, and which is narrower than the peak produced by the 543/563 trichromatic female, A3.

The white-field sensitivity data from the father, B2, exhibit a single peak between 520 and 620 nm, and in Fig. 6 are fitted to the absorbance curve obtained from his long-wave cones ($\lambda_{max} = 556$ nm). Under white-field conditions, the threshold sensitivity function from B4 shows peaks at 450 and 550–560 nm, and a dip at 500 nm. Under blue-field conditions the latter peak is unchanged in shape and position, suggesting this animal was dichromatic.

Family C. The three brothers, C3–C5, all produced similar distributions of MSP records in the long-wave region (Fig. 7 and Table 1). None of the distributions of cone records were significantly non-normal ($a = 0.84$, $P > 0.05$; $a = 0.78$, $P > 0.10$), and all had average λ_{max} values near 556 nm. The distributions of the cone records from the three brothers were not significantly different (one-way randomised ANOVA, $F = 0.685$, d.f. = 2, 70, $P > 0.01$).

Behavioural data were obtained from C3 and C4, and were consistent with their being 556 nm dichromats (Fig. 8). Their white-field data showed two peaks at 450 and 550–560 nm, and a dip at around 500 nm. Under blue-field conditions the position and shape of the long-wave peak was unchanged.

Short-wave cones

Records from 14 short-wave cones were obtained from the members of the 3 families and from other unrelated marmosets. Since records were obtained for a total of 1200 cones, the rarity of short-wave cones is manifest. A similar result was obtained by Travis *et al.* (1988) in their MSP study of the marmoset, and this is consistent with findings in other New World primates (e.g. Mollon *et al.*, 1984), and in Old World primates (e.g. Marc & Sperling, 1977; Dartnall, Bowmaker & Mollon, 1983; MacNichol, Levine, Mansfield, Lipetz & Collins, 1983).

The λ_{max} values of individual short-wave cones are not precise, owing to the low optical density of their outer segments and the low light levels from the tungsten source. However, the mean absorbance curve for the 14 cones gives a λ_{max} of 423.3 nm; and the average of the bleached spectra gives a λ_{maxb} of 423.2 nm. We have used the log-frequency basis (see Methods) to reanalyse the earlier sample of marmoset short-wave cones from Travis *et al.* (1988) and obtain a λ_{max} of 426.1 and a λ_{maxb} of 425.7 nm.

Using conditions that favoured the short-wave cone mechanism at the expense of the long-wave cone mechanisms, behavioural sensitivity functions were obtained from three male animals (Fig. 9). These all produced very similar curves with a peak at around 440 nm, and a very sharp decline in sensitivity on the short-wave side of the peak. Fitted to the behavioural data in Fig. 9 is the average short-wave cone pre-bleach curve, corrected for lens absorbance and for pigment self-screening. The pre-bleach short-wave curve was used since it was pre-bleach data that were fitted to the behavioural data

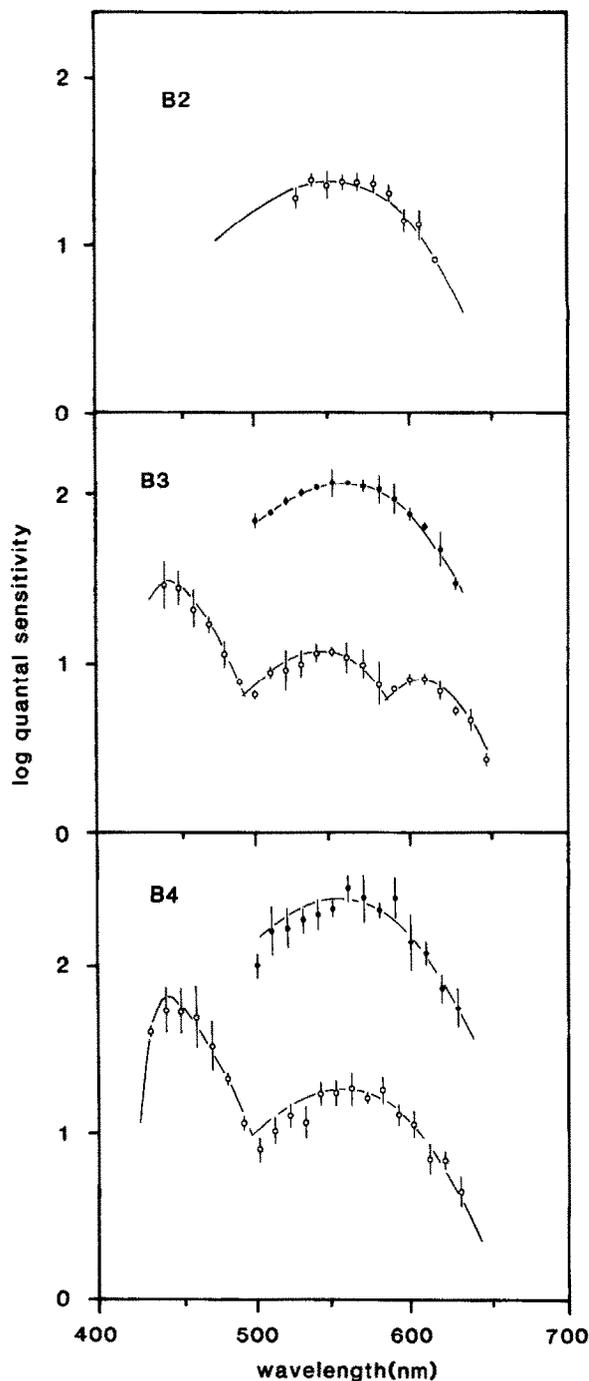


FIGURE 6. Spectral sensitivities obtained behaviourally for 3 members of Family B. Open circles represent sensitivities on white adapting fields, and solid circles represent sensitivities on blue fields. Solid lines represent the sensitivities predicted from MSP measurements for the corresponding animals.

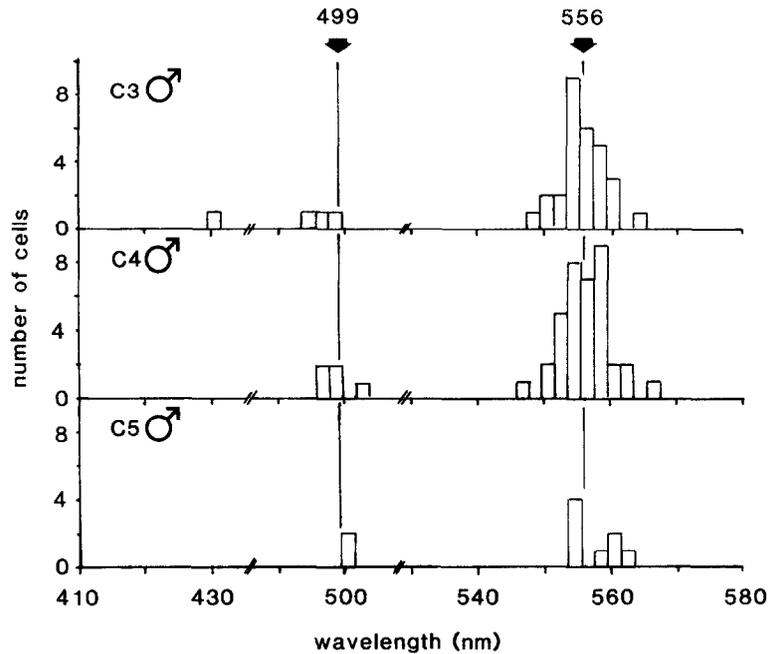


FIGURE 7. Distribution of values of peak sensitivity for individual receptor cells from the members of Family C. The bin size is 2 nm.

for the long-wave region. The lens absorbance data used for each animal are the average values for that animal.

Figure 10 shows the mean absorbance curve for eight marmoset lenses from our sample. The sharp rise in absorbance below 440 nm causes a corresponding reduction in short-wave behavioural sensitivity and has the effect of shifting the behavioural peak to a wavelength longer than the λ_{\max} of the photopigment.

DISCUSSION

The long-wave pigments of the marmoset

Our results show a clear polymorphism of long-wave pigments in the marmoset: at least three distinct pigments can occur, with λ_{\max} values close to 543, 556 and 563 nm. All the male animals were dichromatic, exhibiting a single pigment in the long-wave region, whereas the females were either dichromatic (e.g. A5) or trichromatic (e.g. A3 and B4). And the variations in the photopigments give rise to clear variations in behavioural sensitivity.

The P556 and P563 pigments are very closely placed in the spectrum and Travis *et al.* (1988) were led to ask whether there are in fact just two distinct pigments or whether there is a more finely graded set of pigments in this range. With one exception (B6), the dichromats in the present sample support the existence of two distinct pigments. A one-way randomised ANOVA test on the long-wave MSP records from the five dichromats (B1, B4, C3, C4, C5) classified as having P556 showed no significant difference between the distributions for different animals ($F = -1.127$, d.f. = 4, 112, $P > 0.05$). Nor was there any significant difference between the dichromats classified as having P563 (A6, A7, B5). However,

as did Travis *et al.* (1988), we find one animal (B6) exhibiting a λ_{\max} intermediate between 556 and 563 nm. This animal is discussed further below.

Trichromatic phenotypes

The behavioural data from the two females A3 and B3 offer clear evidence for opponent interactions between two classes of long-wave cone. This interaction is particularly marked in the case of A3, where the peaks of the white-field behavioural sensitivities lie at 520 and 615 nm. The microspectrophotometric data show that the underlying pigments have λ_{\max} values at 543 and 565 nm. So the peak sensitivity of the middle-wave opponent channel has been shifted towards shorter wavelengths by some 20 nm, and the peak of the long-wave channel has been shifted to longer wavelengths by 50 nm. The shape of A3's behavioural function resembles those obtained from human and catarrhine subjects under similar conditions (e.g. Sperling, Sidley, Dockens & Joliffe, 1968), although A3's "middle-wave" pigment, with λ_{\max} at 543 nm, does not lie in the range (530–535 nm) occupied by the middle-wave pigments of man and Old world primates (Dartnall *et al.*, 1983; Schnapf, Kraft, Nunn & Baylor, 1988; Bowmaker *et al.*, 1991). Jacobs (1990) has demonstrated a notch near 580 nm in a female callitrichid (*Saguinus fuscicollis*) with putative pigments at 544 and 557 nm.

The trichromatic female B3 represents a marmoset phenotype not previously described. Her behavioural sensitivity exhibits peaks at 550 and 615 nm. The corresponding microspectrophotometric data reveal cone pigments with closely spaced λ_{\max} values at 554 and 564 nm. The behavioural long-wave peak is shifted to 615 nm as in the previous trichromat, but the middle-wave peak is shifted towards shorter wavelengths by only around 5 nm. Given the spectral proximity of the two long-wave

cone pigments, it is instructive that a female of this phenotype can develop chromatically opponent channels that are sensitive to the difference of the two long-wave signals. In this respect, B3 may offer a model for anomalous trichromacy in man.

The short-wave cone pigment and pre-retinal absorption at short wavelengths

Behavioural sensitivities obtained on yellow fields show no obvious differences between individuals (Fig. 9). The peak sensitivity lies close to 440 nm. The microspectrophotometrically measured short-wave pigment has a λ_{max} at 423 nm, but the high short-wave absorbance of the marmoset lens shifts the maximum sensitivity of the short-wave channel to longer wavelengths. When the lens absorbances for our own animals are used to reconstruct the behavioural sensitivity, a good agreement is obtained (Fig. 9). Notice that at 410 nm the average absorbance for a marmoset lens is twice that for a human lens (Fig. 10). In absolute value and in spectral

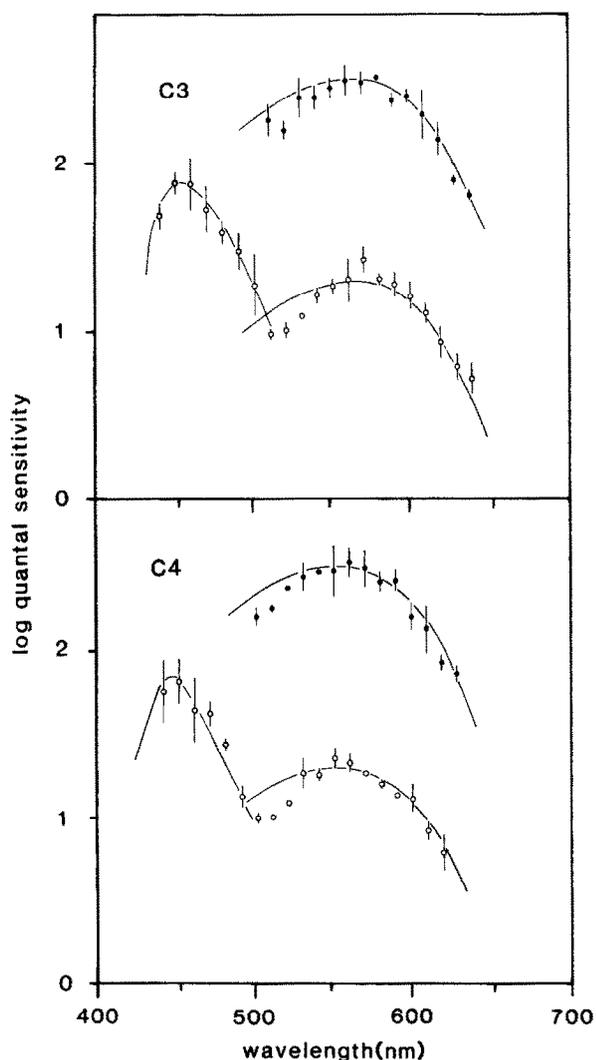


FIGURE 8. Spectral sensitivities obtained behaviourally for 2 members of Family C. Open circles represent sensitivities on white adapting fields, and solid circles represent sensitivities on blue fields. Solid lines represent the sensitivities predicted from MSP measurements for the corresponding animals.

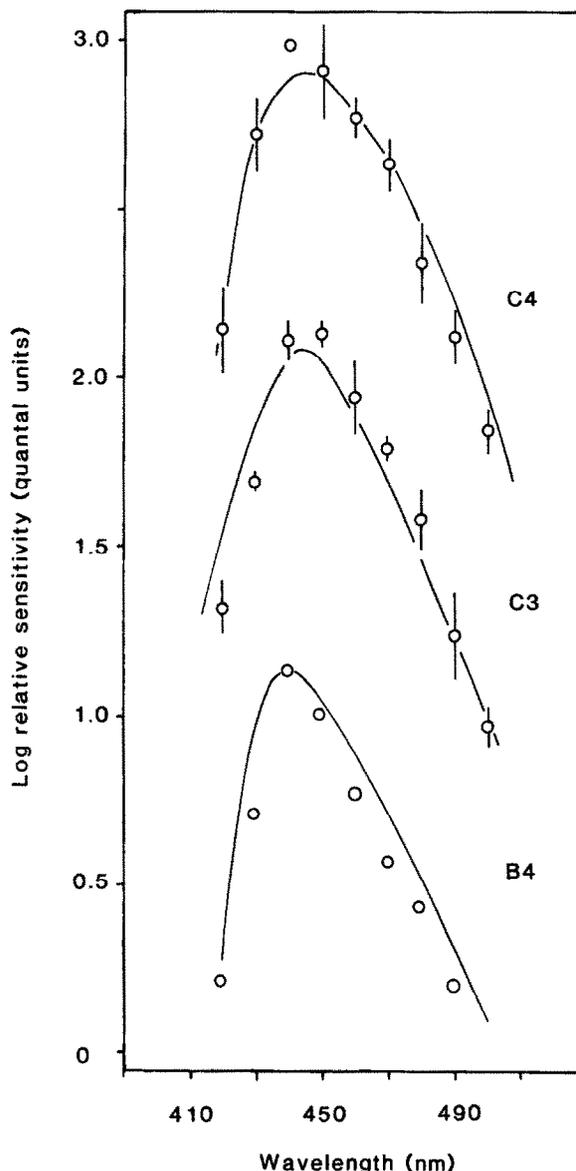


FIGURE 9. Spectral sensitivities on long-wave adapting fields for 3 individual monkeys. The solid lines represent sensitivities predicted from MSP, using a mean short-wave cone curve but using lens absorbances from the individual animals.

variation, the lens absorbances obtained for the present sample of marmosets agree well with those reported by Travis (1986), although different animals and different spectrophotometers were used.

The behavioural sensitivity of the short-wave channel in the marmoset is similar to that measured electroretinographically in the tamarin by Jacobs *et al.* (1987), who calculated that the tamarin's short-wave pigment had its maximum sensitivity between 433 and 436 nm. However, their calculation assumed that the pre-retinal absorbance of the tamarin in the short-wave region was the same as that of the cebid squirrel monkey. The source of their cebid absorbances is unclear, but the values given by Cooper and Robson (1969) are lower, and less sharply changing, than those that we have obtained for marmosets (Fig. 10). Since the tamarin is phylogenetically closer to the marmoset, our marmoset values may be more suitable for estimating the spectral

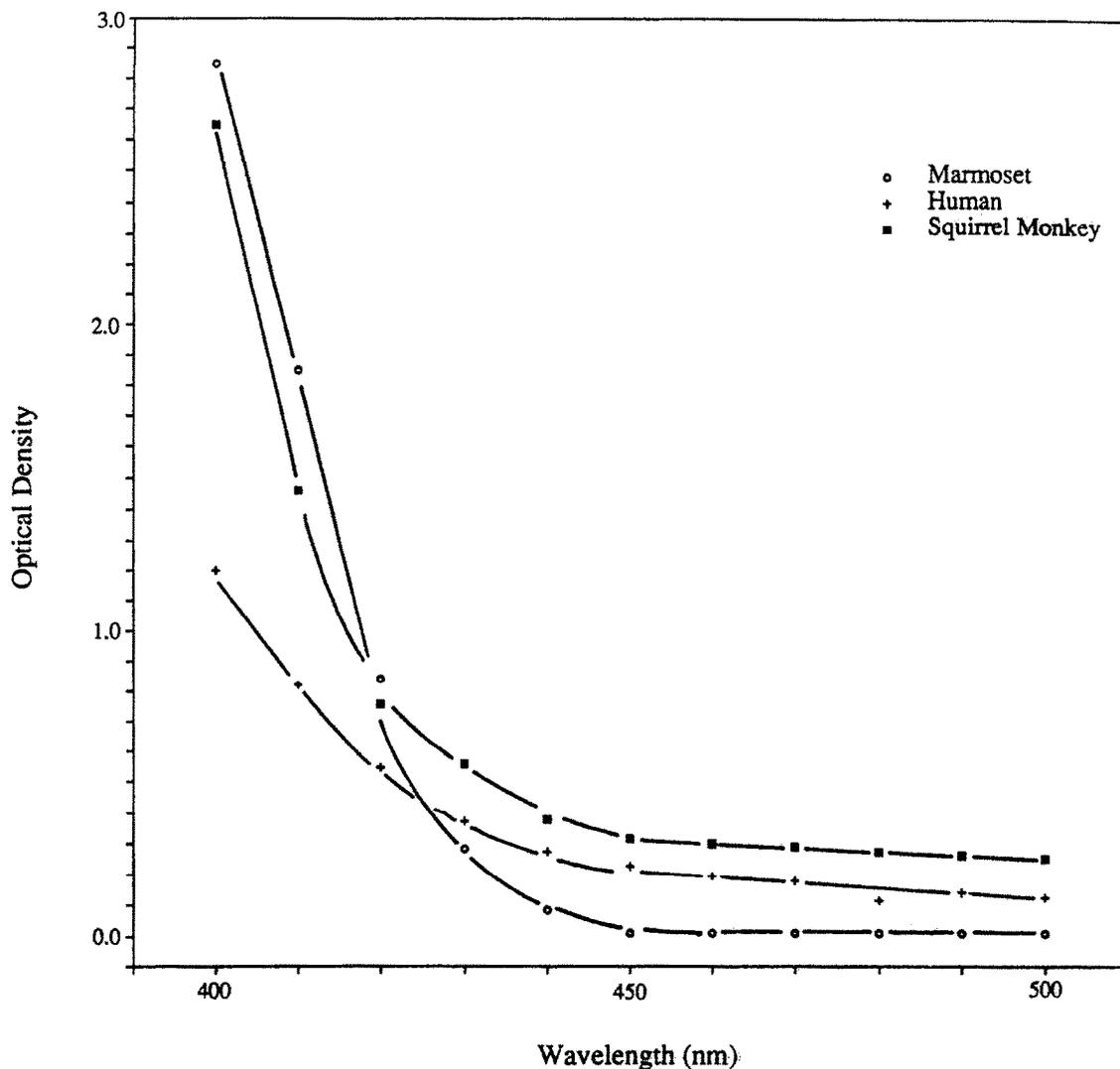


FIGURE 10. Absorbance curves for lenses from marmosets, squirrel monkeys and man. The values for squirrel monkeys are taken from Cooper and Robson (1969) and those for man are taken from Wyszecki and Stiles (1982, p.109). The marmoset measurements were made by Dr R. Douglas, using tissue from animals in the present sample.

position of the short-wave pigment in the tamarin: we suggest the value lies closer to 425 nm than to 435 nm.

The single-locus X-chromosome model

The single-locus X-chromosome model draws strong support from the sex difference in the distribution of phenotypes in this sample. None of 9 male monkeys showed either microspectrophotometric or behavioural evidence of trichromacy; but 3 of the 5 females exhibited three cone pigments and in 2 of these cases we were also able to obtain behavioural evidence of trichromacy.

With one possible exception, none of the details of the pedigrees contradict the model. Consider first Family A (Fig. 2). The behavioural and microspectrophotometric data concur in showing that the males A6 and A7 had a single long-wave pigment, with a λ_{\max} at 563 nm. This requires that their mother, A1, must have had an allele on one of her X-chromosomes for the 563 nm opsin. Behavioural and microspectrophotometric data show that the daughter, A5, had a single long-wave pigment, with a λ_{\max} at 543 nm. So this animal must have been homozygous for the 543 nm pigment allele. Since one of

A5's X-chromosomes came from her mother (A1), one of her mother's X-chromosomes must have carried the 543 nm allele—while the mother's other X-chromosome carried the 563 nm allele inherited by her sons (A6, A7). The microspectrophotometric data obtained directly from the mother do conform to these requirements of the model: she exhibits (Fig. 3) two cone pigments, with λ_{\max} values at 543 and 563 nm. The father A2 died without data being obtained from him, but his dichromatic daughter A2 requires that he himself was a 543 nm dichromat. This inference, required by the theory, is compatible with the results for the other daughter (A3). She is trichromatic, exhibiting the 543 and 563 nm pigments: so she was able to inherit the 543 nm allele from her father and the 563 nm allele from her mother.

No member of Family A exhibits the 556 nm pigment. In contrast, a salient feature of Family B (Fig. 5) is that none of its members exhibits the 543 nm pigment. Microspectrophotometric and behavioural data for the father (B2) suggest that he was a dichromat with a 556 nm pigment, whereas the microspectrophotometric data for the mother (B1) suggest that she had both 556

and 563 nm pigments. According to the single locus X-chromosome theory, female offspring of this pair could be either 556 nm dichromats or 556/563 nm trichromats. In fact, the single female offspring (B3) of this pair was found to have both the 556 and 563 nm pigments, and her trichromacy was confirmed by her behavioural sensitivity on a white field. The X-chromosome theory also predicts that any male offspring could be either a 556 nm dichromat or a 563 nm dichromat. B4 is the former, B5 is the latter. The male B6 is the one difficulty for the single-locus X-chromosome theory: the mean λ_{\max} of his long-wave cones is 560.6 nm and the distribution of individual cells is significantly different both from the distribution of the long-wave cone records for the 563 nm dichromat (B5) and from those for the 556 nm dichromats (B2 and B4). The difficulty for the theory is not so much the presence of a distinct pigment in the case of B6 (it is quite possible that *Callithrix* has more alleles than *Saimiri*), but that the theory requires B6's phenotype to coincide with either that of B4 or that of B5.*

Family C offers only a limited test for the single-locus X-chromosome theory. To have refuted the theory, we should have required a trichromatic son, or three different types of dichromat. In fact, the three sons are all 556 nm dichromats.

Biological origins of the polymorphism

What maintains the remarkable polymorphisms of cone pigments that are found in the marmoset and in other platyrrhine monkeys? One of several explanations considered for the case of *Saimiri* (Mollon *et al.*, 1984) is *heterozygous advantage*: the heterozygote, being trichromatic, may be able to locate coloured fruit when the dappled illumination and variegated background of the forest make it difficult to use form or lightness cues; it is in exactly such a task that the human dichromat experiences difficulty (Mollon, 1987, 1991). By this account the variation among the dichromatic males is maintained simply by the advantage to the female heterozygote and would become unnecessary if two, non-homologous loci were established on the X-chromosome, as in the catarrhine form of trichromacy.

But a uniform, catarrhine-like trichromacy might not be the ideal solution for neotropical monkeys. There

may be visual tasks in which the dichromat enjoys the advantage. There are recurrent reports that human daltonians are able to penetrate military or natural camouflage that defeats the trichromatic observer (Anonymous, 1940; Ford, 1955); and Morgan, Mollon and Adam (1989) have shown formally in the laboratory that human dichromats can detect a perceptual organisation based on texture when the target is masked for normals by a rival organisation based on hue.

If indeed there are complementary advantages to dichromacy and to trichromacy, then the polymorphism could be maintained by *frequency-dependent advantage*. This mechanism has often been invoked to explain polymorphism in prey species (Clarke, 1979), but here it would be used to explain a perceptual polymorphism in the predator: an advantage will be enjoyed by the monkey whose vision allows him to detect prey missed by most of his conspecifics (Mollon *et al.*, 1984; Endler, 1988).

Thirdly, the polymorphism may be maintained by *kin selection*: all members of a genetically related group may gain if there are several forms of dichromats and trichromats within the foraging troop. It is relevant that callitrichids live in family groups, occupying a territory that they defend against neighbouring groups (Rylands, 1982). Menzel and Juno (1985) examined the foraging strategy of saddle-backed tamarins under semi-natural conditions and reported that on entering the test room the group spread out and searched, "as if acting as a team and actively searching for food". When an animal detected a food source it made a distinctive "food call", which drew other members of the group to the scene. This cooperative foraging can be explained by kin selection (Maynard-Smith, 1976): the frequency of a gene will be influenced not only by the effects that gene has on the survival and fertility of the individual carrying it, but also by its effects on the success of that animal's relatives. The kin selection that favours cooperative foraging may also act against the presence of a single form of colour vision within the group.

Also requiring a biological explanation are the actual spectral positions of the marmoset's long-wave pigments. The values we obtain (543, 556 and 563 nm) resemble those found electroretinographically in another callitrichid monkey, the saddle-backed tamarin (Jacobs *et al.*, 1987), but differ from the more widely spaced values found for *Saimiri*, and for a second cebid monkey, *Cebus apella* (Bowmaker & Mollon, 1980; Jacobs & Neitz, 1987a, b). Perhaps the difference is connected with the ratio of fruit to exudates in the diet: the *Callitrichidae*, but not the *Cebidae*, are specialised for eating gums and saps (Coimbra-Filho & Mittermeier, 1976).

REFERENCES

*Since concurrently obtained rod records are not displaced, it is unlikely that the discrepancy arises from experimental error. A very remote possibility is that unequal crossing-over occurred during the formation of the gamete in the mother, so that B6 inherited an X-chromosome carrying either alleles for both the 556 and 563 nm pigments or a hybrid allele that produced a pigment with an intermediate λ_{\max} . A third possibility is that B6 inherited both his mother's X-chromosomes as well as his father's Y-chromosome, a situation known in man as Klinefelter's syndrome (Avers, 1980). Lyonisation would ensure that only one X-chromosome was expressed in a given cone. In man, the probability of Klinefelter's syndrome increases with maternal age and we record that the female B1 was aged 11 and comparatively old for a marmoset.

- Anonymous (1940). Colour-blindness and camouflage. *Nature, London*, 146, 226.
- Avers, C. J. (1980). *Genetics* (Revised edn). Van Nostrand Co.
- Bowmaker, J. K. & Mollon, J. D. (1980). Primate microspectrophotometry and its implications for colour deficiencies. In Verriest, G. (Ed.), *Colour vision deficiencies V*. Bristol: Hilger.

- Bowmaker, J. K., Astell, S., Hunt, D. M. & Mollon, J. D. (1991). Photosensitive and photostable pigments in the retinæ of Old World monkeys. *Journal of Experimental Biology*, *156*, 1–19.
- Campbell, R. A. & Lasky, E. Z. (1968). Adaptive threshold procedures: BUDDTIF. *Journal of the Acoustical Society of America*, *44*, 537–541.
- Clarke, B. C. (1979). The evolution of genetic diversity. *Proceedings of the Royal Society of London, B*, *205*, 453–574.
- Coimbra-Filho, A. F. & Mittermeier, R. A. (1976). Exudate eating and tree-gouging in marmosets. *Nature, London*, *262*, 630.
- Cooper, G. F. & Robson, J. G. (1969). The yellow colour of the lens of man and other primates. *Journal of Physiology*, *203*, 411–417.
- D'Agostino, R. A. (1970). Simple compact portable test of normality; Geary's test revisited. *Psychological Bulletin*, *74*, 138–140.
- Dartnall, H. J. A., Bowmaker, J. K. & Mollon, J. D. (1983). Microspectrophotometry of human photoreceptors. In Mollon, J. D. & Sharpe, L. T. (Eds), *Colour vision: Physiology and psychophysics*. London: Academic Press.
- Douglas, R. H. & McGuigan, C. H. (1989). The spectral transmission of freshwater teleost ocular media—an interspecific comparison and a guide to potential ultraviolet sensitivity. *Vision Research*, *29*, 871–880.
- Endler, J. A. (1988). Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society of London, B*, *319*, 505–523.
- Findlay, J. M. (1978). Estimates on probability functions: A more virulent PEST. *Perception and Psychophysics*, *23*, 181–185.
- Ford, E. B. (1955). *Maths*. London: Collins.
- Giacomelli, F., Wiener, J., Kruskal, J. B., Poweranz, J. V. & Loud, A. V. (1971). Subpopulations of blood lymphocytes demonstrated by quantitative cytochemistry. *Journal of Histochemistry and Cytochemistry*, *19*, 426–433.
- Jacobs, G. H. (1990). Discrimination of luminance and chromaticity differences by dichromatic and trichromatic monkeys. *Vision Research*, *30*, 387–397.
- Jacobs, G. H. & Neitz, J. (1987a). Polymorphism of the middle-wave-length cone in two species of South American monkey: *Cebus apella* and *Callicebus moloch*. *Vision Research*, *27*, 1263–1268.
- Jacobs, G. H. & Neitz, J. (1987b). Inheritance of color vision in a New World monkey (*Saimiri sciureus*). *Proceedings of the National Academy of Sciences, U.S.A.*, *84*, 2545–2549.
- Jacobs, G. H., Neitz, J. & Crognale, M. (1987). Color vision polymorphism and its photopigment basis in a callitrichid monkey (*Saguinus fuscicollis*). *Vision Research*, *27*, 2089–2100.
- Kalmus, H. (1955). The familial distribution of congenital tritanopia with some remarks on some similar conditions. *Annals of Human Genetics*, *20*, 39–56.
- King-Smith, P. E. & Carden, D. (1976). Luminance and colour contributions to visual detection and adaptation and to temporal and spatial integration. *Journal of the Optical Society of America*, *66*, 709–717.
- Knowles, A. & Dartnall, H. J. A. (1977). In Davson, H. (Ed.), *The eye. Vol. 2B, The photobiology of vision*. New York: Academic Press.
- Liebman, P. A. & Entine, G. (1964). Sensitive low-light-level microspectrophotometer: Detection of photosensitive pigments of retinal cones. *Journal of the Optical Society of America*, *54*, 1451–1459.
- Lyon, M. (1962). Sex chromatin and gene action in the mammalian X-chromosome. *American Journal of Human Genetics*, *14*, 135–148.
- MacNichol, E. F. Jr, Levine, J. S., Mansfield, R. J. W., Lipetz, L. E. & Collins, B. A. (1983). Microspectrophotometry of visual pigments in primate photoreceptors. In Mollon, J. D. & Sharpe, L. T. (Eds). *Colour vision: Physiology and psychophysics*. London: Academic Press.
- Mansfield, R. J. W. (1985). Primate photopigments and cone mechanisms. In Fein, A. & Levine, J. S. (Eds), *The visual system*. New York: Liss.
- Marc, R. E. & Sperling, H. G. (1977). Chromatic organization of primate cones. *Science*, *196*, 454–456.
- Maynard-Smith, J. (1976). Group selection. *Quarterly Review of Biology*, *51*, 277–283.
- Menzel, E. W. & Juno, C. (1985). Social foraging of marmoset monkeys and the question of intelligence. *Philosophical Transactions of the Royal Society of London, B*, *308*, 145–158.
- Mollon, J. D. (1982). Color vision. *Annual Review of Psychology*, *33*, 41–85.
- Mollon, J. D. (1987). On the origins of polymorphisms. In *Frontiers of visual science*. Washington, D.C.: Committee on Vision, National Academy Press.
- Mollon, J. D. (1991). The uses and evolutionary origins of primate colour vision. In Cronly-Dillon, J. R. & Gregory, R. L. (Eds), *Vision and visual dysfunction, Vol. 2, Evolution of the eye and visual system*. London: MacMillan.
- Mollon, J. D., Bowmaker, J. K. & Jacobs, G. H. (1984). Variations of colour vision in a New World primate can be explained by a polymorphism of retinal photopigments. *Proceedings of the Royal Society, B*, *222*, 373–399.
- Morgan, M. J., Mollon, J. D. & Adam, A. (1989). Dichromats break camouflage of textural boundaries. *Investigative Ophthalmology and Visual Science*, *30* (Suppl.), 220.
- Rylands, A. B. (1982). The behaviour and ecology of three species of marmosets and tamarins (*Callitrichidae*, primates) in Brazil. Ph.D. thesis, University of Cambridge.
- Schnapf, J. L., Kraft, T. W., Nunn, B. J. & Baylor, D. A. (1988). Spectral sensitivity of primate photoreceptors. *Visual Neuroscience*, *1*, 255–261.
- Sperling, H. G. & Harwerth, R. S. (1971). Red–green cone interactions in the increment threshold spectral sensitivity of primates. *Science*, *171*, 179–184.
- Sperling, H. G., Sidley, N. A., Dockens, W. S. & Joliffe, C. L. (1968). Increment threshold of the rhesus monkey as a function of the spectral composition of the background field. *Journal of the Optical Society of America*, *58*, 263–268.
- Tovée, M. J., Mollon, J. D. & Bowmaker, J. K. (1990). The polymorphism of the middle- to long-wave photopigments in the common marmoset may not conform to the single-locus X-chromosome theory. *Perception*, *19*, A34.
- Travis, D. S. (1986). Polymorphism of retinal pigments and behavioural sensitivity in the common marmoset, *Callithrix jacchus*. Ph.D. thesis, University of London.
- Travis, D. S., Bowmaker, J. K. & Mollon, J. D. (1988). Polymorphism of visual pigments in a Callitrichid monkey. *Vision Research*, *28*, 481–490.
- Wyszecki, G. & Stiles, W. S. (1982). *Color science* (2nd edn). New York: Wiley.

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