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# POLYMORPHISM OF VISUAL PIGMENTS IN A CALLITRICHID MONKEY

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**Abstract**—Microspectrophotometric measurements of visual pigments have been obtained for a large sample of New World monkeys of the species *Callithrix jacchus jacchus*. These animals exhibit a polymorphism of visual pigments. The rods ($L_{max}$ 499 nm) and the short-wave receptors ($L_{max}$ 423 nm) appear to be common to all animals but individuals differ in the number and spectral position of pigments in the green-yellow spectral region. The latter pigments cluster near 545, 559 and 567 nm. Male monkeys draw one pigment from this set and female monkeys may draw one or two. The results are generally consistent with a genetic theory that postulates in *Callithrix* three possible alleles for a single locus in the X-chromosome. It appears that polymorphisms of cone pigments may be widespread among neotropical primates.

Colour vision Microspectrophotometry Visual pigments Genetics Polymorphism Marmosets *Callithrix jacchus jacchus*

## INTRODUCTION

The study of New World primate colour vision has recently become of some comparative interest with the discovery of significant within-species variations in both the colour vision and the cone visual pigments of a Cebid monkey, the squirrel monkey *Saimiri sciureus* (Jacobs, 1984; Mollon *et al.*, 1984). Behavioural experiments have shown that animals can be either dichromatic or trichromatic; and within these broad classes various sub-types may be distinguished. Independent microspectrophotometric measurements on animals for whom behavioural data were available have shown that the sub-types can be accurately defined by the cone pigment complement as measured by microspectrophotometry.

The microspectrophotometric results have shown that at least four different cone pigments are available to squirrel monkeys. All animals appear to share the short-wave pigment ($L_{max}$ 433 nm); but in the middle- to long-wave part of the spectrum there is a polymorphism of visual pigments. Males appear to draw only one of three available pigments (with $L_{max}$ at either 536 or 549 or 564 nm) whereas females draw either one or two. On the basis of these results, it has been suggested (Mollon *et al.*, 1984; Jacobs and Neitz, 1985) that the inheritance of the middle- and long-wave cone pigments in squirrel monkeys is governed by a single locus on the X-chromosome. One of three alleles, corresponding to the protein moiety of the three longer-wave pigments, may be inherited at this locus. Males, having one X-chromosome, may be only dichromatic; whereas females, having two, may be either homozygous and dichromatic or heterozygous and trichromatic.

The purpose of the present study was to examine another species of New World primate, the common marmoset *Callithrix jacchus jacchus* by microspectrophotometry in order to determine whether polymorphism of visual pigments is peculiar to *Saimiri* or is widespread among neotropical monkeys. Marmosets are members of the *Callitrichidae*, one of the two Platyrrhine families; whereas the genus *Saimiri* belong to the second, the *Cebidae*.

Little behavioural work has addressed the issue of colour vision in marmosets, although there is some suggestion that they can make red/green discriminations (Miles, 1958). More recently, Travis (1986), using Stiles' two-colour threshold technique, established that a pair of

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male marmosets were dichromatic and comparable to a human deuteranope. This was confirmed by subsequent microspectrophotometric measurements, showing the animals to have two cone pigments only (with $L_{max}$ values at about 423 and 567 nm). *Saquinous fuscicollis*, another species of the *Callitrichidae*, has been studied behaviourally and by ERG flicker photometry (Neitz *et al.*, 1985). The behavioural experiments showed three males to be dichromatic and two females to be trichromatic; the ERG measurements identified two cone pigments having maximal absorbance at 543 and 555 nm.

## METHODS

### *Animals*

The animals were 21 marmosets of the species *Callithrix jacchus jacchus*, 9 males and 12 females. Eight pairs of animals were dizygotic twins.

### *Microspectrophotometry*

Measurements were made with a dual-beam Liebman microspectrophotometer described elsewhere (Knowles and Dartnall, 1977). Full details of the procedures and the analysis of results are given by Mollon *et al.* (1984). In many cases the fovea was readily visible as a dark spot and the first preparation was taken from this region. When the fovea could not be identified, its position was estimated by reference to the optic disc. The tissue was dispersed on a microscope slide and a narrow beam of light, about 2 $\mu$m in cross-section, was passed through the pigment-containing outer-segment of a rod or cone cell. On finding a putative cone outer segment under infra-red illumination, the operator oriented the microscope stage so that the measuring beam passed through the structure and the reference beam fell in a clear area of slide. The absorbance of the outer segment was sampled at 2-nm intervals between 700 and 390 nm and then a return scan was made measuring absorbances at the interleaved wavelengths.

### *Analysis of results*

A standardised computer program was used to estimate the $L_{max}$ of the resulting absorbance spectrum (see Mollon *et al.*, 1984). The program first calculated a 2-nm averaged curve and then normalised the resulting spectrum around the mean of seven points centred on the highest individual point on the averaged curve. Twenty relative absorbance values taken from the right-hand limb of this curve were next referred to a visual pigment template curve to establish the $L_{max}$. A second estimate of the $L_{max}$ was made in the same way by taking the 25 values either side of the highest point (51 points in total). The template curve used was Dartnall's standard spectrum for frog rhodopsin (Knowles and Dartnall, 1977, their Table 1) with $L_{max}$ at 502 nm but expressed on an abscissal scale of fourth root of wavelength (Barlow, 1982). Selection criteria were used to discard records whose shapes were distorted by abnormally high or low short-wave absorbance. Records from rods and long- or middle-wave cones were discarded if:

—The standard deviation of the $L_{max}$ estimates obtained from fitting the template to the right-hand limb of the curve was greater than 10 nm.

—The two independent estimates of the $L_{max}$, obtained from the right-hand limb and the top of the curve, differed by more than 5 nm.

—They displayed negative short-wave absorbance. Short-wave absorbance was calculated from 11 values on the 2-nm averaged curve between 420.5 and 440.5 nm and expressed as a percentage of the absorbance of the top of the curve.

Additionally, records from longer-wave cones were discarded if short-wave absorbance was greater than 40% of maximum absorbance.

### *Short-wave receptors*

After measurement of any structure absorbing maximally in the short-wave end of the spectrum, the following routine was followed. The structure was re-measured twice and then exposed for five minutes to white light. Measurements were continued on structures only that proved photosensitive, in order to establish a post-bleach absorbance spectrum. No further selection criteria were placed on the records from short-wave receptors.

Two independent estimates of the $L_{max}$ of short-wave receptors were made. First, the three measurements of the cell made before bleaching were averaged together and the $L_{max}$ estimated. Second, the three measurements of the cell made after bleaching were averaged together and this post-bleach record was subtracted from the pre-bleach record: i.e. a difference spectrum was calculated. The $L_{max}$ of this record was then

estimated. These two estimates were obtained because light scatter in the short-wave end of the spectrum may bias estimation of the $L_{max}$ from the absorbance spectrum towards shorter wavelengths; whereas the possible presence of photoproducts formed after bleaching may bias the estimation of the $L_{max}$ from the difference spectrum towards longer wavelengths (Mansfield *et al.*, 1984).

## RESULTS

Absorbance spectra from a total of 291 rods and 446 cones were considered for further analysis. This represents about 70% of the rods that were measured, and about 65% of the cones. Histograms of the $L_{max}$ values for the rods and cones from the individual animals are given in Figs 1–4 and show the distribution of the $L_{max}$ of the cells as a function of wavelength. The values of $L_{max}$ are those estimated from the right-hand limb of the absorbance curve (see Methods). All the animals shown in these figures have a rod pigment with $L_{max}$ close to 499 nm.

### *Middle- and long-wave cones*

The 15 animals shown in Figs 1 to 3 exhibit only a single photopigment in the middle- to

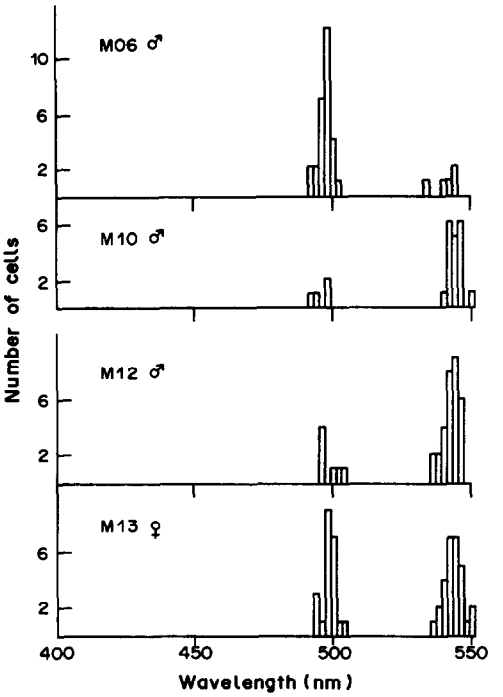


Fig. 1. Distribution of values of peak sensitivity ($L_{max}$) of individual microspectrophotometric records. Distributions are shown separately for each monkey. Additionally, each animal's laboratory number and sex is identified on the figure. The histograms for these four animals show evidence for a population of rods and only one class of longer-wave cone.

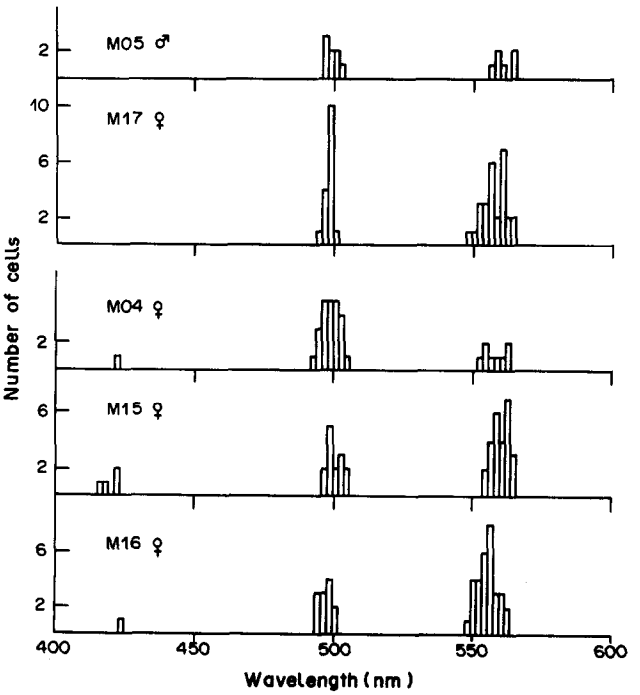


Fig. 2. As Fig. 1. The histograms for these five animals show evidence for a population of rods and only one class of longer-wave cone. Note the short-wave receptors in *M*04, *M*15 and *M*16.

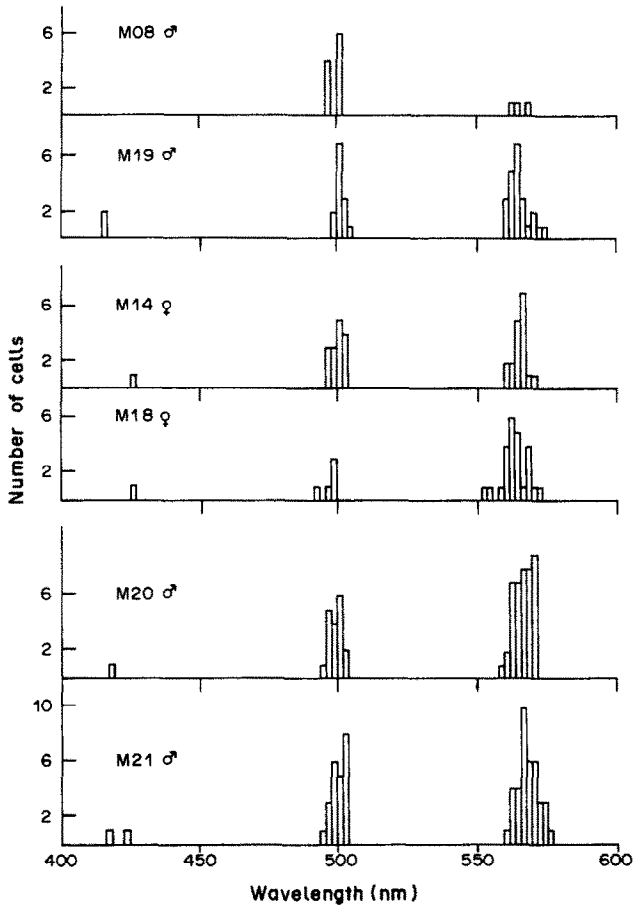


Fig. 3. As Fig. 1. The histograms for these six animals show evidence for a population of rods and only one class of longer-wave cone. Note the short-wave receptors in all animals except *M*08.

long-wave spectral region, but it is clear that the spectral position of this pigment varies substantially between animals. Thus the four animals shown in Fig. 1 have mean $L_{max}$ values in the range 542–547 nm, whereas those shown in Figs 2 and 3 have mean $L_{max}$ values at longer wave-

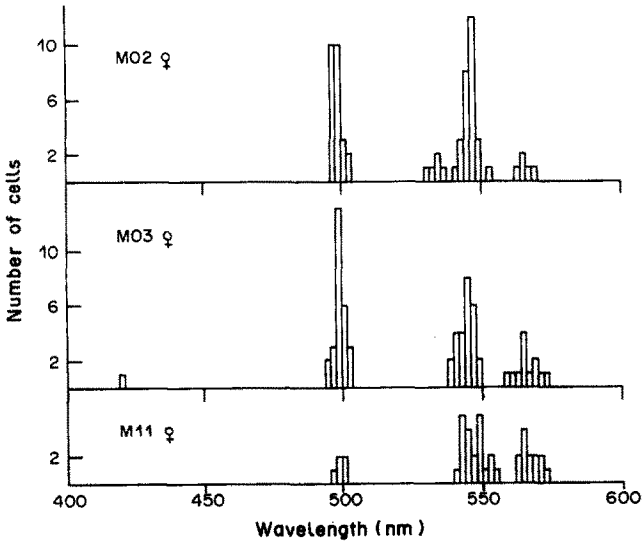


Fig. 4. The histograms for these three animals show evidence for a population of rods and two different classes of longer-wave cone. Note the short-wave receptor in *M*03.

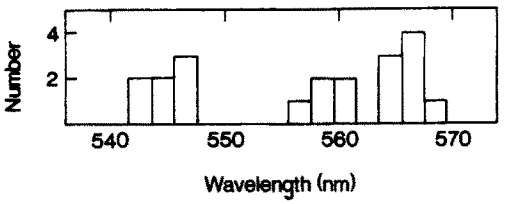


Fig. 5. Summary histogram of the average $L_{max}$ values from the middle- and long-wave visual pigments of each animal. The $L_{max}$ plotted is the estimate obtained from fitting the template to the right-hand limb of the average curve (i.e. $L_{max_2}$ estimates).

lengths, in the range 556–567 nm. Both male and female monkeys are found amongst the putatively dichromatic animals of Figs 1–3.

Figure 4 shows results for three monkeys that exhibit more than one pigment in the middle- to long-wave spectral region. These animals were all female. The $L_{max}$ values for one cone class cluster between 544 and 547 nm whereas the values for a second class cluster between 565 and 568 nm. In the case of *M*02 there is a further cluster of five cells close to 533 nm: if these cells are grouped with the 545-nm cells of *M*02, the combined distribution is shown to be non-normal by the Shapiro and Wilk (1965) test ($P < 0.01$) (see Discussion).

Figure 5 is a summary histogram of the average $L_{max}$ values for the visual pigments from each animal plotted against wavelength. These values have been placed in 2-nm bins in the same way as the data from individual cells. These average $L_{max}$ values cluster into three groups ranging from 542 to 547 nm, from 556 to 561 nm and from 565 to 568 nm. It is on the basis of this *prima facie* clustering into three classes of cone that we have grouped the data presented in Figs 1, 2 and 3 and in Table 2. We do not exclude the possibility that further research will distinguish subtypes within the three clusters.

The mean $L_{max}$ values and the number of cells analysed are given for each animal in Tables 1 and 2. Figure 6 shows mean, normalised absorbance spectra for the rods and for the three classes of cone in the middle- to long-wave range. In three animals (*M*01, *M*07, *M*09) too few cells were measured to determine their complement of visual pigments, but the data from these animals are included in Tables 1 and 2 and in Fig. 6. The 533-nm cluster of five cells

Table 1. The mean $L_{max}$ of the rods and the short-wave receptors measured in 21 marmosets

| | | Rods | | | Short-wave receptors | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Absorbance | | Difference | |
| Animal | Sex | *N* | $L_{max_1}$ | $L_{max_2}$ | *N* | $L_{max_1}$ | $L_{max_2}$ | $L_{max_1}$ | $L_{max_2}$ |
| *M*01 | ♀ | 0 | | | 0 | | | | |
| *M*02 | ♀ | 25 | 498.2 ± 1.7 | 498.6 ± 2.3 | 0 | | | | |
| *M*03 | ♀ | 27 | 498.9 ± 1.8 | 499.1 ± 1.2 | 1 | 421.4 | | 423.1 | |
| *M*04 | ♀ | 24 | 498.7 ± 2.9 | 499.1 ± 1.6 | 1 | 423.7 | | 422.0 | |
| *M*05 | ♂ | 8 | 498.8 ± 2.0 | 498.3 ± 1.9 | 0 | | | | |
| *M*06 | ♂ | 28 | 497.9 ± 2.0 | 498.4 ± 1.2 | 0 | | | | |
| *M*07 | ♂ | 2 | 497.5 ± 0.6 | 499.0 ± 4.6 | 0 | | | | |
| *M*08 | ♂ | 10 | 499.1 ± 1.8 | 500.4 ± 2.2 | 0 | | | | |
| *M*09 | ♀ | 13 | 502.4 ± 2.1 | 501.8 ± 1.4 | 0 | | | | |
| *M*10 | ♂ | 4 | 496.0 ± 2.1 | 496.9 ± 3.2 | 0 | | | | |
| *M*11 | ♀ | 5 | 498.6 ± 1.2 | 499.2 ± 2.4 | 0 | | | | |
| *M*12 | ♂ | 7 | 499.1 ± 2.9 | 499.5 ± 2.5 | 0 | | | | |
| *M*13 | ♀ | 22 | 499.0 ± 2.3 | 499.8 ± 1.4 | 0 | | | | |
| *M*14 | ♀ | 15 | 499.7 + 2.1 | 500.5 + 2.8 | 1 | 428.8 | | 421.7 | |
| *M*15 | ♀ | 14 | 500.1 ± 2.7 | 500.2 ± 1.1 | 4 | 420.6 ± 2.6 | 421.0 ± 3.6 | 418.2 ± 4.1 | 421.9 ± 4.4 |
| *M*16 | ♀ | 12 | 496.7 + 2.1 | 496.6 + 1.4 | 1 | 418.7 | | 432.1 | |
| *M*17 | ♀ | 16 | 498.2 ± 1.3 | 497.9 ± 1.1 | 0 | | | | |
| *M*18 | ♀ | 5 | 497.0 ± 2.1 | 497.4 ± 2.1 | 1 | 426.2 | | 422.4 | |
| *M*19 | ♂ | 13 | 500.8 ± 1.6 | 500.8 ± 1.2 | 2 | 419.8 ± 0.3 | 420.0 ± 2.8 | 416.5 ± 2.2 | 422.8 ± 8.8 |
| *M*20 | ♂ | 18 | 498.8 ± 2.1 | 499.1 ± 1.4 | 1 | 423.5 | | 411.6 | |
| *M*21 | ♂ | 23 | 499.9 ± 2.3 | 500.6 ± 1.5 | 2 | 421.6 ± 4.1 | 422.3 ± 4.1 | 421.5 ± 3.2 | 425.9 ± 12.5 |

Parentheses next to the laboratory numbers of a pair of animals indicate that they were twins. Two different estimates of the $L_{max}$ are shown for each putative pigment: these are the average $L_{max}$ of the individual $L_{max}$ values of a particular class of cells ($L_{max_1}$); and the $L_{max}$ of the averaged curve ($L_{max_2}$). The $L_{max_1}$ values were obtained by simply averaging together the individual $L_{max}$ values shown in the histograms; in the table these values are expressed as the mean value ±1 SD. The $L_{max_2}$ values were obtained by averaging together the individual spectra for a particular class of cells and then estimating the $L_{max}$ as for a single record (see Methods). For the $L_{max_2}$ values, the table thus shows the mean $L_{max}$ estimated from 20 relative absorbance values taken from the right-hand limb of the averaged curve and the associated standard deviation of those 20 estimations. The averaged curve used in the $L_{max_2}$ estimate was computed from the raw absorbance values for each individual cell and not from the normalised spectrum of each cell.

Table 2. The mean $L_{max}$ of the three classes of longer-wave cones measured in 21 marmosets. For full explanation see Table 1 legend

| Animal | Sex | P545 N | P545 $L_{max_1}$ | P545 $L_{max_2}$ | P559 N | P559 $L_{max_1}$ | P559 $L_{max_2}$ | P567 N | P567 $L_{max_1}$ | P567 $L_{max_2}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| M01 | ♀ | 3 | 538.2 ± 1.5 | 538.1 ± 3.9 | 2 | 559.3 ± 2.8 | 558.0 ± 5.8 | | | |
| M02 | ♀ | 28[a] | 545.7 ± 2.3 | 546.5 ± 2.3 | | | | 5 | 565.0 ± 1.7 | 565.9 ± 4.1 |
| M03 | ♀ | 26 | 543.9 ± 2.6 | 544.4 ± 2.1 | | | | 12 | 565.5 ± 3.7 | 564.9 ± 3.0 |
| M04 | ♀ | | | | 8 | 557.8 ± 3.6 | 558.7 ± 2.0 | | | |
| M05 | ♂ | | | | 6 | 560.3 ± 2.9 | 560.7 ± 3.1 | | | |
| M06 | ♂ | 5 | 541.3 ± 2.8 | 541.7 ± 4.2 | | | | | | |
| M07 | ♂ | | | | | | | | | |
| M08 | ♂ | | | | | | | 3 | 565.2 ± 2.0 | 566.9 ± 5.2 |
| M09 | ♀ | 1 | 547.2 | | | | | | | |
| M10 | ♂ | 19 | 544.4 ± 2.3 | 543.8 ± 2.3 | | | | | | |
| M11 | ♀ | 21 | 546.5 ± 3.8 | 546.9 ± 1.2 | | | | 16 | 567.1 ± 3.2 | 567.7 ± 1.5 |
| M12 | ♂ | 31 | 542.7 ± 2.9 | 542.8 ± 2.6 | | | | | | |
| M13 | ♀ | 29 | 543.5 ± 3.3 | 544.7 ± 2.1 | | | | | | |
| M14 | ♀ | | | | | | | 18 | 564.9 ± 2.7 | 565.4 ± 1.7 |
| M15 | ♀ | | | | 26 | 559.9 ± 3.0 | 561.0 ± 2.1 | | | |
| M16 | ♀ | | | | 31 | 555.4 ± 3.6 | 556.4 ± 1.5 | | | |
| M17 | ♀ | | | | 27 | 557.7 ± 4.1 | 558.3 ± 1.8 | | | |
| M18 | ♀ | | | | | | | 26 | 563.8 ± 4.5 | 564.7 ± 1.6 |
| M19 | ♂ | | | | | | | 23 | 565.1 ± 3.8 | 566.0 ± 1.6 |
| M20 | ♂ | | | | | | | 42 | 566.3 ± 3.4 | 567.0 ± 1.2 |
| M21 | ♂ | | | | | | | 38 | 567.9 ± 3.9 | 566.7 ± 0.9 |

[a]In addition, a third cluster of cells were found ($N = 5$) with a $L_{max_1}$ of 533.1 ± 2.1.

from *M*02 have not been included in the average curve for the *P*545 pigment.

### *Short-wave receptors*

Short-wave cones were rare in *Callithrix*, comprising 14 out of a total of 460 outer segments. This proportion is similar to that reported for other primate species by microspectrophotometry (Bowmaker *et al.*, 1983; Dartnall *et al.*, 1983; Mollon *et al.*, 1984; Mansfield *et al.*, 1984). In 11 of the present animals no unambiguous evidence for short-wave receptors was found, but in our classification of animals as dichromatic or trichromatic we assume the short-wave pigment common to all.

In the histograms (Figs 1–4) the $L_{max}$ values of individual short-wave receptors are plotted as the average of the absorbance and bleaching-difference spectra. In Table 1, the $L_{max}$ values for both types of spectra are given for individual animals. Figure 6 shows the normalised, mean absorbance spectrum, the mean post-bleach spectrum, and the mean difference spectrum. The mean $L_{max}$ for the 14 cells was 423 nm. This value is closer to that found for man (419 nm; Dartnall *et al.*, 1983) than to the value obtained (using similar techniques) for squirrel monkeys (431 nm; Mollon *et al.*, 1984) and for baboons (433 nm; Bowmaker *et al.*, 1983).

## DISCUSSION

The present results reveal a polymorphism of cone pigments that is as striking as the polymorphism found earlier in the squirrel monkey (Mollon *et al.*, 1984). Some female marmosets are evidently trichromatic, having two pigments in the green-yellow spectral region. Other marmosets, both male and female, are evidently dichromatic, exhibiting only one pigment in this region; and the spectral position of this single pigment varies in the range 545–567 nm. Since squirrel monkeys and marmosets are drawn from two distinct families of New World monkeys, the *Cebidae* and the *Callitrichidae* respectively, it appears that intra-specific variations of cone pigments may be widespread among the Platyrrhine monkeys and not peculiar to *Saimiri*.

However, the present results suggest that the commonly occurring marmoset pigments lie at approximately 545, 559 and 567 nm, whereas the microspectrophotometrically estimated values for *Saimiri* are 537, 550 and 565 nm. Only a pigment near 565 nm seems to be common to the two species. This contrast between Cebid and Callitrichid species is also seen in the electroretinographic measurements of Jacobs and Neitz (1987) and Neitz *et al.* (1985) who report values of 538, 551 and 561 nm for the squirrel monkey and 543, 555 and 561 nm for the Callitrichid species *Saguinus fuscicollis*.

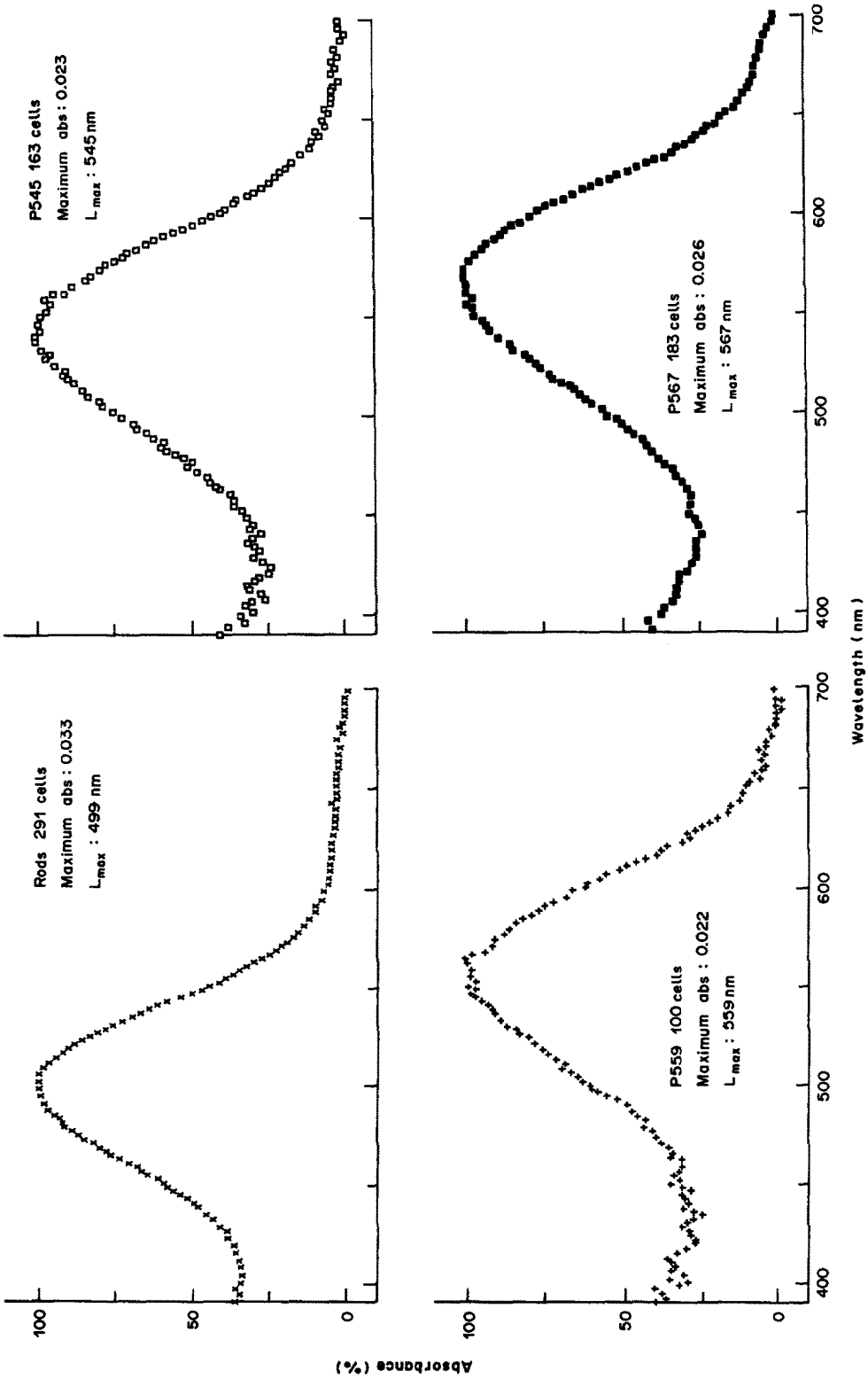


Fig. 6. The averaged, normalised absorbance spectra of the rods and three classes of longer-wave cone measured in marmosets. The individual absorbance values were averaged before being normalised. Shown next to each spectrum is the number of cells of that class that were suitable for analysis; the average transverse density at $L_{max}$ and the approximate $L_{max}$. The precise $L_{max}$ of these curves are: rods, $499.4 \pm 1$ nm; $P545$, $545.1 \pm 1.1$ nm; $P559$, $559.2 \pm 0.8$ nm; $P567$, $566.7 \pm 0.9$ nm (the standard deviations are those of the template fit to the right-hand limb of the curve).

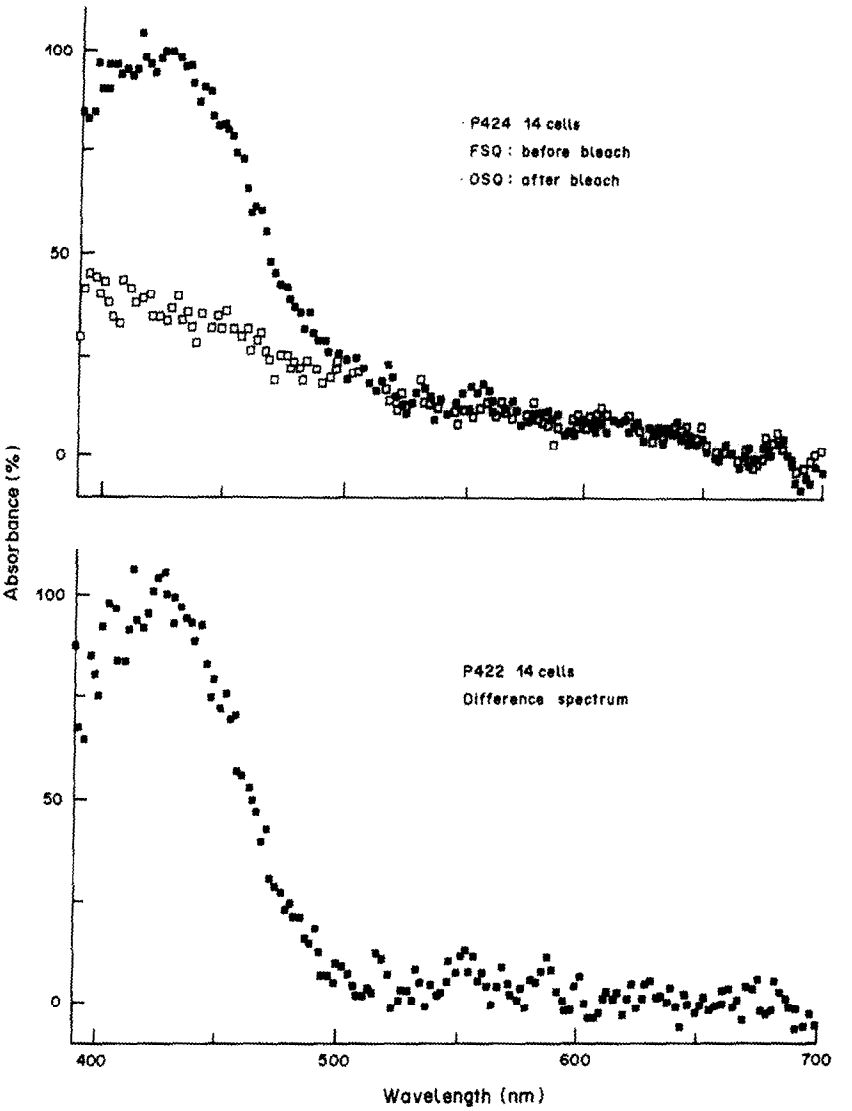


Fig. 7. The averaged, normalised absorbance and difference spectra of the short-wave cones in marmosets. The individual absorbance values were averaged before being normalised. In the upper panel is shown the average absorbance spectrum (solid squares) and the subsequent bleach (open squares). The $L_{max}$ of the absorbance spectrum is at about 424 nm; the averaged transverse density at peak absorbance was 0.013. In the lower panel is shown the difference spectrum. This has a $L_{max}$ at about 422 nm and an averaged transverse density at peak absorbance of 0.009.

Thus there exist in New World monkeys at least five different cone pigments in the middle- to

trasts with the stability seen in Old World monkeys, which consistently exhibit a pair of pigments close to 535 and 565 nm; the latter values correspond roughly to the two extremes of the range so far recorded in Platyrrhine species. A pigment close to 565 nm occurs in all primate species so far examined by microspectrophotometry, although the pigment may not be found in each individual member of the species.

aly in the present results is the ster of cells near 533 nm in animal M02. None of the dichromatic marmosets exhibit a pigment with so short a $L_{max}$ value. All five of the discrepant cells were measured in the same preparation, and in roughly the same area; but this does not necessarily indicate a general contamination of the preparation, since *P*545 and *P*567 cells were concurrently found in the same area. The aberrant cells were morpho-

logically indistinguishable from other adjacent cells and, since the records pass our selection criteria, we have no grounds to discard them.

*The one-locus model for long-wave pigments in Platyrrhini*

It is well established that the common forms of human colour deficiency are sex-linked: that is, are due to some abnormality of the X-chromosome (Pokorny *et al.*, 1979). There are two X-chromosome loci associated with human colour vision (Siniscalco *et al.*, 1964; Kalmus, 1965): one gene is thought to specify the opsin of the long-wave human pigment, and one the opsin of the middle-wave pigment (Nathans *et al.*, 1986a, b). This model appears inappropriate for the marmoset, since the males are invariably dichromatic. But our results are consistent with a model which supposes that in New World monkeys there is only a single X-chromosome locus for a cone photo- pigment (Mollon *et al.*, 1984; Jacobs and Neitz, 1985).

The model postulates that at least three different alleles can occur at the single locus: each allele specifies a slightly different sequence for the opsin and, when combined with 11-*cis* retinal, gives rise to a photopigment with a different spectral sensitivity. Male monkeys, being hemizygous, would necessarily be dichromatic; but females, having two X-chromosomes, may be heterozygous and thus potentially trichromatic. The phenomenon of random X-chromosome inactivation, or "Lyonisation", would ensure that only one allele was expressed in any given cell (Gartler and Riggs, 1983); this mechanism would prevent the occurrence of mixtures of pigments in the cones of heterozygous females. Females can of course inherit the same allele on both X-chromosomes and so may be dichromatic.

In the case of *Saimiri*, strong support for such a model comes from Jacobs and Neitz (1987), who have established the distribution of cone phenotypes for a large sample ($n = 78$) of squirrel monkeys, including several families, using an electroretinographic technique. Frequencies of dichromatic and trichromatic females were predicted almost perfectly from the one-locus model, as was the inheritance of the cone phenotypes. In the case of the marmoset, however, the model must remain tentative. For firstly, the model requires two types of female trichromat that we have not yet observed, one combining the *P*545 and *P*559 pigments and one combining the *P*559 and *P*567 pigments. Secondly, the female monkey *M*02 (Fig. 4) may exhibit three, rather than two, pigments in the middle- to long-wave region. Thirdly, the model does not predict the occurrence of twin females (*M*14 and *M*15, Table 2) exhibiting different types of dichromacy (although the mean values of $L_{max}$ differed by little more than 4 nm, and we have only the supplier's report that these animals were from the same litter).

It should also be said that our results are compatible with a more complex, but genetically plausible model, in which individuals vary in the number of X-chromosome loci for cone pigments. Suppose there are only two frequent alleles in the population, one specifying the *P*545 pigment and one specifying the *P*567 pigment. But suppose also, through a misalignment at the time of crossing-over, two different alleles sometimes become established on a single chromsome—rather as some human X-chromosomes exhibit more than one copy of the gene for the middle-wave pigment (Nathans *et al.*, 1986a, b). If both alleles are expressed within a given cell, this "two-locus" chromosome will produce cones with intermediate spectral sensitivity. Since the two genes will be very close together, it is likely to be many generations before they are separated. So they will behave as if they constituted the third of the alleles of our one-locus model; and if heterozygous females are at an advantage (see below), this pseudo-allele will be selected for, as it it were a newly arisen allele. Thus the two models make similar predictions. It might be thought that a broadening of the absorbance curve would betray the mixture of pigments generated by the pseudo-allele of the "two-locus" chromosome; but in fact it would be practically impossible to distinguish between a true pigment and a mixture of two pigments that were a little more than 10 nm apart in their spectral position (see Knowles and Dartnall, 1977, pp. 84–85). Intense chromatic bleaches, however, might reveal a wavelength-dependent change in the behavioural or electroretinographic sensitivity of those male monkeys that exhibited the pseudo-allele.

*The biological function of the polymorphism*

Now that polymorphisms of cone pigments have been demonstrated in two distinct families of neotropical monkeys, it becomes increasingly of interest to ask whether there is a biological factor that maintains the polymorphisms. One possibility, considered by Mollon *et al.* (1984),

is that the polymorphism has the function of providing the very mechanism of trichromatic vision in the *Platyrrhini*: that is to say, the polymorphism is established, and is maintained, by the advantage to the heterozygous female. The heterozygote has two cone pigments in the green-yellow range and Lyonisation ensures that they are segregated in different cells. Behaviourally, she seems to be able to compare the rates of quantum catch in these two subsets of cones and so she comes to enjoy trichromatic discrimination. In the dappled environment of a tropical forest, where luminance edges are masked, she can use chromatic differences to lead her troop towards fruit that is concealed amongst foliage; and she can readily judge the relative ripeness of fruit. To explain the polymorphism we need only allow that such trichromatic discriminations lend a true advantage to the heterozygote (see Bowmaker *et al.*, 1987).

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