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Variations of colour vision in a New World primate can be explained by polymorphism of retinal photopigments

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The squirrel monkey (*Saimiri sciureus*) exhibits a polymorphism of colour vision: some animals are dichromatic, some trichromatic, and within each of these classes there are subtypes that resemble the protan and deutan variants of human colour vision. For each of ten individual monkeys we have obtained (i) behavioural measurements of colour vision and (ii) microspectrophotometric measurements of retinal photopigments. The behavioural tests, carried out in Santa Barbara, included wavelength discrimination, Rayleigh matches, and increment sensitivity at 540 and 640 nm. The microspectrophotometric measurements were made in London, using samples of fresh retinal tissue and a modified Liebman microspectrophotometer: the absorbance spectra for single retinal cells were obtained by passing a monochromatic measuring beam through the outer segments of individual rods and cones. The two types of data, behavioural and microspectrophotometric, were obtained independently and were handed to a third party before being interchanged between experimenters.

From all ten animals, a rod pigment was recorded with λ_{max} (wavelength of peak absorbance) close to 500 nm. In several animals, receptors were found that contained a short-wave pigment (mean $\lambda_{max} = 433.5$ nm): these violet-sensitive receptors were rare, as in man and other primate species. In the middle- to long-wave part of the spectrum, there appear to be at least three possible *Saimiri* photopigments (with λ_{max} values at about 537, 550 and 565 nm) and individual animals draw either one or two pigments from this set, giving dichromatic or trichromatic colour vision. Thus, those animals that behaviourally resembled human protanopes exhibited only one pigment in the red–green range, with $\lambda_{max} = 537$ nm; other behaviourally dichromatic animals had single pigments lying at longer wavelengths and these were the animals that behaviourally had higher sensitivity to long wavelengths. Four of the monkeys were behaviourally judged to be trichromatic. None of the latter animals exhibited the two widely separated pigments (close to 535 and 567 nm) that are found in the middle- and long-wave cones of macaque monkeys. But the spread of λ_{max} values for individual cones was greater in the trichromatic squirrel monkeys than in the dichromats; and in the case of three, behaviourally deuteranomalous, trichromats there was

clear evidence that the distribution of λ_{max} values was bimodal, suggesting photopigments at approximately 552 and 565 nm. The fourth, behaviourally protanomalous, trichromat exhibited a spread of individual λ_{max} values that ranged between 530 and 550 nm.

Good quantitative agreement was found when the microspectrophotometrically measured absorbance spectra were used to predict the behavioural sensitivity of individual animals to long wavelengths. The concordance of the two sets of measurements places beyond question the existence of a polymorphism of colour vision in *Saimiri sciureus* and suggests that the behavioural variation arises from variation in the retinal photopigments. Heterozygous advantage may explain the polymorphism.

Introduction

In Caucasian populations, as many as 8% of men inherit abnormal colour vision. It is far from clear why these genetic variations occur with such high incidence (Ford 1964; Kalmus 1983), and it is relevant to ask whether within-species variations in colour vision are present in non-human primates as they are in man. In the case of Old World monkeys it seems that colour deficiency or anomaly, if it occurs at all, has an incidence lower than that found in Caucasian man. Although only a relatively small number of individuals and species have been tested, there is no published report of an individual Old World monkey whose colour vision diverged markedly from the trichromacy that is known to characterize the typical macaque monkey (De Valois *et al.* 1974; Jacobs 1981, 1983*a*). However, it has recently become apparent that one New World species, the squirrel monkey (*Saimiri sciureus*), exhibits a striking polymorphism of colour vision. Some animals are dichromatic, whereas others are trichromatic. More than one subtype can be distinguished in each of these groups, and there exist subtypes that resemble each of the most common variant forms of human dichromatic and trichromatic vision (Jacobs 1983*a*, *b*, 1984). Female squirrel monkeys are either trichromatic or dichromatic, but it appears that all male squirrel monkeys are dichromats (Jacobs 1984).

In the case of man, it has long been predicted that the main forms of variant colour vision will prove to be caused by individual differences in the photopigments of the retinal cone cells. By the technique of microspectrophotometry, in which a very narrow, monochromatic beam is passed through the outer segment of an individual receptor, it is possible to measure the spectral absorbance of the photopigment within a single retinal cone (Brown & Wald 1964; Marks *et al.* 1964; Liebman 1972; Bowmaker *et al.* 1980); and microspectrophotometry has recently advanced to the stage where it allows a quite large number of records to be secured from an individual primate retina, enough perhaps to predict the behavioural capacities of that individual animal. It thus promises to be possible to test directly whether individual variations in colour vision are indeed associated with variations in the cone photopigments. We have therefore performed a collaborative study in which behavioural measurements of visual performance and microspectrophotometric measurements of cone photopigments were both obtained for each of ten squirrel monkeys. The behavioural tests were carried out in Santa Barbara,

California, and the microspectrophotometric measurements were made in London. The two sets of results for each animal were obtained independently and were handed to a third party before being interchanged between experimenters. Preliminary reports for subsets of the animals have been published (Jacobs *et al.* 1981, 1982).

Methods

(*a*) *Subjects*

The subjects were ten adult squirrel monkeys (*Saimiri sciureus*), seven female and three male. Throughout this paper, individual animals are identified by the numbers listed in table 1. The monkeys were obtained from the Perrine Primate Center of the U.S. National Institutes of Health and had previously been imported from Peru under the auspices of the Pan American Health Organization. Four different types of squirrel monkey are currently distinguished, each type reflecting a different location of origin (Ariga *et al.* 1978). We verified by karyological tests that each monkey used in the present study had five acrocentric pairs of chromosomes, the pattern distinctive of animals of Peruvian origin (Ariga *et al.* 1978). These animals correspond to the type described earlier as having the 'Roman Arch' phenotype (McLean 1964).

(*b*) *Behavioural testing*

During the period of behavioural testing, each animal was housed in a separate cage in a constant-temperature colony room, which was kept on a daily cycle of 12 h light–12 h dark. The animals were fed immediately after a testing session and were then deprived of food for about 22 h before the next session.

Apparatus

All the behavioural tests were conducted in a forced-choice discrimination apparatus, which has been described in detail elsewhere (Blakeslee & Jacobs 1982). One wall of a small test chamber contained three circular (25 mm diameter), translucent stimulus panels, mounted in a row (centre-to-centre distance; 50 mm). The monkey's task on a given trial was to touch that panel that was illuminated differently from the other two. The panels were touch-sensitive and a slight pressure on the appropriate panel resulted in the delivery of food reinforcement (a 97 mg banana-flavoured pellet) into a cup beneath the panel. The panels were transilluminated by light sources located outside the test chamber. Each panel could be illuminated by light from its own tungsten-filament lamp and these lights are referred to here as background lights. The spectral characteristics of the background lights were controlled by inserting Kodak Wratten or Ilford Cibachrome filters into the beams; and the intensity of each was controlled by Kodak neutral density step filters. Any one of the three panels could also be illuminated by a test beam, which was drawn either from a Bausch and Lomb high-intensity grating monochromator or from an anomaloscope. These latter two sources were mounted on a motor-driven platform, which could be moved laterally to positions where the test beam illuminated any one of the three windows. The light source for the anomaloscope was a tungsten filament lamp. The output of this lamp illuminated

two juxtaposed Schott glass filters (dominant wavelengths, 536 and 625 nm), which were mounted on a microscope stage, so that translations of the stage gave variable proportions of red and green light. After passing through these filters the light was further mixed in an integrating sphere and then directed towards one of the three stimulus windows. The intensity of the test beam was varied by means of neutral density filters. Electronically controlled shutters were placed in the test and background beams, so that the test beam could be added to one of the background lights (sensitivity tests) or, alternatively, could replace one of the background lights (colour tests). The interior of the test chamber was lined with white formica and diffusely illuminated by a fluorescent lamp mounted in the ceiling (mean illuminance, 18 lx).

General procedures

The position of the positive (that is, uniquely illuminated) panel varied randomly over trials, with two restrictions: all three windows were positive in approximately equal proportions in each session, and no single position was positive for more than two consecutive trials. These two restrictions discouraged the animals from developing position biases. A cueing tone delivered through a small speaker mounted inside the test chamber was used to signal the occurrence of a test trial. The trial continued until the animal responded, or until 2 s had elapsed. The intertrial interval was usually 4 s, although some variation in this figure was required to accommodate idiosyncracies of particular subjects. During the actual running of problems a non-correction procedure was used. Under these conditions the monkeys completed an average of about 75 trials per session. Animals were initially trained on a very easy brightness discrimination. Once that discrimination was well learned the actual tests were initiated. Three types of measurement were obtained.

(i) *Sensitivity test.* First, increment thresholds were measured for 540 and 640 nm test lights. These test stimuli were produced by the monochromator (half-energy band-width, 10 nm) and they were superimposed on continuously present, achromatic (4800 K) backgrounds (luminance, 3.4 cd m^2). On each trial the test stimulus was added to just one of the panels. To determine thresholds, the 540 and 640 nm lights were presented over a range of intensity so selected for each animal as to span a performance range from 100 % correct to chance (33 % correct). Each intensity range was covered in steps of 0.3 % lg unit and intensity–wavelength combinations were presented in blocks of five trials. The ordering of these blocks was random across test session. Usually between four and eight intensity steps were tested at each wavelength, the exact number varying according to the subject's sensitivity. Monkeys were trained over the full span of intensity values at both wavelengths until their performance showed no further improvement. At that point an additional 25 trials were run at each intensity–wavelength combination. From these final data, psychometric functions were constructed and thresholds were then taken as the intensity necessary to support discrimination at an average level of 50 % correct.

(ii) *Wavelength discrimination.* To test wavelength discrimination, Kodak Wratten filters were inserted into the background beams. These lights served as standards.

The test stimulus was drawn from the monochromator (half-energy band-width, 20 nm). Over trials the wavelength of this light was varied around that of the standards so as to determine the wavelength difference required for successful discrimination. Discrimination was examined at 11 standard values: 452, 471, 491, 510, 538, 555, 576, 586, 606, 633 and 656 nm. All of these were set to a luminance of 3.4 cd m^2, except for the three shortest wavelengths where luminance values of 0.7 cd m^2 were used.

In tests of colour vision with animals it is imperative to ensure that discriminations are not based on luminance rather than on colour differences. To this end, the monkeys were first required to produce a brightness match between the test and standard beams. The wavelength of the monochromator was set to the dominant wavelength of the standard. The luminance of the test light was initially set much higher than that of the standard, so the animal could easily discriminate it. The test luminance was then decreased in steps of 0.1 log unit until it was, finally, very much less than that of the standard. The performance of the animal reflected this variation, being 100 % correct when the test light was much brighter, dropping to chance when the two were equally bright, and then rising toward 100 % correct when the test light was much dimmer. The point of poorest discrimination was taken as an estimate of the luminance match. Once this value was obtained, the wavelength of the monochromator was varied in steps of 5 nm away from that of the standard. At each wavelength, the luminance of the test light was varied over a range of 0.8 log units (in steps of 0.1 log unit) with the middle of this range corresponding to the value estimated as the luminance match. Over those portions of the spectrum where the sensitivity of the squirrel monkey changes relatively rapidly (Jacobs 1963, 1972), the entire luminance range of the test light was shifted so as to try to keep it centred about the point of equal luminance. With this procedure, when the monkey accurately discriminated a particular test wavelength at all its luminance values, it could be concluded that the discrimination must be based on colour cues alone.

The luminance equation procedure was followed for each animal at each standard light. Tests were run on both sides of all standard values except for the two located at the spectral extremes, where variation in the test wavelength was limited to movement toward the centre of the spectrum. The order in which the test wavelengths were run was randomized. A minimum of 25 trials were accumulated at each wavelength–intensity combination. The wavelength difference required to support discrimination at a level of 70 % correct was taken as threshold.

(iii) *Rayleigh match.* The yellow light used in this classical test of colour vision was produced by inserting Ilford Cibachrome Y filters (dominant wavelength 585 nm) into the background beams. The test beam came from the anomaloscope. The anomaloscope was first set by a normal human observer so that the appearance of the test beam matched that of the yellow backgrounds. At that setting, the luminances of all panels were adjusted to 6.1 cd m^2. In the discrimination test, the luminance of the anomaloscope beam was varied over a range of 0.8 log units in steps of 0.1 log unit, with the value of 6.1 cd m^2 representing the centre of this range. The centre point of the luminance range was then adjusted at the various

mixture settings between pure red and pure green for each animal in accord with how their equal luminance settings (see (ii) above) had varied between these wavelengths in the wavelength–discrimination test relative to the variation seen for a normal human trichromat.

Monkeys were first trained to discriminate pure red and pure green light from the yellow. If they succeeded at this discrimination, they were then tested for their ability to discriminate a large range of red–green mixture combinations (minimum, 15) from the yellow. These different mixture values were presented in random order. Testing continued until at least 25 trials were accumulated at each mixture–luminance combination. A Rayleigh match was defined as the midpoint of the mixture range over which discrimination did not differ significantly from chance ($p < 0.05$).

(*c*) *Microspectrophotometry*

Preparation of tissue

After the completion of behavioural measurements, animals were flown to the United Kingdom, where microspectrophotometric measurements were performed. Animals were killed with an i.p. injection of nembutal or of pentabarbitone sodium, after first being narcotized with ketamine hydrochloride. The eyes were then immediately enucleated, wrapped in metal foil and placed on ice. The microspectrophotometric measurements began between 45 and 120 min after death and typically continued for 10 h. Under dim red light, an equatorial section was made of the globe, the vitreous was removed from the posterior section, and the eye-cup was placed in ice-cold mammalian Ringer (pH 7.1). For each preparation a small piece (approximately 1 mm^2) of retinal tissue was cut out with scissors, lifted on to a cover slip, and deliberately dispersed with a few strokes of a razor blade. A drop of Ringer solution was placed on the tissue and the preparation was then squashed under a second cover-slip and sealed with paraffin wax. 5% Dextran (by volume) was added to the Ringer to minimize movement of the tissue during measurements. Several preparations were made from each eye: the first was usually from the foveal region, although we were not able to locate the fovea in every eye.

Absorbance measurements

Measurements were made with a modified Liebman microspectrophotometer (Liebman & Entine 1964; Knowles & Dartnall 1977, pp. 562–566), which had been placed under the control of a MNC-11 computer (Digital Equipment). Under infra-red inspection, the measuring beam of the instrument was arranged to pass through the structure of interest (usually the outer segment of an isolated photoreceptor) while a reference beam was passed through adjacent clear space in the preparation. Typically the beams were each 2 by 3 μm in size, but they could be conjointly adjusted to accommodate different cells. By means of a stepping motor, the monochromator of the microspectrophotometer was advanced from 700 to 390 nm in 2 nm increments, taking wavelengths with even values, and was then returned through the spectrum taking the interleaved values. In the Liebman microspectrophotometer the signals from the measuring and reference beams are sampled alternately by an electromechanical chopper, and the reference signal is

fed back to control the gain of the photomultiplier by regulation of the dynode voltage. Thus the reference signal is held constant as the spectrum is scanned and the sample signal reflects the spectral variation in the proportion of the measuring beam that is transmitted. In our modified instrument the sample signal is converted to absorbance (optical density) by the computer. (That is to say, the signal is now tapped off before amplifier A4 in the circuit diagram shown by Knowles & Dartnall (1977), p. 566, and the remaining electronics of the original microspectrophotometer are by-passed). The measuring beam is usually passed transversely through an outer segment, but, in the present experiments, we also occasionally made near-axial measurements when a matrix of end-on receptors presented itself (see below). To increase the proportion of light absorbed in transverse measurements, the beams were polarized so that the *e*-vector of the measuring beam was perpendicular to the long axis of the outer segment. After an individual cell had been scanned, a check was made that the preparation had not moved during the measurement. To allow for constant differences between measuring and reference beams, a baseline was obtained by adjusting the preparation so that both the sample and the measuring beam fell into clear space. The final absorbance spectrum for the cell was obtained by subtracting this baseline from the trace obtained when the measuring beam passed through the outer segment. In the case of putative short-wave receptors, after completing the initial measurement of absorbance, we always exposed the structure to white light for 5 min and repeated the measurements, to establish that the cell did contain a photolabile pigment.

Analysis of individual records

Individual records were stored on disk for later analysis. Approximately 5 % of the stored records were not subjected to detailed analysis, either because the maximum absorbance was less than 0.01 or because the curve was distorted by very high absorbance at short wavelengths. In the case of each of the remaining records, a standardized computer program was used to estimate the wavelength of peak sensitivity (λ_{max}). First, the absorbance values at pairs of adjacent wavelengths were averaged to obtain a mean curve from the outward and return traces. The resulting absorbances were re-expressed as percentages of the peak absorbance, the latter being taken as the mean of seven points centred on the highest individual point of the mean curve. Each of 20 relative absorbance values on the long-wavelength limb of the curve (corresponding to a 40 nm segment of the trace and to absorbances in the range approximately 45–90 % of the maximum for that cell) was then referred to a standard template curve (see below) to obtain an estimate of the λ_{max}; this operation amounts to finding the spectral location of the standard curve that gives the percentage absorbance value under consideration. The 20 individual estimates were then averaged to give the values entered in the histograms of figures 3 and 5. Three considerations guided our decision to estimate the spectral location of individual records from a segment of the right-hand limb: (i) our primary purpose in this study was to establish for each animal the number and spectral positions of its photopigments rather than to derive definitive measurements of the absorbance spectra; (ii) the right-hand limb includes the steepest part of a photopigment's absorbance spectrum and so small changes in

wavelength correspond to large changes in absorbance; and (iii) the short-wavelength region of a microspectrophotometrically measured absorbance spectrum is known to be the most variable, owing perhaps to wavelength-dependent scattering and the presence of photoproducts (MacNichol *et al.* 1983). Variation of short-wave absorbance can be clearly seen in the mean absorbance spectra plotted in figures 4 and 6 of the present paper.

It is no longer acceptable to use a single template curve to estimate the λ_{max} of any A1 pigment plotted in wavenumber units: accumulated evidence shows that bandwidth, if expressed in frequency units, increases as wavenumber of peak absorbance increases (Ebrey & Honig 1977; Knowles & Dartnall 1977) and this relation is now known to hold for the four photopigments of a macaque retina (Bowmaker *et al.* 1980; MacNichol *et al.* 1983). In recent studies, therefore, (Bowmaker *et al.* 1980; Dartnall *et al.* 1983) distinct templates have been used to analyse the different classes of photoreceptor. However, a nice problem arises in the analysis of the long-wave pigments of the three deuteranomalous animals that were included in the present study. If we use different templates for analysing the longer and the shorter of the two pigments that are apparently present in the long-wave range for these animals, we run the risk of artificially exaggerating a difference between the two subpopulations of receptors. On the other hand, the mean absorbance spectra for the two putative subpopulations do have systematically different shapes, and thus, if we use a single template to analyse all individual records, we must knowingly distort slightly the true values of λ_{max} for part of the total population of long-wave receptors. A solution to this dilemma is offered by Barlow's (1982) insight that the absorbance spectra of different classes of photoreceptor have approximately the same shape when plotted against the fourth root of wavelength. The theoretical basis of this relation is not understood and it has so far been tested only against microspectrophotometric measurements for macaque and human pigments (see Barlow 1982; Dartnall *et al.* 1983), but for our present purpose it offers a suitable tool with which to analyse two adjacent classes of receptor without artificially creating a bimodality. Therefore, to obtain the values of λ_{max} plotted in the histograms of figures 3 and 5, the individual absorbance values on the right-hand limb of each record (see above) were referred to a single template expressed in units of $\lambda^{\frac{1}{4}}$. The template that was used was Dartnall's standard spectrum for rhodopsin (Knowles & Dartnall 1977, table 1) placed with its λ_{max} at 502 nm and expressed as a function of $\lambda^{\frac{1}{4}}$. We emphasize that different methods of analysis produce only small changes in the distributions of λ_{max} and do not alter our assessment of any individual animal. We have in fact analysed all the present records by the earlier method of distinct templates expressed in wavenumber units: the interested reader will find summary histograms plotted in this way in a conference report (Bowmaker *et al.* 1983).

The mean absorbance spectra shown in figures 4, 6 and 7 do not depend on the methods used to prepare histograms of individual records (except in so far as the individual λ_{max} values were used to assign records to different classes). These mean spectra were obtained by averaging all the absorbance curves for a given class and then normalizing. The individual records were not normalized before averaging.

Results

(*a*) *Behavioural results*

Increment sensitivity

Table 1 shows for each animal the lg difference between sensitivity at 540 nm and at 640 nm. There are substantial individual differences in this ratio: the range between the most and the least sensitive monkeys was 1.11 log units. It has been established elsewhere that (i) the within-species variation seen in this test is due to variation in sensitivity to the 640 nm light, and (ii) that increment thresholds measured for individual animals are repeatable to better than 0.2 log unit (Jacobs 1983*b*). Thus we can be confident that the large individual differences in the present sample reflect true variations in sensitivity to the 640 nm test light.

Wavelength discrimination

Complete wavelength-discrimination functions were obtained from eight monkeys. These results are plotted in the conventional manner in figure 1. Striking differences are seen in the functions for different animals. Five animals, whose results are summarized in the bottom panel of figure 1, gave a function with a single minimum at about 500 nm. Away from this spectral region, discrimination worsened towards both shorter and longer wavelengths, yielding the U-shaped discrimination function. None of these five monkeys was able to show any significant discrimination for standard values greater than about 540 nm. The remaining animals, S2, S3 and S7, gave wavelength-discrimination curves with two minima, close to 500 and 575 nm (top two panels of figure 1). These three animals have extremely similar wavelength discrimination from 450 nm to about 590 nm; but at wavelengths longer than this S7 is slightly but consistently poorer than the other two animals, which are in turn very similar to each other.

Wavelength-discrimination data were not obtained for animals S19 and S25. However, by means of similar procedures, S19 was tested for the presence of a spectral neutral point: there proved to be an intensity of a 495 nm test light at which she could not distinguish the test from an achromatic (4800K) standard, whereas she was always able to perform at a better than chance level when the test was of shorter or longer wavelength.

Rayleigh matches

The results from the Rayleigh match tests are given in figure 2. To provide a comparative framework, matches were also obtained from 46 human observers, who were tested in the same apparatus as that used for the monkeys. Forty of these observers were normal trichromats. The average Rayleigh match (solid circle), and the entire range of matches (horizontal bar), obtained from this group are given in the bottom panel of figure 2. Six human subjects were categorized as anomalous trichromats (four deuteranomalous, two protanomalous) on the basis of their performance on the Farnsworth–Munsell 100-hue test. Their Rayleigh matches are shown in the second panel of figure 2.

The performances of the ten individual monkeys are shown in the upper panel of figure 2. For each animal the horizontal bar represents the range of red–green

TABLE 1. SUMMARY OF BEHAVIOURAL AND MICROSPECTROPHOTOMETRIC RESULTS FOR INDIVIDUAL MONKEYS

animal	sex	lg ratio of sensitivity 540/640 nm	behavioural diagnosis	λ_{max} for short-wave cones nm	λ_{max} for rods nm	s.d. for rods nm	λ_{max} for middle-wave cones nm	λ_{max} for 'anomalous' cones nm	λ_{max} for long-wave cones nm	s.d. for all cones of λ_{max} > 525 nm nm
S1	F	1.27	severe protan	431.2 (2)	497.8 (13)	3.6	538.5 (18)	—	—	3.2
S2	F	0.36	deuteranomalous	431.9 (1)	498.8 (22)	5.3	—	551.7 (13)	564.4 (10)	8.0
S3	F	0.36	deuteranomalous	436.0 (1)	502.5 (11)	3.2	—	552.4 (36)	566.0 (13)	6.7
S5	M	0.91	deuteranope	430.0 (2)	501.7 (12)	2.2	—	551.0 (63)	—	3.8
S7	F	0.86	protanomalous	428.0 (1)	498.6 (14)	2.5	536.5 (18)	546.5 (30)	—	5.7
S19	F	0.40	deuteranomalous	430.2 (2)	500.9 (13)	1.7	—	552.9 (18)	565.3 (24)	7.0
S21	M	1.28	protanope	431.9 (3)	497.2 (15)	2.8	536.4 (60)	—	—	3.3
S22	M	1.26	protanope	—	496.7 (20)	3.7	536.2 (47)	—	—	3.1
S24	F	0.91	deuteranope	—	501.7 (13)	2.6	—	—	563.1 (56)	4.7
S25	F	1.47	protanope	433.0 (2)	495.9 (11)	2.0	536.5 (64)	—	—	3.5

The entries under 'behavioural diagnosis' are given as shorthand labels for the reader's convenience. These labels should not be taken to imply that the animals are necessarily identical in their colour vision to the corresponding types of human observers; and reference should be made to the text for full details of the behavioural measurements.

The values of λ_{max} in this table represent means of estimates from individual records; numbers in brackets after λ_{max} values indicate the number of cells contributing to each mean. s.d., The estimated standard deviation of the population from which the sample is drawn.

Average transverse absorbances at the wavelengths of peak sensitivity were 0.016 for short-wave cones, 0.029 for rods, and 0.023 for each of the remaining classes of cone.

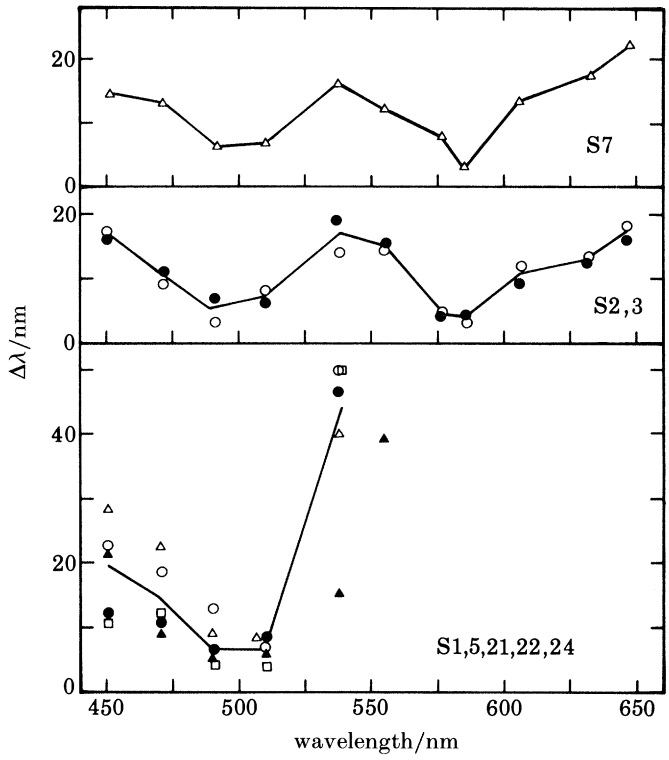

FIGURE 1. Behaviourally measured wavelength-discrimination functions for individual monkeys. The ordinate indicates the magnitude of the wavelength change required at each spectral location to support discrimination at a criterion level of 70% correct. The values are averages for differences in both spectral directions, except at 452 nm. The bottom panel shows results for five animals that in this test resemble human dichromats: S1 (▲), S5 (□), S21 (○), S22 (●), and S24 (△). The middle panel gives results for two animals, S2 (○) and S3 (●), that resemble human trichromats but make Rayleigh matches (figure 2) on the deutan side of the normal human setting. The uppermost panel gives results for S7, which appears trichromatic by this test and which (figure 2) makes Rayleigh matches on the protan side of the normal human match.

mixtures over which performance did not differ significantly from chance (see Methods). None of the five monkeys whose results appear at the top was able to learn to discriminate the pure red or the pure green test lights from the yellow standard. Consequently, each of these animals is indicated as being unable to make a Rayleigh match. The remaining five animals were able to discriminate various red–green mixtures from yellow. The Rayleigh match values for these monkeys fell into two patterns. Relative to the results obtained from the normal human trichromats, S1 and S7 had match values significantly displaced towards the red side, whereas the matches for S2, S3 and S19 were displaced in the other direction, towards the green.

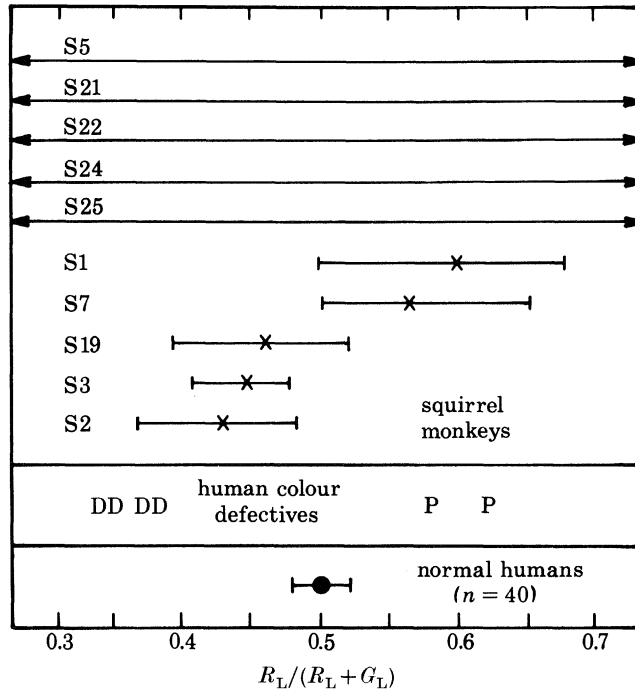

FIGURE 2. Rayleigh matches for squirrel monkeys, human colour defectives, and normal human trichromats tested in the same apparatus. The techniques used for measurement are described in the text. For the individual monkeys (upper panel) the horizontal lines mark the range of the acceptable Rayleigh matches and the crosses mark the mid-points. The middle panel shows the individual matches of four human deuteranomalous observers (D) and two protanomalous human observers (P). The solid circle in the lowermost panel shows the average match of 40 normal human observers and the horizontal bar shows the entire range of matches.

Diagnosis of colour vision

On the basis of the behavioural results it is possible to categorize the colour vision of these monkeys. It is mnemonically helpful to use here the criteria and terminology that are used to categorize defective colour vision in man (e.g. Pokorny *et al.* 1979); but we explicitly leave open the question of whether *Saimiri* provides exact models for the several forms of human colour deficiency. This issue is further examined elsewhere (Jacobs 1984).

Five of the monkeys (S5, S21, S22, S24, S25) appear to have dichromatic colour vision. None of these animals showed any discrimination in the Rayleigh match test and their wavelength-discrimination functions have the single minimum (*ca.* 500 nm) that characterizes most human dichromats (S25 was not run on the wavelength-discrimination test, but in the Rayleigh match test and the sensitivity test, her performance was essentially similar to that of S22 and S24). However, although these animals all appear dichromatic, the sensitivity measurements suggest that their dichromacies may be distinguishable: from table 1 it is clear that the 540–640 nm threshold differences for the five animals cover a rather large range

(nearly 0.6 log unit). Three of the dichromats (S21, S22, S25) have very large 540–640 nm sensitivity ratios and their performance resembles that of human protanopes. The other two dichromats (S5 and S24) show a somewhat lower ratio and might be thought to resemble human deuteranopes.

Four of the ten monkeys (S2, S3, S7, S19) probably have trichromatic colour vision, since (i) they can set discrete Rayleigh matches (a result implying access to two pigments in the red–green range) and since (ii) their wavelength-discrimination functions contain the twin minima characteristically seen in trichromatic human observers. (Although S19 was not run on the wavelength-discrimination problem, her Rayleigh match setting and sensitivity test results were indiscriminable from those of S2 and S3.) Although these four appear to be trichromatic, they would by human standards be judged anomalous trichromats. The Rayleigh match setting of S7 is well to the red side of the normal trichromats, and her wavelength-discrimination data show some loss in the long wavelengths. She would thus be classified as protanomalous. S2, S3 and S19 made Rayleigh matches that were slightly, but significantly, to the green side of the normal human setting. On this ground these animals might be categorized as mildly deuter-anomalous trichromats.

The colour vision of one of the ten subjects, S1, was difficult to categorize with certainty on the basis of the behavioural tests. After long training she was able to discriminate some red–green mixtures from yellow and produced a Rayleigh match well-displaced towards the red side. On that basis she might be thought a protanomalous trichromat. However, her wavelength-discrimination function (figure 1) has the U shape characteristic of the human dichromat. Perhaps attesting further to S1's borderline status is the fact that of all the animals who had a U-shaped wavelength-discrimination function she had the most acute wavelength discrimination at the longer test wavelengths (S1 is represented by the triangles in the bottom panel of figure 1). On the basis of these contradictory indications we conclude only that S1 is a severe protan, leaving open the question of whether she is anomalous or a true dichromat.

(*b*) *Microspectrophotometry*

Appearance of cells

Under infra-red inspection, the outer segments of cones in this species had a characteristically granular appearance. In our preparations the outer segment was usually separated from the inner segment. We were not able to distinguish one class of cone from another by appearance, nor indeed were we able reliably to distinguish rods from cones. The majority of structures that contained a pigment peaking near 500 nm did have a classically rod-like appearance, with parallel sides, but we often recorded a 500 nm pigment in a structure that we had hoped was a cone. Conversely, in the case of animal S2, a record with $\lambda_{max} = 558$ nm was obtained from a cell that was described in advance by the operator as a 'classical rhesus rod'. When, elsewhere in this paper, we refer to 'rods' and 'cones' we are making no histological claim, but are basing our classification merely on the spectra obtained. The retinal eccentricity of a given preparation was often clear from its appearance: in foveolar preparations we occasionally came upon intact fragments

of a regular matrix of cones (see below), whereas with peripheral preparations we sometimes saw 'rosettes' consisting of a single cone surrounded by one or more rings of rods.

Absorbance spectra for long- and middle-wave cones

Absorbance spectra were analysed for a total of 474 cones and 144 rods from the ten monkeys. Histograms of the λ_{max} values for each animal are shown in figures 3 and 5. In the green–yellow range of the spectrum there are clear differences in the photopigments that are found in different animals.

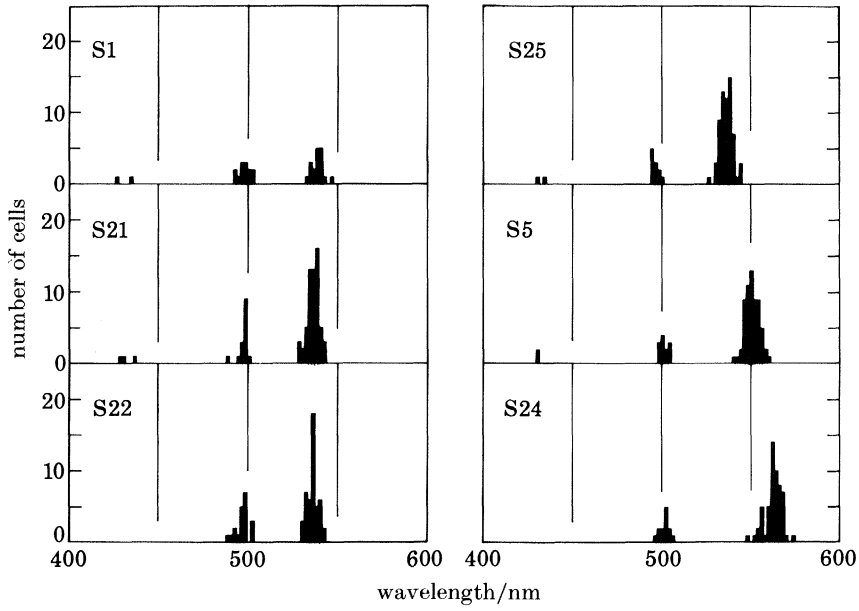

FIGURE 3. Distributions of values of peak sensitivity (λ_{max}) of all individual microspectrophotometric records from the five behaviourally dichromatic monkeys and the 'severe protan', S1. The animals S1, S21, S22 and S25 all exhibit a cone pigment with λ_{max} close to 537 nm. The animals S5 and S26 also appear to exhibit only a single pigment in the green–yellow part of the spectrum, but the distributions of λ_{max} values are clearly shifted to longer wavelengths. The bin size in the histogram is 2 nm.

(i) *Behavioural dichromats.* In six of the ten animals (S1, S5, S21, S22, S24, S25) there appears to be only one pigment in the green–yellow range (figure 3). These animals correspond to the five animals identified behaviourally as dichromats, plus the marginal animal, S1 (see above). In the green–yellow part of the spectrum the distribution of λ_{max} values for each of these animals has a unimodal form and has a standard deviation in the range 3.0–4.7 nm (table 1). However, the spectral position of the putative single pigment is not constant for all six animals. For four monkeys (S1, S21, S22 and S25) the mean values of λ_{max} are very similar (538.5, 536.4, 536.2, and 536.5 nm respectively) and the reader will recall that these were all behaviourally protan. In spectral position and in standard deviation, these distributions resemble those typically found for the middle-wave cones of macaques

(Bowmaker *et al* 1980; Hárosi 1982; MacNichol 1983), but the spectral position occupied by the long-wave cones of macaques is completely unoccupied in these four animals. It is interesting to note that the distribution for S1 (the severe protan animal whose colour vision was difficult to diagnose) has one outlying record at 545.9 nm, a λ_{max} greater than any recorded for S21, S22, or S25.

For the remaining behavioural dichromats (S5, S24) there again appears to be only a single distribution of λ_{max} values in the green–yellow range, but the distributions clearly lie at longer wavelengths. In the case of S5 the mean λ_{max} value is 551.0 nm and in the case of S24 it is 563.1 nm (figure 3, right-hand panels).

By using Geary's test (Pearson & Hartley 1954; D'Agostino 1970), which is based on the ratio of the sample mean deviation to the standard deviation, we have tested the normality of the distribution of λ_{max} values in the green–yellow range for each of the five behavioural dichromats and the 'extreme protan', S1. In no case was there evidence to reject the null hypothesis that the distribution was normal.

Figure 4 shows for each of these six animals the mean absorbance spectrum of the single pigment that is found in the green–yellow range.

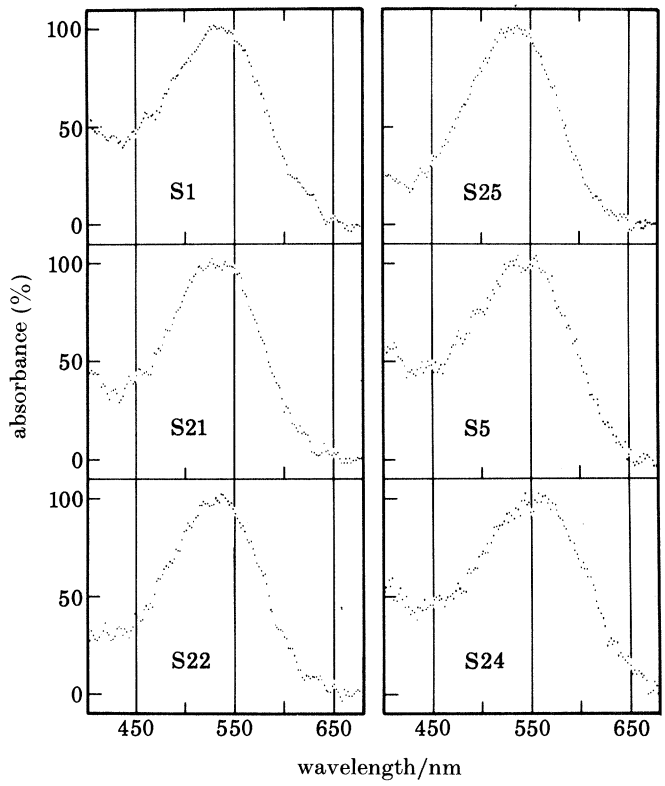

FIGURE 4. Mean absorbance spectra for the long-wave cones of the five behaviourally dichromatic squirrel monkeys and the 'severe protan', S1. Each datum point corresponds to the average of values obtained at two adjacent wavelengths, one recorded in the descending scan of the microspectrophotometer, one recorded in the ascending scan. Absorbance values for different receptors were averaged before being normalized.

(ii) *Behavioural trichromats.* Figure 5 shows the distribution of λ_{max} values for the remaining four animals, S2, S3, S7 and S19. These are the monkeys that enjoyed good wavelength discrimination in the red–green range and were classified behaviourally as trichromatic. None of these monkeys show the two clearly separated pigments at 535 nm and 567 nm that would characterize a macaque retina (Bowmaker *et al.* 1978, 1980; Hárosi 1982; MacNichol *et al.* 1983), but the distributions of λ_{max} values in this part of the spectrum are clearly broader than for the behavioural dichromats: the standard deviations range from 5.7 to 8.5 nm (table 1). There is no overlap between the range of standard deviations for the behavioural trichromats and those for the behavioural dichromats. And in the case of two of the trichromats, S7 and S19, the distribution of λ_{max} values in the green–yellow range was found to be significantly non-normal by Geary's test ($p < 0.05, p < 0.01$ respectively). The departure from normality was in the direction of positive kurtosis.

Three of the four animals, S2, S3 and S19, show very similar distributions extending over the range 540–570 nm. These are in fact the three deuteranomalous animals, which performed very similarly on the Rayleigh match test (figure 2). In the case of S3 and S19 there is an indication that the distribution of long-wavelength cones is bimodal; and the bimodality is particularly clear in the pooled distribution for the three behaviourally deuteranomalous animals (figure 5, lower right panel).

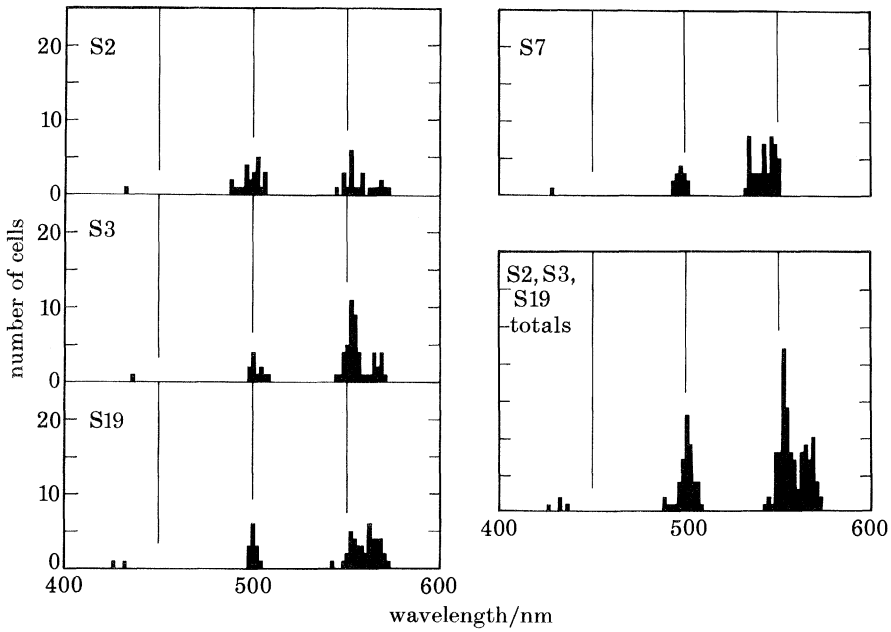

FIGURE 5. Distributions of values of peak sensitivity (λ_{max}) of all individual microspectrophotometric records from the four monkeys that were shown behaviourally to be trichromatic. S2, S3 and S19 were behaviourally deuteranomalous and are thought to have two pigments in the green–yellow range, with λ_{max} values at approximately 552 nm and 565 nm (see pooled histogram on right). S7 was the behaviourally protanomalous animal. The bin size in the histograms is 2 nm.

A statistical test for bimodality has been described by Kruskal (Giacomelli *et al.* 1971) and we have applied this test to the pooled distribution: the null hypothesis, that the distribution is unimodal, was rejected at the 1 % level.

If we divide the long-wavelength cones of the deuteranomalous animals into two groups according to whether the λ_{max} lies above or below 559 nm, we obtain two putative pigments with λ_{max} values at approximately 552 and 565 nm. The lower of these values resembles that of the single long-wavelength pigment of S5, whereas the upper value resembles that for the single long-wavelength pigment of S24.

The fourth of the behaviourally trichromatic animals, the protanomalous S7, shows a distribution of λ_{max} values that is quite different from the distributions for the other three trichromats: for this animal there is a range of cells extending from 530 to 550 nm, with a standard deviation of 5.7 nm (figure 5, table 1). A tentative division of this distribution into two subpopulations (table 1) gives putative pigments at approximately 537 and 547 nm; the first of these would correspond to the single middle-wave pigment of S1, S21, S22 and S24, while the second might correspond to the single pigment of S5.

If, for each of the behavioural trichromats, we take all the individual records in the green–yellow range and divide them into two groups as proposed in the two preceding paragraphs, then we obtain the pairs of mean absorbance spectra shown in figure 6. Although there are marked differences *between* animals in the relative

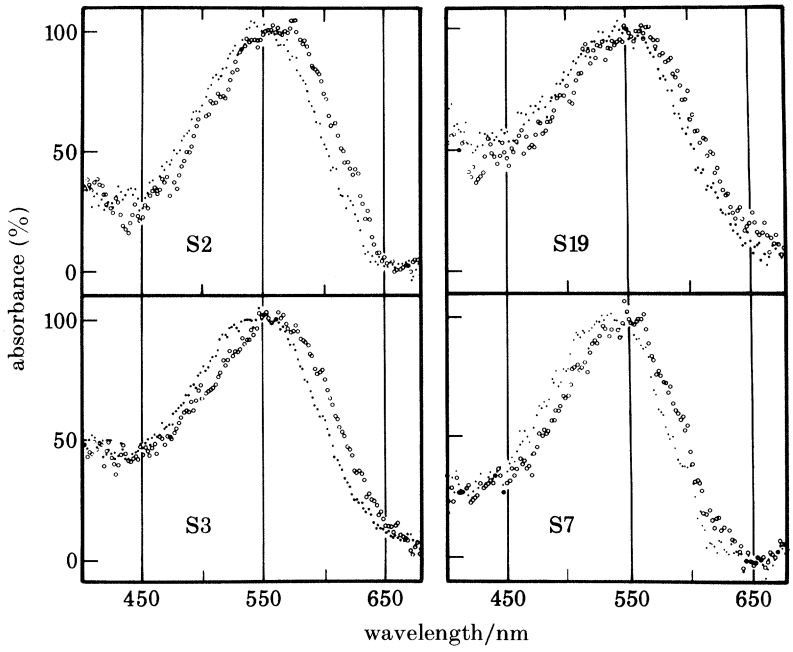

FIGURE 6. Mean absorbance spectra for the two putative types of long-wavelength receptor thought to be present in each of the monkeys found to be behaviourally trichromatic. In the case of each animal the two absorbance spectra show similar values at short wavelengths; this would be unlikely to be the case if the apparent presence of two groups of receptors arose from variations in optical scattering or in the presence of photoproducts.

absorbance measured at short wavelengths, the short-wave absorbance for any given animal is very similar for the two curves; this observation adds to our confidence that the variation in λ_{max} values of individual records reflects the presence of more than one pigment. For the factors that distort estimates of λ_{max} are usually associated with variation in short-wave absorbance (MacNichol *et al.* 1983).

Short-wave receptors

Cones absorbing maximally at short wavelengths were rare, only 14 being identified out of a total of 474 cones and none being identified in two of the ten animals (figures 3 and 5). These 14 cells had a mean λ_{max} of 431.4 ± 3.2 nm. Their mean absorbance spectrum is shown in figure 7 (open circles).

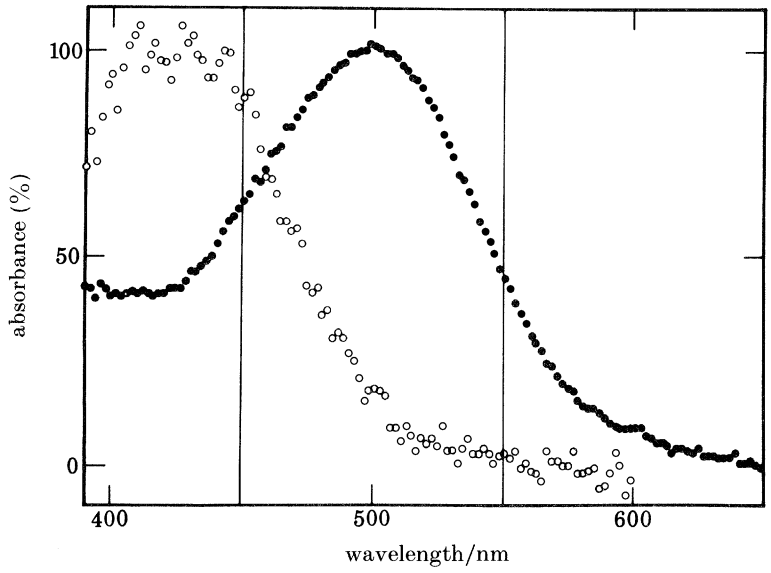

FIGURE 7. Mean absorbance spectra for short-wave cones and for rods from squirrel monkeys. Data have been pooled from all ten animals to yield these curves. Absorbance spectra for individual cells were averaged before being normalized.

Rods

The 144 rods analysed had a maximum absorbance at approximately 500 nm. The mean values of λ_{max} for individual animals ranged from 496 nm for S25 to 502.5 nm for S3 (see table 1). Histograms of the individual λ_{max} values are shown in figures 3 and 5 and the mean absorbance spectrum for the 144 cells is shown in figure 7 (solid circles).

Foveolar matrices

On a number of occasions we came upon a fragment of apparently foveolar tissue where large numbers of cone outer segments lay in a regular array with their long axes approximately parallel to the axis of the measuring beam. Where such arrays

were tightly packed, we were unable to make measurements, owing to the difficulty of placing the reference beam in tissue-free space; but where the array was slightly splayed, we were able to measure every cell within a small piece of regular lattice. Figure 8 shows examples of such foveolar fragments. The drawings are based on sketches made at the time, and against individual cells are shown λ_{max} estimates for the individual microspectrophotometric records. The dashed circles in the figure indicate cells that were sketched but could not be measured. Notice that in the behaviourally anomalous animal, S19, there is no hint of a systematic alternation between the two putative classes of long-wave cone that appear to be present; and there are not frequent gaps in the lattice that might correspond to missing short-wave cones.

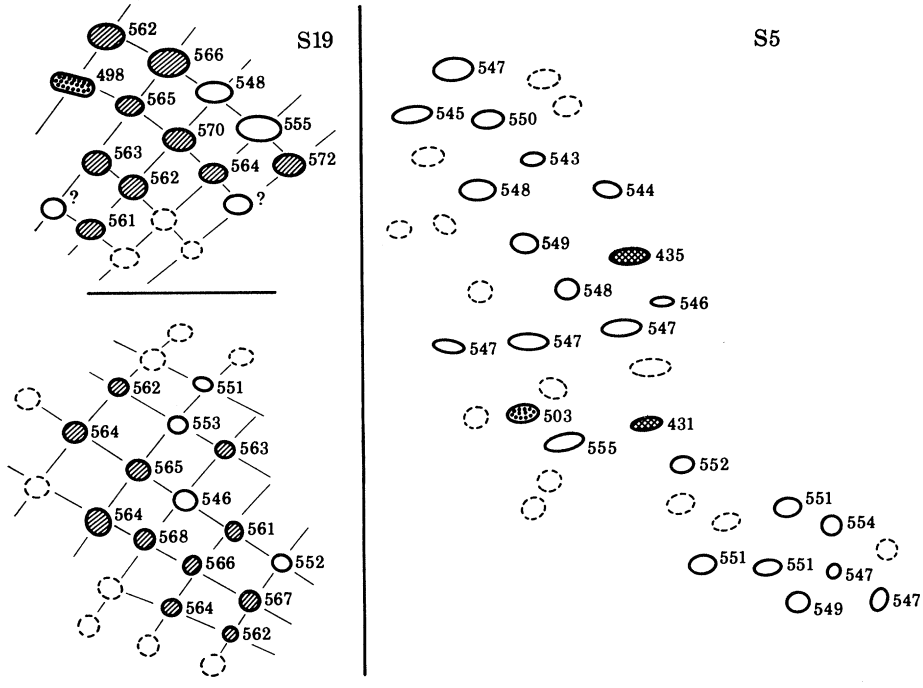

FIGURE 8. Fragments of foveolar arrays found in preparations from a behavioural trichromat, S19, (left-hand panel) and a dichromat, S5. The diagrams show the spatial arrangements of different classes of receptor and are based on sketches made at the time of microspectrophotometric recording. Rods are indicated by stippling, short-wave cones by double hatching, and P_{565} cones by single hatching; P_{550} cones are left unfilled. Where it is known, the λ_{max} actually recorded is given against the cell. Question marks indicate that the microspectrophotometric record is adequate to classify the cell but not adequate to yield a precise λ_{max}. Broken contours indicate the position of outer segments that were sketched but for which microspectrophotometric records were not obtained. In each case the fragment shown was part of a larger array visible to the operator.

Discussion

Concordance of results

The behavioural and the microspectrophotometric results of this study were obtained independently under double-blind conditions. Each type of measurement has separately revealed individual differences that are too large to be attributed to experimental error. The concordance of the two sets of measurements places beyond question the existence of a polymorphism of colour vision in *Saimiri sciureus* and suggests that the behavioural variation arises from variation in retinal photopigments. On the basis of the microspectrophotometric results, the behavioural dichromats were all clearly distinguished from the behavioural trichromats (the animal S1 being marginal in both sets of results). The microspectrophotometric results also distinguished clearly between behavioural protans and behavioural deutans. The one minor discrepancy is that two of the dichromats, S5 and S24, were microspectrophotometrically distinct but gave very similar sensitivity ratios on the increment-threshold test (see table 1).

The agreement between the behavioural and densitometric measurements is quantitative as well as qualitive. From the absorbance spectra that were obtained microspectrophotometrically for individual animals (figures 4 and 6), we have derived estimates of the 540/640 nm sensitivity ratios that would have been expected in the behavioural measurements (table 1). In the case of the dichromatic animals, the ratio of sensitivity should have been determined by the single photopigment found in the red–green part of the spectrum. In the case of the four clearly trichromatic animals we made the simplifying assumption that the ratio was determined by the cone pigment with the longest λ_{max}. (This is to ignore any opponent processes, which might be expected to decrease the ratio for the large, long-lasting stimuli that were used to measure increment thresholds for the monkeys (see, for example, King-Smith & Carden 1976; Mollon 1982.) To estimate the axial *absorptances* (fractions of light absorbed) from the transversely measured absorbances for 540 and 640 nm, the effective length of the outer segments was taken to be 35 μm and the specific absorbance was taken to be 0.015 μm^{-1} (see Knowles & Dartnall 1977, chapter 3; Bowmaker *et al.* 1980). It was assumed that pre-receptoral absorption did not vary between 540 and 640 nm. Figure 9 shows the relation between the sensitivity ratios that were behaviourally measured and the values estimated from the actual absorbance data for individual animals. Despite the uncertainties that accompany a reconstruction of this kind and despite the fact that the two sets of measurements were made by different techniques in different countries, the estimated ratios are strongly and significantly correlated with the empirically measured values ($r = 0.91$; $t = 6.208$; $p < 0.001$). Moreover, the regression line (figure 9) comes close to passing through the origin (y intercept, 0.16); and the slope (0.85) is similar to the theoretically required value of unity.

Number of pigment types. Comparison with Old World monkeys and man

In the green–yellow range, there appear to be at least three possible *Saimiri* photopigments, with λ_{max} values at about 535, 550 and 565 nm. Individual

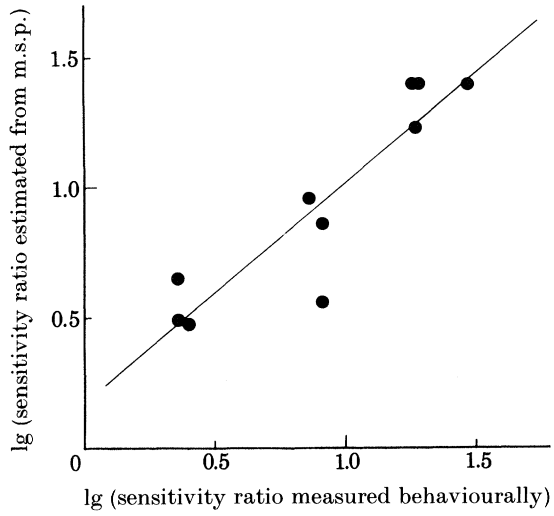

FIGURE 9. The relation between the 540/640 sensitivity ratio, behaviourally measured, (see table 1 and Methods) and estimates of this ratio reconstructed from the absorbance spectra of figures 4 and 6. Each datum point corresponds to an individual animal. The solid line represents a least squares fit to the data. m.s.p., microspectrophotometry.

animals appear to draw either one or two pigments from this set, giving dichromatic or trichromatic colour visiom. Two of the three pigments resemble closely the middle and long-wave cone pigments of macaques and it is curious that we have not yet found a squirrel monkey that combines the two most disparate of the three *Saimiri* pigments to give the two well-separated distributions seen in macaques, baboons, and man (Bowmaker *et al.* 1983; Dartnall *et al.* 1983). It is instructive to find that two pigments separated by 15 nm or less can sustain good hue discrimination in the red–green range (animals S2, S3, S7, S19). Owing to the very different incidences of different forms of colour vision in squirrel monkey and man, it would be improper to extrapolate from one species to the other; but our results are consistent with those explanations of human anomaly that postulate a shift in the spectral position of one of the cone pigments (König & Dieterici 1895; Boynton 1979, chapter 10; Pokorny *et al.* 1979, chapter 7).

In the case of man, a celebrated question has been whether protanomalous and deuteranomalous observers share a common 'anomalous' pigment in the red–green range. Cited as evidence has been the rare type of human dichromat who is said to be able to accept the colour matches of both protanomalous and deuteranomalous observers – and who therefore should have two cone pigments in common with each type of anomalous observer (De Vries 1984). One of these two pigments would be the short-wave pigment, the other would be the 'anomalous' pigment. The long-wave pigment ($\lambda_{max} = 547$ nm) of our one protanomalous animal is *similar* to the 552 nm pigment of the three deuteranomalous animals and to the 551 nm pigment of one of the dichromats (S5). But our data are not yet precise enough to show definitely whether a single 'anomalous' pigment is common to three behavioural types.

Explanations of polymorphism

Until recently it was often supposed that all New World monkeys were either protanopic or severely protanomalous. This traditional view is clearly mistaken: some squirrel monkeys enjoy good colour discrimination in the red–green range and some are deutan rather than protan in type. But why should several forms of colour vision exist within the same species? Why should male squirrel monkeys be dichromatic when trichromatic discrimination is available to many of their female conspecifics? In the brief discussion that follows we make the assumption that the individual differences are of genetic origin, although there is not yet available the direct proof that would be provided by pedigrees. The variation in colour vision in *Saimiri* is an attractive problem for biological and genetic study, since, as the present results suggest, the large variations in sensory capacity result from variations in the spectral absorbance curves of retinal photopigments; and the latter variations, it is thought, arise from small changes in the protein moiety of the photopigment molecule (the 'opsin'), while the chromophore remains common to all A_1 pigments (Knowles & Dartnall 1977).

In attempting to give a biological explanation of the polymorphism of colour vision in *Saimiri*, we should remember that the genes that determine the photopigments could influence an individual's survival not by their effects on colour discrimination but by some quite other, pleiotropic, effect (Kalmus 1983). It is also possible, though implausible, that there are neither selective advantages nor selective disadvantages associated with the different phenotypes apparently present in the *Saimiri* population. These possibilities apart, there are several classes of mechanism that may maintain a polymorphism within a population (Sheppard 1975; Shorrocks, 1978) and specific forms of four of these could be invoked in the present case.

Group selection

Where animals live in a shoal, flock or troop it might be advantageous to the group to have members with several forms of colour vision. Camouflaged predators and cryptic food are likely to be visible to some subset of the group, who can then attract the attention of other members of the group. In the case of man, it has been reported that observers with variant colour vision may successfully penetrate military camouflage that deceives the colour-normal†. A group-selection hypothesis

† The paper traditionally cited in support of this claim is that by Judd (1943), but Judd considers only the theoretical possibility and gives no empirical support. An unpublished military study by Whittenburg & Lowenhaupt (1974) showed that colour-deficient observers were at no advantage in penetrating the particular set of camouflaged targets used in the study. In assessing the theoretical possibility of an advantage, we should consider anomalous and dichromatic observers separately. Since stimuli that are metamers for a protanomalous monkey will not always be metamers for a deuteranomalous monkey (and vice versa), there must in principle exist pairs of stimuli that appear indistinguishable to one animal but are discriminable by the other; so the two observers together ought, in principle, to penetrate more instances of camouflage than can either alone. It is less clear that a dichromat will be able to penetrate camouflage that deceives a trichromat with whom he shares two cone pigments. In the case of man, the standard argument has been that the dichromat will be more sensitive to variations

has been advanced by Bridges (1964) to explain the presence of individual differences in the pigments of schooling fish: in such a case, if individual members of a tightly coordinated school differed in the ratio of A_1 and A_2 pigments, the overall spectral sensitivity of the school would be broadened.

Frequency-dependent selection

This is now a generally accepted way of accounting for the stable presence of more than one phenotype within a population (Clarke 1979). 'In a population dominated by millers, individual farmers prosper, while in a population dominated by farmers it pays to be a miller.' (Dawkins 1983). The classical biological examples are provided by polymorphism among prey species. It is supposed that predators have a searching image of their prey and that this leads to overpredation of those members of the prey species that exhibit the most frequent phenotype. It is advantageous for an individual member of the prey species to be, say, of a different colour from that of the majority of its conspecifics; but the advantage is lost as soon as the frequency of the variant phenotype rises too high. Thus, opposing advantage and disadvantage maintain the minority allele at a stable frequency. In the present case, we might apply the principle of frequency-dependent selection to the *predatory* behaviour of *Saimiri* and suppose that minority phenotypes among the population are able to feed upon cryptic fruit and cryptic insects that are passed over by other phenotypes.

Spatial heterogeneity of environment

It is possible that different phenotypes occupy different locations within the jungle. For example, daylight is strongly modified in its spectral composition as it penetrates dense jungle, becoming dominated by the green light that escapes absorption by foliage (Lythgoe 1972), and we might suppose that protan animals occupy niches where long-wave light is more attenuated, whereas deutan animals occupy niches where long-wave light is less attenuated, for example, the canopy or relatively open areas at the forest edge. (Note that the wavelength of peak transmission of chlorophyll, 540 nm, corresponds closely with the peak sensitivity of protan squirrel monkeys.) This class of hypothesis recalls the correlation between visual pigments and depth of habitat that is well established – *between species* – in fish (MacNichol *et al.* 1958; Lythgoe 1972; Levine & MacNichol 1979).

Heterozygous advantage

The most frequently cited example of heterozygous advantage is provided by sickle-cell anaemia in man (Allison 1964). In certain African populations there occur two forms of haemoglobin, A and S. The homozygotes (Hb^s Hb^s) suffer severe anaemia, owing to the inefficiency of their red blood cells, and frequently die before reaching reproductive age. However, despite the severe disadvantage of the Hb^s allele, it survives at frequencies of up to 20 %, because the heterozygotes (Hb^A Hb^S),

in lightness (for example Adam 1969). There is some laboratory evidence that some human dichromats are more sensitive than normals to flicker in some parts of the spectrum (see, for example, Alpern *et al.* 1982, figure 4).

who have both types of haemoglobin, enjoy a resistance to malaria that is greater than that of the homozygote (Hb^A Hb^A). Heterozygous advantage offers a particularly interesting type of explanation of the polymorphism of cone pigments in *Saimiri*. We make the following assumptions:

(i) in this species there is only one genetic locus for a photopigment in the red–green range;

(ii) this locus is on the X chromosome;

(iii) by a process of Lyonization, either the paternal or the maternal X chromosome is suppressed in individual cone cells of female monkeys (Lyon 1962), so that any individual cone manufactures only the opsin specified by one X chromosome.

Now, if several alleles are present in the population, the female must have a good likelihood of drawing different alleles from her two parents and thus being able to manufacture two pigments in the red–green range; but the male, having only one X chromosome, can never manufacture more than one such pigment. To complete our argument we must make explicit two further assumptions:

(iv) the nervous system of the heterozygous female is versatile enough to allow her to exploit the presence of a third cone pigment, so that she comes to enjoy trichromatic colour vision;

(v) the heterozygous female, since she is able to discriminate hue in the red–green range, is at a selective advantage relative to the homozygous female.

In this explanation of polymorphism, we are proposing that it is the resulting advantage *to the heterozygous females* that accounts for the presence of multiple alleles among the dichromatic male population. The more genotypes that exist in the male population, the more likely it is that any individual female will be heterozygous and thus able to make colour discriminations in the red–green range. In constructing the hypothesis, we have not had to include any implausible assumptions. The first and second of our assumptions are strongly implied by the absence of male trichromats, and by the proportion of female dichromats, found in behavioural studies (Jacobs 1984; Jacobs & Neitz 1984); and assumption (ii) is consistent with the fact that in man it is the X-chromosome that carries the two loci responsible for the long- and middle-wave pigments (see, for example, Pokorny *et al.* 1979). There is extensive genetic evidence for the Lyon hypothesis (iii); and the bimodality of our microspectrophotometric results for deuteranomalous monkeys (figure 5) suggests that individual cone cells may contain either a P_{550} or a P_{565} pigment but not a range of intermediate mixtures of pigment. If, like the retinae of Old World primates, the *Saimiri* retina contains ganglion cells that draw their field-centre input from only one, or from only a small number, of cones, then assumption (iv) is also a reasonable one; for individual ganglion cells will vary in their spectral sensitivities, and our knowledge of the versatility and plasticity of the visual system suggests that the later stages will be able to extract the spectral information that is present in the retinal signals. We have made assumption (v) explicit, since it is always possible that the trichromacy of some female squirrel monkeys is selectively neutral, arising when the animal is forced in the laboratory to discriminate on the basis of minimal cues but not lending any advantage in the wild. The latter possibility seems unlikely to us, since the ability to discriminate

in the red–green range cannot but assist, say, the detection of fruit among foliage and the judgement of ripeness.

Rarity of short-wave receptors

Short-wave cones proved to be very rare in our microspectrophotometric records and in this result *Saimiri sciureus* resembles Old World monkeys (Bowmaker *et al.* 1980, 1983; Hárosi, 1982; MacNichol *et al.* 1983) and man (Dartnall *et al.* 1983). Although microspectrophotometry is one of the few direct ways of identifying short-wave cones, we have hitherto had to acknowledge that this class of receptor might be selectively missed through a sampling bias; for example, the outer segments of short-wave receptors might differentially adhere to the pigment epithelium when the retina was lifted away or they might decay more rapidly after enucleation or they might be selectively vulnerable to the barbiturate used to kill the animal. Some direct, though preliminary, evidence against these possibilities is provided by our present observations on fragments of foveolar matrix (figure 8): where outer segments lay in a regular lattice, there did not appear to be gaps corresponding to absent short-wave cones. These observations are consistent with indirect histological evidence for the paucity of short-wave cones (Sperling 1980; De Monasterio *et al.* 1981; McCrane *et al.* 1983) and deserve to be replicated more extensively using a single-beam microspectrophotometer.

Validity of microspectrophotometric measurements

Primate microspectrophotometry has been hampered by the lack of any independent estimate of experimental error. It has been difficult to estimate even the internal repeatability of measurements, since the object of measurement, a visual pigment, is destroyed by the act of measurement. The chance of collating microspectrophotometric data with a quite separate type of measurement was one of our explicit reasons for undertaking this double-blind study. The results show that mean λ_{max} values for separate samples from the same behavioural phenotype are consistent to within 2–3 nm, while systematic differences can be shown between behavioural types. And it has proved possible (figure 9) to reconstruct behavioural sensitivities with some precision.

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