Proc. R. Soc. Lond. B **231**, 383–390 (1987)
Printed in Great Britain

Polymorphism of photopigments in the squirrel monkey: a sixth phenotype

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(*Communicated by H. B. Barlow, F.R.S. – Received* 2 *December* 1986)

We describe here a trichromatic type of squirrel monkey that resembles Old World monkeys in having two well-separated photopigments in the red-green part of the spectrum; the cones of this phenotype have peak sensitivities close to 430, 536 and 564 nm. The existence of such animals is predicted by a genetic model that postulates three alleles for a single locus on the X-chromosome of the squirrel monkey. The three alleles correspond to three different photopigments in the red–green spectral range. A male monkey, or a homozygous female, will be dichromatic, combining short-wave cones with just one of the cone types in the red–green range. But a female monkey, if heterozygous at the locus, draws any two of the three alleles from the set. X-chromosome inactivation ensures that the two alleles are expressed in different subpopulations of retinal cone, giving the monkey the basis for trichromatic colour vision. This model requires three trichromatic types of female squirrel monkey. The photopigment complements of two types have previously been reported and microspectrophotometric data are now given for the third type. Behaviourally, this third type of trichromat gives precise Rayleigh matches that are intermediate between those of the other two types of trichromat.

The polymorphism of photopigments in the squirrel monkey may be maintained by the heterozygous advantage enjoyed by the trichromatic females. This would be an instructive instance of heterozygous advantage because it is a case where X-chromosome inactivation plays a crucial role in segregating the two different gene-products into different cells.

INTRODUCTION

The squirrel monkey, *Saimiri sciureus*, exhibits a remarkable intraspecific variation in its colour vision (Jacobs 1984); in earlier papers we have shown, by microspectrophotometric measurements of isolated receptor cells, that the behavioural variation in colour vision arises from a polymorphism of retinal photopigments (Mollon *et al.* 1984; Bowmaker *et al.* 1985). In the samples hitherto tested, all the male monkeys, and some of the females, were behaviourally

dichromatic. These dichromatic animals proved to have only one photopigment in the red–green spectral region, but three subtypes of dichromat have been recorded: all are thought to have a short-wave cone pigment with its wavelength of peak sensitivity (λ_{max}) in the region of 430 nm, but this pigment is combined with a second that can have its peak sensitivity near 536 nm in some animals, near 549 nm in others and near 564 nm in the third subtype. Two subtypes of trichromatic monkey were found: one subtype combined the 549 nm photopigment with the 536 nm pigment (see figure 2, upper panel), while the other combined the 549 nm pigment with the 564 nm pigment (see figure 2, middle panel).

In the present paper we report a third subtype of trichromatic monkey, a phenotype missing from our earlier samples but demanded by the genetic model that we provisionally proposed (Mollon *et al.* 1984; Jacobs & Neitz 1985). The model incorporates the following assumptions:

(*a*) in the squirrel monkey there is only one genetic locus for a photopigment in the red–green spectral range;

(*b*) there are at least three alleles that can occur at this locus, the three alleles corresponding to three slightly different versions of the protein moiety (the 'opsin') of the photopigment;

(*c*) the locus is on the X-chromosome;

(*d*) in those females that are heterozygous at this locus, only one of the two pigments is manufactured in any given cone cell, owing to the phenomenon of Lyonization (the inactivation of either the maternal or the paternal X-chromosome that occurs in every somatic cell (Lyon 1972; Gartler & Riggs 1983)).

By this account, in the middle- to long-wave region of the spectrum, at least three photopigments are potentially available to the squirrel monkey. Male monkeys, we suppose, can draw only one pigment from the set, because they have only one X-chromosome; so males are obliged to be dichromatic. Female monkeys may draw either one or two pigments from the set; if they inherit the same alleles on their maternal and paternal X-chromosomes then they will be dichromatic, but if they inherit two different alleles then they will be trichromatic and will enjoy good discrimination in the red–green spectral region. As it stands, our model requires the existence of females that draw the 536 and 564 nm pigments from the pool; but no animals of this type were found in our earlier microspectrophotometric studies. Two examples of the missing phenotype are here described.

Methods

The subjects were two adult female squirrel monkeys (*Saimiri sciureus*). The behavioural and microspectrophotometric procedures, and the method of analysing the microspectrophotometric records have been described previously (Jacobs 1984; Mollon *et al.* 1984; Bowmaker *et al.* 1985) and only a summary is given here.

Rayleigh matches (Rayleigh 1881) were obtained for each animal by a forced-choice method: the monkey was required to choose which one of three, transilluminated panels was differently illuminated from the other two and, across successive trials, the experimenter determined the proportion of red (625 nm) to green (536 nm) light that the animal could not discriminate from a yellow light (585 nm).

Post mortem microspectrophotometric measurements were made on samples of fresh retinal tissue from the same monkeys. The measuring beam of a modified Liebman microspectrophotometer (Knowles & Dartnall 1977, pp. 562–566) was passed transversely through the outer segments of individual photoreceptors while a reference beam was passed through adjacent clear space in the preparation.

The λ_{max} for an individual receptor was estimated by fitting a template to the right-hand limb of the absorbance spectrum. Except in the case of (very rare) short-wave cones, microspectrophotometric records were excluded from further analysis if they failed any one of the criteria specified by Bowmaker *et al.* (1985), i.e. if the peak value of the transverse absorbance was less than 0.01, or if the standard deviation of the 20 estimates of λ_{max} was greater than 10 nm, or if there was a discrepancy of more than 10 nm between the estimate of λ_{max} obtained by fitting the template to the right-hand limb of the absorbance curve and an estimate derived by fitting the template to both long- and short-wave regions.

Results

Figure 1 shows results for the Rayleigh match test. Unlike their dichromatic conspecifics, S21F and S34 were behaviourally able to discriminate between yellow and all but a small range of red–green mixtures. In comparison with normal human trichromats (lowermost symbol), they required slightly more red light in the red–green mixture to match the yellow light. However, the matches of these

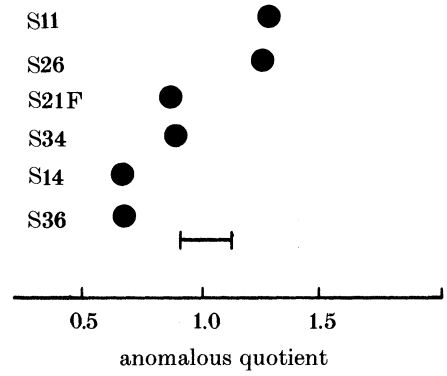

Figure 1. Results of behavioural tests to establish Rayleigh matches for S21F and S34. The matches are expressed as anomalous quotients, that is, as the ratio of green light to red light in the match, divided by the corresponding mean ratio for normal human observers. Results are also shown for two behaviourally 'protanomalous' animals (S14, S36) and two 'deuteranomalous' animals (S11, S26). The horizontal bar indicates the total range of matches by colour-normal observers in the same apparatus.

animals are intermediate between the matches of the two types of trichromatic monkey included in our previous microspectrophotometric samples (Mollon *et al.* 1984; Bowmaker *et al.* 1985). It was on the basis of these matches that S21F and S34 were selected for microspectrophotometric study.

In the case of S21F, 49 microspectrophotometric records met the criteria

specified in methods (see above); in the case of S34, this number was 52. The estimated λ_{max} values for individual cells are plotted as histograms in the lower two panels of figure 2. The single short-wave cone found in animal S34 had a λ_{max} of 430.6 nm. The mean λ_{max} values for the rods were 498.2 nm for S21F and 499.1 nm for S34.

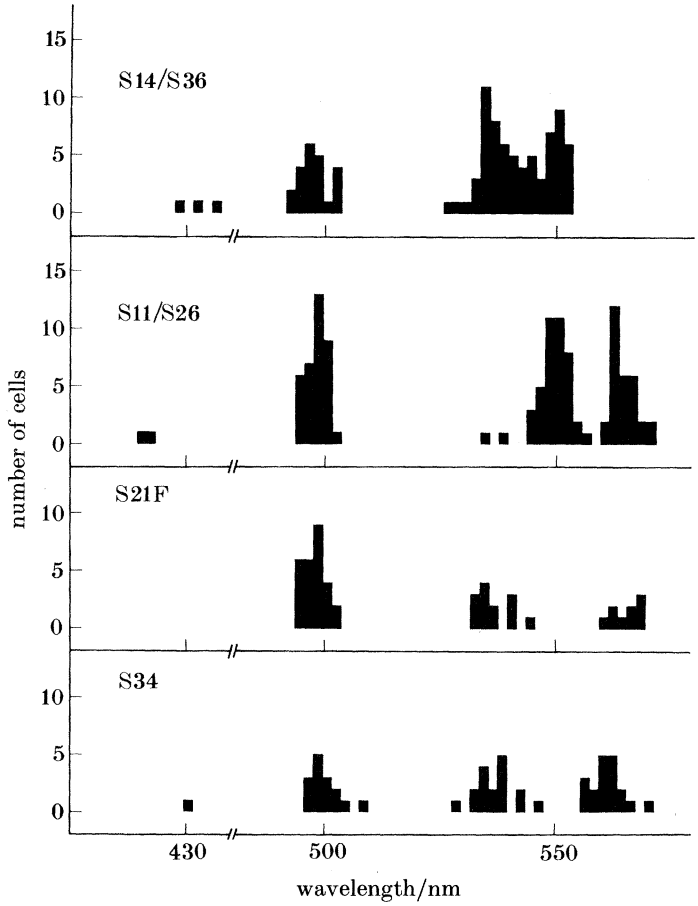

FIGURE 2. Distribution of values of peak sensitivity (λ_{max}) for individual cells from monkeys S21F and S34 (lower panels). Shown for comparison in the upper panels are pooled results for two 'protanomalous' animals (S14, S36) and for two 'deuteranomalous' animals (S11, S26).

In the red–green region of the spectrum, the λ_{max} values for individual cells fall into two well-separated groups. The means for the two groups of cells are 536.3 (3.9) nm and 565.3 (3.3) nm for S21F, and 537.0 (4.4) nm and 561.3 (3.7) nm for S34, where the values shown in parentheses are standard deviations.

For comparison, the upper panels of figure 2 show data from two animals (S14, S36) that we have earlier called 'protanomalous' and from two animals (S11, S26) that we have earlier called 'deuteranomalous'. The data for these four animals are

taken from the study of Bowmaker *et al.* (1985); the experimental procedures and the criteria for inclusion of records were the same as those used in the present study. It was shown in the earlier study that the data for S11, S14, S26 and S36 in the red–green range could be described by a statistical model that assumed only three underlying distributions, two of which were present in each type of monkey. The fit of this model was as good as one in which a 'double normal' distribution was fitted individually to the data for each animal.

Figure 2 suggests that S21F and S34 share a middle-wave cone pigment with S14 and S36, and share a long-wave cone pigment with S11 and S26. The mean values of λ_{max} for the middle- and long-wave cones of S21F correspond closely to the values of 536.2 and 564.0 nm, which were derived from the 'anomalous' monkeys in the statistical model of Bowmaker *et al.* (1985). Similarly, the value for the middle-wave cones of S34 is very close to that of the earlier model; the mean λ_{max} of the long-wave cones of S34 is slightly shorter than that derived in the model.

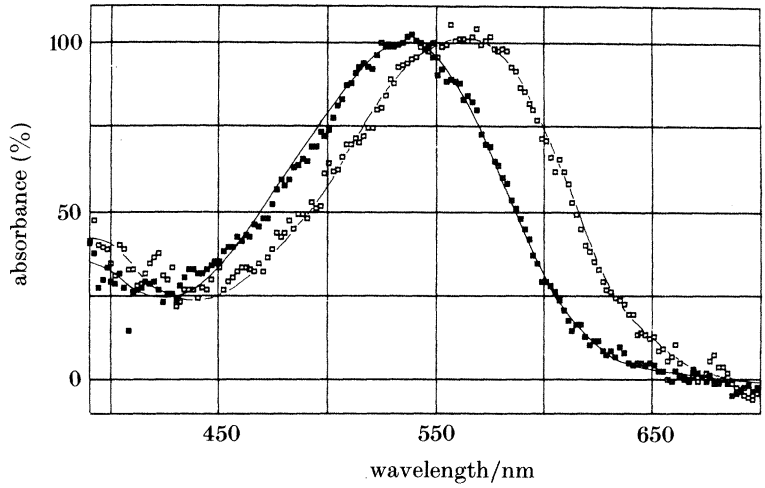

FIGURE 3. Mean absorbance spectra for middle-wave (solid squares) and long-wave (open squares) receptors from monkeys S21F and S34. The solid lines represent the mean absorbance spectra for the middle-wave receptors of 'protanomalous' animals and the long-wave receptors of 'deuteranomalous' animals (see Bowmaker *et al.* 1985, figure 6).

In figure 3 the solid squares show the mean absorbance spectrum for the middle-wave cones from the present animals, S21F and S34. The records from individual cones were averaged before normalization. The open squares show the mean absorbance spectrum for the long-wave cones. The solid lines superimposed on these data are not derived from the present measurements but are taken from our earlier study of 'protanomalous' and 'deuteranomalous' squirrel monkeys (Bowmaker *et al.* 1985, figure 6). They represent two of the three pigments that our statistical model identified in the 'anomalous' phenotypes, the middle-wave curve being derived from the 'protanomalous' animals and the long-wave curve from

the 'deuteranomalous'. The excellent fit of the solid lines to the new data supports our conclusion that the present animals share a middle-wave pigment with the 'protanomalous' phenotype and a long-wave pigment with the 'deuteranomalous' phenotype.

Discussion

It has often been supposed that the apes and the Old World monkeys are the only mammals to share with man a form of trichromatic vision in which there are two well-separated photopigments in the red–green spectral region. The squirrel monkeys described here are remarkable in that their long- and middle-wave photopigments are almost identical to those found in macaques and baboons (Marks *et al.* 1964; Bowmaker *et al.* 1978, 1980, 1983; Hárosi 1982; MacNichol *et al.* 1983) and are not dissimilar from those of man (Dartnall *et al.* 1983). These 'macaque-like' squirrel monkeys add to the three types of dichromatic squirrel monkey and the two types of trichromat previously described.

The trichromacy found in some squirrel monkeys has a genetic basis distinct from that of human trichromacy. The present evidence for a sixth *Saimiri* phenotype supports our one-locus model for the long- and middle-wave pigments of squirrel monkeys. The model postulates three alleles at a single locus on the X-chromosome. Each of the three alleles generates a slightly different version of the opsin of the photopigment molecule. If a female monkey inherits different alleles from her two parents, then she has the capacity to manufacture three different cone pigments (one of them being the short-wave pigment common to all members of the species). The process of X-chromosome inactivation ensures that only one allele is expressed in any given cell and thus gives rise to three classes of cone with distinct spectral sensitivities. The phenotypic evidence for this model would have been incomplete without the evidence, reported here, for female squirrel monkeys that combine the 536 and 564 nm types of photopigment.

The evolution of polymorphous colour vision

Polymorphism of cone pigments may be widespread in New World monkeys: intraspecific variations have been found in two callitrichid species, although the set of photopigments is different from that found in squirrel monkeys (Bowmaker *et al.* 1984; Neitz *et al.* 1985). Do such polymorphisms survive because no especial advantage attaches to any one of the alleles or are they maintained by selective pressures?

We have earlier suggested that the polymorphism of cone pigments in *Saimiri* may be maintained by heterozygous advantage (Mollon *et al.* 1984). To understand how a stable polymorphism could arise, consider first an ancestral, dichromatic population within which there is only one gene for a photopigment in the red–green spectral region. Suppose now a mutant allele enters the gene pool. When it is inherited by a female monkey, the rare allele will almost invariably be paired with the common allele. Females that inherit the rare allele will thus be trichromatic, and thereby will be the more able to detect fruit, cryptic insects, or conspecifics against the dappled background of the forest; and they will be the more able to

discriminate ripe fruit from unripe. And if these discriminative abilities do increase the biological fitness of the heterozygous female, then the frequency of the new gene will rise. But the more common the new allele becomes, the greater the likelihood that a given female will be homozygous for the new allele – and thus be dichromatic. So, other factors being equal, the frequency of the new allele should never rise above 0.5. It is easy to generalize this argument to the case where a third allele enters the population: the new equilibrium should occur when each of the three alleles has a frequency of 0.33. At the level of the whole animal, heterozygous advantage and frequency-dependent selection are alternative explanations of polymorphisms (see, for example, Clarke 1979), but if we consider the individual gene (Dawkins 1982), then a heterozygous advantage will usually imply a frequency-dependent advantage: the rarer an allele the greater its advantage because it is the more likely to be paired with a different allele in the female monkey.

However, there are two noteworthy properties of the heterozygous advantage that we are postulating. First, the different alleles do not have different roles, as they do in the classical example of sickle-cell anaemia (Allison 1964). Rather, the advantage lies simply in inheriting two alleles that are different. Second, X-chromosome inactivation serves to segregate, in different cells, the products of the two alleles. Herein may lie an unrecognized function of X-chromosome inactivation. There may be other physiological systems where the heterozygous female can take advantage of two different alleles only if their products are segregated in different cells. This might be the case, for example, if the alleles specified membrane receptor molecules.

Evolution of human trichromacy

We have suggested how a polymorphism of cone pigments might arise in an essentially dichromatic population. Such a polymorphism might in turn have been an intermediate stage in the evolution of human trichromacy. As a result of unequal crossing over, the polymorphic locus could have been duplicated, so that alleles for the middle- and long-wave pigments were both established on a single X-chromosome. A recent duplication of this kind is strongly suggested by the proximity and the extreme similarity of the human genes for the middle- and long-wave pigments (Nathans *et al.* 1986*a*,*b*); these two genes lie close together on the distal portion of the *q* arm of the X-chromosome and exhibit a 96% mutual identity. But such a duplication will not automatically allow the whole species to become trichromatic. There must be also a mechanism for segregating the gene products into different cone cells. X-chromosome inactivation provides the heterozygous squirrel monkey with a mechanism of segregation; she loses this mechanism if, by duplication, the same two genes become established on each X-chromosome.

This work was supported by National Institutes of Health Grant EY-02052 and Medical Research Council Grant 8206715N. We are grateful to J. Neitz and M. Downham for experimental assistance, M. W. Smith for veterinary supervision and Professor H. B. Barlow for comments on the text.

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