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Inheritance of color vision in a New World monkey (*Saimiri sciureus*)

(photopigments/polymorphism/X chromosome/evolution)

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ABSTRACT **Squirrel monkeys (*Saimiri sciureus*) have a striking color-vision polymorphism; each animal has one of six different types of color vision. These arise from individual variation in the presence of three different middle- to long-wavelength cone pigments. The distribution of cone phenotypes was established for a large sample of squirrel monkeys, including several families, through analysis of a retinal gross potential. The results indicate that the inheritance of color vision in the squirrel monkey can be explained by assuming that the three middle- to long-wavelength cone pigments are specified by three alleles at a single locus on the X chromosome. This arrangement is discretely different from that found in Old World monkeys and humans.**

It has long been known that humans have color-vision polymorphism, but it has only more recently been discovered that some other primate species share this characteristic. An individual squirrel monkey (*Saimiri sciureus*) has one of six distinct types of color vision; there are three dichromatic and three trichromatic variants (1). In squirrel monkeys, studies involving microspectrophotometric (MSP) measurements of single cones established that these color-vision variations arise from individual variations in cone-pigment complement (2, 3). Four classes of cones are found in this species. These have average spectral peak absorption (λ_{max}) of ≈434, 536, 549, and 564 nm. The retinas of all squirrel monkeys are believed to contain 434-nm cones, but there is individual variation in the representation of the other three cone classes. Dichromatic monkeys have any one of the three longer-wavelength cone types, while trichromatic monkeys have any pair.

A characteristic feature of squirrel monkey color vision is its unequal variation in the two sexes. Among 21 female monkeys examined in behavioral tests there were both trichromatic and dichromatic individuals, but of 15 males identically tested all were dichromatic (1, 4). One plausible explanation for this disparity is that the three middle- to long-wavelength cone pigments arise from activity of genes at a single locus on the X chromosome with three alleles corresponding to the three cone pigments (2, 5). If so, males would receive only one of the three middle- to long-wavelength cone types and thus have no better than dichromatic color vision. Females, having two X chromosomes, would have the possibility of inheriting alleles that code for two different middle- to long-wavelength pigments. In such animals, random X chromosome inactivation (6) would produce two populations of middle- to long-wavelength cones providing the basis for trichromatic color vision. We now report results that suggest this mechanism correctly accounts for the inheritance of color vision in the squirrel monkey.

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MATERIALS AND METHODS

The subjects were a heterogeneous collection of adult and young-adult squirrel monkeys. Most were feral animals housed in two colonies. The taxonomy of *Saimiri* remains controversial (7, 8). A large majority of the monkeys tested in this investigation were of the so-called "Roman Arch" and "Gothic Arch" subtypes (9). We found no differences in the distribution of cone types in these two groups.

Cone-pigment complements were determined by analyzing a retinal gross potential, the electroretinogram (ERG), recorded from a corneal contact-lens electrode fitted to an anesthetized monkey. This procedure is described in detail elsewhere (10, 11). The procedure is a variation of a classical psychophysical technique, flicker photometry. In this variation, the radiance of a flickering monochromatic test light is adjusted until the ERG it produces best nulls the (inverted) ERG produced by a flickering reference light. The reference light is of fixed radiance and spectral content; the test and reference lights are presented as an interleaved train of flashes. The use of high-frequency flicker (62 Hz) ensures that neither rods nor the 434-nm cones contribute to the response (10). Establishment of such equations for a range of monochromatic test lights that cover the spectrum thus permits the determination of a spectral sensitivity curve for the middle- to long-wavelength cone(s) present in the retina.

RESULTS

We first distinguished between trichromatic and dichromatic squirrel monkeys by determining whether an ERG photometric equation established for a 540-nm test light and a 630-nm reference light still held when the eye was concurrently adapted to a bright light of either 540-nm or a 630-nm light or whether the equation varied in accord with the chromatic adaptation state of the eye. Fig. 1 illustrates the procedure with results obtained from a trichromatic monkey. Note that the equation made under one chromatic adaptation condition fails to hold under the other condition of adaptation. The conventional interpretation of a result of this type is that there must be two classes of middle- to long-wavelength cone operative in this eye (12). To the contrary, when this test was run on monkeys known from behavioral examination to have dichromatic color vision, the photometric equation was unchanged regardless of the adaptation condition (10).

Results from chromatic adaptation tests of the type just described are summarized in Fig. 2 that shows, for 52 squirrel monkeys, the difference required to complete the photometric equation under the two chromatic adaptation conditions. The animals fall into two groups: those who showed no clear chromatic adaptation effect (Fig. 2 *Top*) and those whose equations under the two adaptation conditions were system-

Abbreviations: MSP, microspectrophotometric; ERG, electroretinogram.

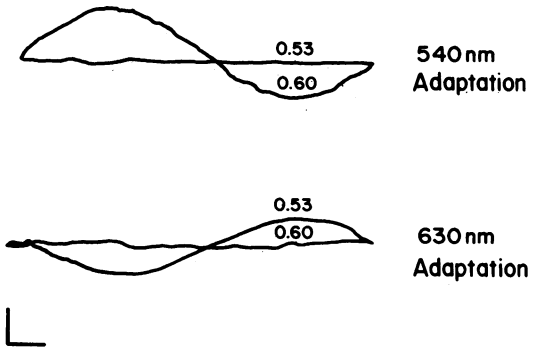

FIG. 1. Effects of chromatic adaptation on the flicker-photometric ERG of the squirrel monkey. Illustrated is the test used to determine if one or two cone types contributed to the ERG. Each trace represents the averaged ERG response produced from a comparison of a 540-nm test light and a 630-nm reference light. The numbers on each trace are settings from a density wedge placed in the beam from the test light. A flat trace results when the test light has been so adjusted in intensity that the ERG it produces is identical to that produced by the reference light; trace deviations of opposite phase occur when the test light is either more or less effective than the reference light (10, 11). (*Upper*) Results for two wedge settings recorded in the presence of intense 540-nm adaptation (adjusted so that it elevated the test-light threshold by 0.5 log unit); (*Lower*) results for the same wedge settings recorded when the eye was adapted to an equally effective 630-nm light. Note that more 540-nm light is required for the equation in the presence of 540-nm adaptation than in the presence of 630-nm adaptation. (Calibration: 1 μV and 10 ms.)

atically different (Fig. 2 *Middle* and *Bottom*). The former must be dichromatic monkeys; the latter are trichromats.

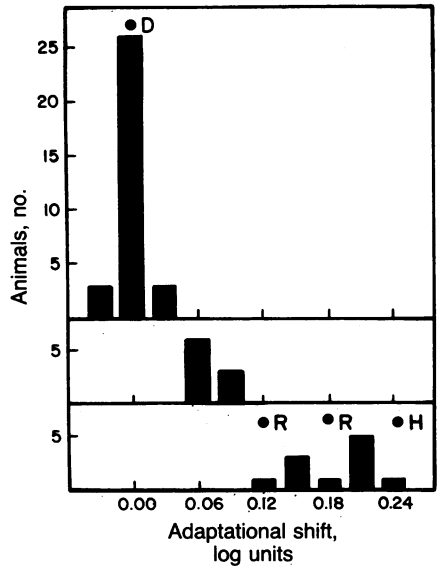

FIG. 2. Magnitude of the chromatic adaptation effect in the squirrel monkey ERG. Each entry in the graph was obtained from an experiment of the type illustrated in Fig. 1. Plotted for each of 52 squirrel monkeys is the difference in wedge density required for the photometric equation of 540-nm and 630-nm lights alternately determined with 540-nm and 630-nm adaptation. Animals that showed no clear chromatic adaptation effect are shown on the *Top*. Animals showing an adaptation effect are in the *Middle* and *Bottom*. This group has been subdivided by the post hoc discovery that animals showing an adaptation effect in excess of 0.10 log unit (*Bottom*) have the 536-nm and 561-nm cones while those who had a smaller adaptation effect (*Middle*) had either the 536/551 or the 551/561 cone pair. Results are also given from identical tests run on a human deuteranope (D), a normal human trichromat (H), and two rhesus monkeys (R).

There are two other sources of validation for this conclusion. First, several of the animals, whose results are shown in Fig. 2 *Top* and were judged dichromatic, had been diagnosed as dichromatic in behavioral tests. As well, two monkeys diagnosed as trichromatic in these same behavioral tests gave significant chromatic adaptation values (Fig. 2 *Bottom*). The two latter animals were also examined by MSP; both were found to have two types of cone in the middle-to-long wavelengths. The results of the adaptation test are, therefore, in accord with both behavioral results and with direct measurements of cone pigments. Second, this test appears to provide a generally valid means for distinguishing between trichromatic and dichromatic individuals. Four other primate subjects were tested in the same way. One was a dichromatic human (a deuteranope), and his flicker-photometric ERG yielded no evidence for a chromatic adaptation effect ("D" in Fig. 2 *Top*). On the other hand, a second human subject, a normal trichromat, did show significant chromatic adaptation ("H" in Fig. 2 *Bottom*) as did two rhesus monkeys (*Macaca mulatta*), a species generally acknowledged (13) to have excellent trichromatic color vision ("R" in Fig. 2 *Bottom*).

Further sorting of dichromatic squirrel monkeys into the three categories indicated by the earlier behavioral investigations was done through examination of spectral sensitivity functions obtained from the flicker-photometric ERG. In this case an achromatic reference light was used. The spectral sensitivity data were corrected and fit to visual-pigment nomograms using described procedures (10, 11). The resulting functions from all dichromatic animals fell into one of three groups. Average spectral sensitivity functions for each of the types are given in Fig. 3. That the variation in spectral peak location among individuals of each of the three types is

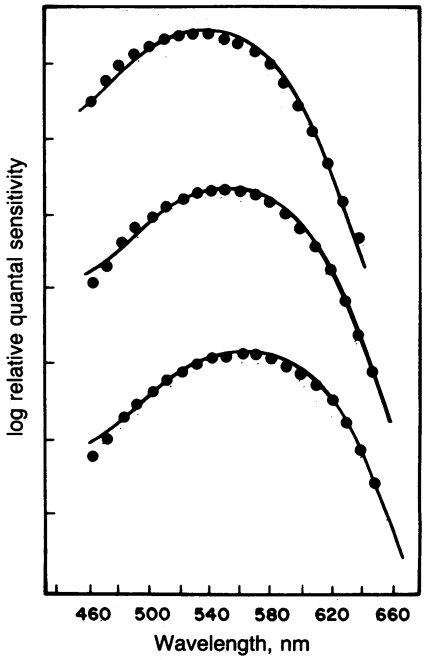

FIG. 3. ERG-flicker photometric spectral sensitivity for dichromatic squirrel monkeys. Shown are the average spectral-sensitivity functions for each of the three forms of squirrel monkey dichromacy. The solid circles are sensitivity values obtained by equating the effectiveness of monochromatic test lights and an achromatic reference light. The lines are curves derived for the best-fitting visual-pigment nomogram (10, 11). The three functions are arbitrarily positioned on the ordinate (step size = 0.5 log unit). Top curve: mean values for 11 monkeys; λ_{max} = 538 nm. Middle curve: mean values for 10 animals; λ_{max} = 551 nm. Bottom curve: mean values for 15 animals; λ_{max} = 561 nm.

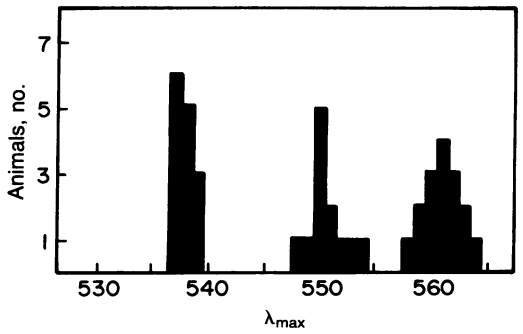

FIG. 4. Frequency distribution of λ_{max} values for a total of 42 dichromatic squirrel monkeys. Each entry was obtained from a spectral-sensitivity function of the type illustrated in Fig. 3. Bin width is 1 nm.

small may be seen in Fig. 4 that provides the distribution of λ_{max} values obtained from 42 dichromatic monkeys. The mean peak locations for individuals in these three groups are 537.8 nm (SD = 0.8), 550.6 nm (SD = 1.59), and 561.1 nm (SD = 1.59). These values are, within the errors of measurement of the respective techniques, indistinguishable from the λ_{max} values obtained from direct MSP measurements (2, 3).

The three varieties of trichromacy in the squirrel monkey arise from the three pairwise combinations of the individual cone mechanisms whose sensitivity curves are shown in Fig. 3. Those animals showing significant chromatic adaptation effects (Fig. 2 *Middle* and *Bottom*) must each represent one of these three classes. To distinguish among them, we examined flicker-photometric spectral sensitivity assuming that the sensitivity function for each animal reflects a pairwise combination of the outputs from two cone types. To determine which two cone classes were present in any individual, each spectral-sensitivity function was fitted with each of the three possible pairwise combinations of cone types. On the grounds that the spectral-sensitivity functions given in Fig. 3 should provide the most accurate ERG reflection of the individual cone classes, these three averaged spectral-sensitivity functions were employed as fundamentals in the fitting procedure. A computer was used to test each of the derived trichromatic spectral-sensitivity functions to determine which linear sum of two cone types best fit the derived function. The three pairs were tested in all possible proportions in steps of 1%, and the pair providing the best fit was taken as specifying the type of trichromacy present in any animal.

Examples of spectral-sensitivity functions obtained from each of the three types of trichromatic monkey are shown in Fig. 5. This sorting procedure appears adequate for two reasons: (*i*) Four trichromatic monkeys examined in this way had had their color vision established in behavioral tests; the psychophysical and electrophysiological diagnosis of phenotype always matched. (*ii*) Two trichromatic animals examined in this way were later subjects in MSP experiments; again, there was concordance of results from the two procedures.

Table 1 shows the distribution of the six squirrel monkey color-vision phenotypes among the animals tested. Included are all of the animals that could be unambiguously categorized using the two ERG procedures described above. To these are added the results from 14 other squirrel monkeys whose phenotypes were earlier determined by behavioral measurements and MSP examination (1–4). There are thus 76 squirrel monkeys (31 male and 45 female) whose cone phenotypes were securely established. Numerous representatives of each of the six types were found in this sample. In the sample of dichromatic monkeys the three subtypes were not differentially represented [$\chi^2 = 0.37$; degrees of freedom

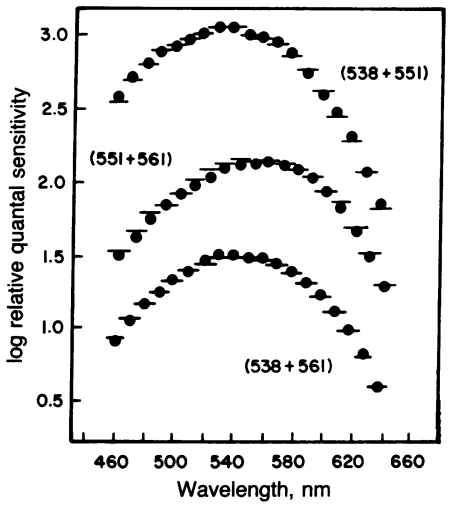

FIG. 5. Flicker-photometric spectral-sensitivity functions obtained from three squirrel monkeys, each a representative of one of the three varieties of trichromacy found in this species. The solid circles are sensitivity values obtained by equating the effectiveness of monochromatic test lights and an achromatic reference light. The horizontal dashed lines are the best fit values obtained from a linear summation of two cone sensitivity curves. The cone combinations illustrated here are 538 nm and 551 nm (top curve), 551 nm and 561 nm (middle curve), and 538 nm and 561 nm (bottom curve).

(*df*) = 2; not significant (ns)]. The hypothesis that the three trichromatic types are equally frequent also cannot be rejected ($\chi^2 = 1.55$; *df* = 2; ns). The most parsimonious interpretation of the results for both trichromats and dichromats is that the three pigment alleles are approximately equally frequent in this population of squirrel monkeys.

The frequency of dichromacy and trichromacy found in male and female monkeys is given in Table 2. Two monkeys whose vision could be classified as trichromatic, but whose subtype could not be confidently determined, have here been added to the sample; thus the total number of animals classified is 78. Of the 31 males examined, all were dichromatic as would be required by the single-locus model. Among 47 females, 18 (38%) were dichromats. This too is very close to what would be predicted (33%) on the basis of the single-locus model assuming, as suggested by the results from Table 1, that the three pigment alleles occur at equal frequency.

To determine cone-pigment pedigrees, 25 squirrel monkeys from nine different families were tested. The pigment complements of the members of these families as identified by the ERG-flicker-photometry tests described above are summarized in Fig. 6. Each of the six color-vision phenotypes found in this species is represented in this sample of squirrel monkeys. An examination of Fig. 6 indicates that in each individual case the pigment complement of the offspring is appropriate based on the assumption that the inheritance of cone pigments in the squirrel monkey arises as the result of

Table 1. Distribution of the six squirrel monkey color vision phenotypes

Phenotype	Animals, no.
Dichromatic	
538 nm	18
551 nm	14
561 nm	17
Trichromatic	
538/551 nm	6
551/561 nm	11
538/561 nm	10

Table 2. Frequencies of trichromatic and dichromatic squirrel monkeys

Sex	Animals, no.	
	Dichromatic	Trichromatic
Female	18 (16)	29 (31)
Male	31 (31)	0 (0)

The numbers in parentheses are the frequencies predicted from the single-locus model.

activity at a single X-chromosome locus having three alleles. Note for instance that all male offspring do inherit a photopigment from the mother's complement and that male–male transmission is excluded in all cases where the father's pigment is known. Consider also family 3. According to the single-locus model, a dichromatic daughter would have to inherit the same pigment allele from each parent. The two parents share in common the 538-nm pigment, and the daughter of family 3 does express only the 538-nm cone as the model requires.

DISCUSSION

Results from these experiments strongly support a single-locus model for the inheritance of color vision in the squirrel monkey. The mechanism for the genetic transmission of cone pigments and color vision in the squirrel monkey appears, thus, fundamentally different from that of the human. Although there has been much disagreement on the matter (14), there is now direct evidence that the opsins necessary for the production of the middle- to long-wavelength cone pigments of humans arise from the activity of genes at two (or more) loci on the X chromosome (15). Each locus is conceived to have multiple alleles, and these allelic forms account for the principal variations of human color vision. Polymorphism led to the current understanding of the inheritance of human color vision, just as it now has for the inheritance of squirrel monkey color vision. The squirrel monkey cone-pigment genome is fundamentally different from that of the human in two ways: (*i*) The alleles responsible for the production of different cone pigments are approximately equally frequent in the squirrel monkey, whereas among humans the same allele at each X-chromosome locus is found in the vast majority of individuals with other alleles present at only low frequency in the population. (*ii*) The squirrel monkey has only a single photopigment locus on the X chromosome as compared to two or more in the case of the human.

Although the results are more sparse, there is evidence that several other species of platyrrhine monkey also show a color-vision polymorphism. Two other species of Cebid monkey, *Cebus apella* and *Callicebus moloch* (16), and two species of Callitrichid monkey, *Callithrix jacchus* (17) and *Saguinus fuscicollis* (18), have a color-vision polymorphism. Although the photopigments are not the same in all of these species, in each case it appears all males are dichromatic, just as in the squirrel monkey. This suggests that the single-locus model proposed for the squirrel monkey is apt also to account for the inheritance of color vision in these other South American monkeys. Whether all platyrrhine primates follow this pattern is not known.

The discoveries about squirrel monkey color vision, and the extension of this work to other South American monkeys, contain implications for understanding the evolution of primate color vision. To date, studies of color vision have been completed on only a small fraction of all primate species, and, with the exception of the squirrel monkey, only a few representatives from each species have been tested. Subject to this qualification, the generalization usually offered is that Old World monkeys have trichromatic color vision greatly similar to that of normal humans (13). There is no evidence to indicate the presence of a color-vision polymorphism in any of the Old World monkey species, certainly not at the high frequency seen in the squirrel monkey and perhaps not even at the lower frequency characteristic of the human.

If trichromacy is universal in Old World monkeys it seems reasonable to conclude that those species, like man, have at least two photopigment loci on the X chromosome. That fundamental difference from the squirrel monkey (and perhaps other platyrrhine species) indicates that platyrrhine and catarrhine monkeys are at different stages in the evolution of a color vision capacity. One can speculate that the situation in the squirrel monkey is a contemporary representation of a stage through which those primates that have acquired widespread trichromatic color vision have evolved. One scenario for this process would include an ancestral stage in which a photopigment locus had been established on the X chromosome. In conjunction with the presence of a short-wavelength pigment, presumably produced by the action of an autosome as it is in man (15), and the establishment of chromatic-opponent interactions in the nervous system, this would allow dichromatic color vision. To then gain trichromatic color vision would require only that an allele for an additional photopigment type be established at the X-chromosome locus. In conjunction with random X-chromosome inactivation this would allow heterozygous females the op-

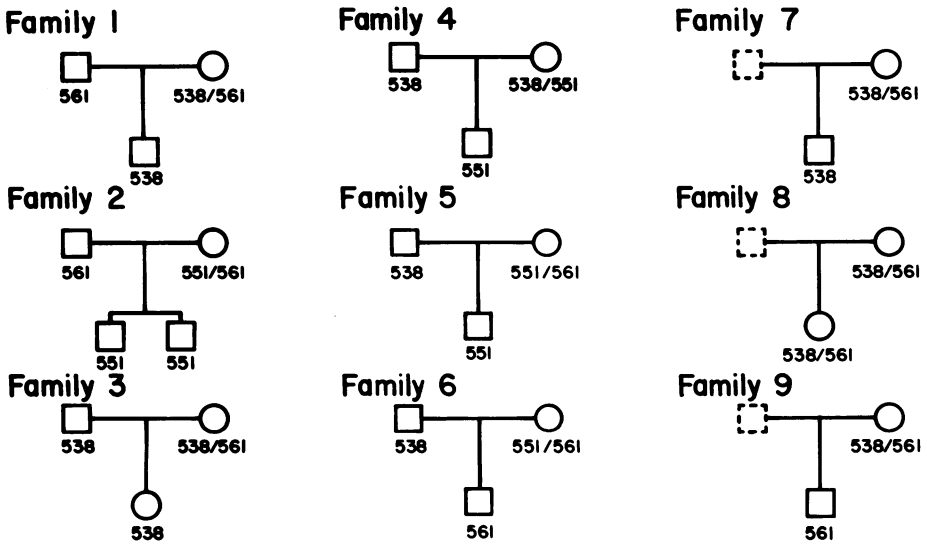

FIG. 6. Cone-pigment pedigrees for nine families of squirrel monkey. The cone complement of each individual was determined through the use of ERG-flicker photometry. The identity of the male parent in families 7, 8, and 9 is not known.

portunity to be trichromatic. An increase in the number of alleles at this locus, and then bringing these alleles to equal frequency in the population, would increase the incidence of female trichromacy. That very situation has apparently been achieved for three alleles in the squirrel monkey. A next step would involve gene duplication (19) so that the alleles might be incorporated into the genome. This would make trichromacy a species characteristic, presumably similar to what Old World monkeys and man have achieved. This scheme would suggest that photopigment gene duplication occurred sometime after the platyrrhine/catarrhine divergence; that is, more recently than 35–40 million years ago (20). The fact that Old World monkeys appear to show no color-vision polymorphism, while humans do, may reflect differences in the selection pressures against color-vision variations in the two lines.

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