Trichromatic colour vision in New World monkeys

Gerald H. Jacobs*, Maureen Neitz†, Jess F. Deegan‡ & Jay Neitz†

* Neuroscience Research Institute and Department of Psychology, University of California, Santa Barbara, California 93106, USA
† Departments of Ophthalmology and Cellular Biology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA
‡ Department of Psychology, California State University, Bakersfield, California 93311, USA

TRICHROMATIC colour vision depends on the presence of three types of cone photopigment. Trichromacy is the norm for all Old World monkeys, apes and humans, but in several genera of New World monkeys, colour vision is strikingly polymorphic[1]. The difference in colour vision between these New and Old World primates results from differing arrangements of the pigment genes on the X chromosome. In Old World primates the three photopigments required for routine trichromatic colour vision are encoded by two or more X-chromosome pigment genes and an autosomal pigment gene. New World monkeys typically have only one X-chromosome pigment gene; multiple alleles allow different types of dichromatic colour vision and, in females heterozygous at this locus, variant forms of trichromatic colour vision. Here we report that multiple X-chromosome pigment genes and trichromatic colour vision are the norm for one genus of platyrrhine monkey, the howler monkey, *Alouatta*.

As a part of a survey of cone photopigments in New World monkeys we used a non-invasive electrophysiological index, the electroretinogram (ERG), to determine the number of types of cone photopigment and their spectral properties in howler monkeys (*Alouatta*). We first measured spectral sensitivity functions under test conditions designed to isolate activity from middle- and long-wavelength cones. The results for two females, one black (*A. caraya*) and one red (*A. seniculus*) howler monkey, and for one black male howler monkey, are shown at the top of Fig. 1. We found that the spectral sensitivity functions are too broad to reflect the sensitivity of a single cone, and that there are no obvious differences between the three monkeys. Spectral sensitivity measured in this way reflects the combined signals from middle- and long-wavelength cone pigments[2], and was essentially identical for these female and male howler monkeys.

An explicit test to determine whether these monkeys had more than one type of X-chromosome-encoded photopigment involved a search for differential effects of chromatic adaptation. Green and red monochromatic lights (540 and 630 nm) flickering in alternation were adjusted in relative intensity until they produced ERGs that were equal in amplitude (a flicker-photometric equation.) These equations were made in the presence of two separate adaptation lights, at 540 and 630 nm. If the retina contains more than a single type of photopigment sensitive to lights from this portion of the spectrum, the equations determined under the two different adaptations should differ, but if there is only a single photopigment the equation value will not change for the two adaptation conditions. Our data (Fig. 1 inset) demonstrates the differences in the equations determined under the two adaptation conditions for samples of dichromatic and trichromatic human subjects, all tested in the same way as the howler monkeys. Note that dichromatic subjects have only a single photopigment sensitive to lights drawn from this part of the spectrum, and show no significant change in the equation values obtained for the two adaptation conditions. Conversely, the human trichromats have two X-chromosome-encoded photopigments, and have significant differences in their equations. The results for the three howler monkeys were very similar to one another, and to the results of the human trichromats.

These measurements of cone sensitivity in howler monkeys contrast sharply with previous findings on New World monkeys. Among animals drawn from species representing nine genera, no single male monkey (from a sample >100 individuals) has previously been found to have more that one X-chromosome encoded photopigment[2–6]. This group includes at least one species from each of the six subfamilies of *Platyrrhini*[7]. However, our results strongly imply that howler monkeys have routine trichromatic colour vision (Fig. 1), probably much like that of the catarrhine primates. To investigate the genetic basis of their trichromacy, we examined the X-chromosome photopigment genes of both red and black howler monkeys.

The spectral tuning of primate photopigments is determined by a small number of amino-acid substitutions in the transmembrane portion of the pigment molecule[8–12]. Changes in the nucleotide sequence in exon 5 of the X-chromosome pigment genes are the main reason for the substantial spectral separation of the middle- and long-wavelength pigments underlying trichromacy in many

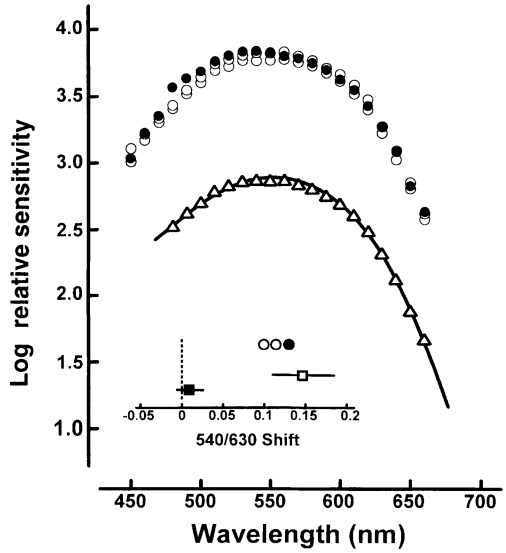

FIG. 1 ERG evaluations of the X-chromosome-linked photopigments in howler monkeys. Top, spectral sensitivity values obtained for three howler monkeys (females, open circles; male, filled circles). Middle, triangles represent average spectral sensitivity data for the three monkeys. The continuous line is the best-fitting summative combination of absorption curves for the two putative cone photopigments of the howler monkey; these two pigments have peak values of 562 and 530 nm. The relative proportions of the two curves were varied in steps of 1% to determine the best fit. The best fit was obtained for a 562/530 weighting of 2.22. To minimize the effect of absorption by potential pre-retinal filters, the sensitivity values for test wavelengths shorter than 480 nm were ignored. Inset, assessment of the number of X-chromosome-linked photopigments, showing the changes in equations for two test lights (540 and 630 nm) determined when the eye was alternately adapted to 540 and 630 nm lights; the scaling is logarithmic. Absence of a chromatic adaptation effect results in no shift in the equation (vertical broken line). Results are shown for three groups of subjects: howler monkeys (circles), normal human trichomats (open square) and human dichromats (solid square). For the human subjects, the plots are mean values (±1 s.d.) for 14 normal trichromats and 27 dichromats (11 protanopes, 16 deuteranopes).
METHODS. ERGs were recorded from sedated monkeys using contact-lens electrodes, and fibre electrodes for the human subjects. Stimuli flickering at 31.25 Hz were presented in maxwellian view. A flicker-photometric procedure was used in which the intensity of a flickering monochromatic light was adjusted in intensity until it produced an electrical response equivalent to that produced by the presentation of a flickering achromatic reference light[25,26]. The two stimuli were interleaved in a temporal train. In the chromatic-adaptation experiment, the 540- and 630-nm adaptation lights were initially adjusted in intensity so that each produced a 0.5 log elevation in threshold for a 540-nm test light flickering at 31.25 Hz.

Old World primates. Polymerase chain reaction (PCR) amplification of exon 5 of the X-linked pigment genes from individuals having both long- and middle-wavelength pigment genes results in the formation of homoduplexes as well as heteroduplexes[13–15]. In individuals with only one type of pigment gene, only homoduplexes are formed. Heteroduplex analysis of amplified exon 5 of a male howler monkey (*A. seniculus*) was performed to see whether two different exon 5 sequences are present (Fig. 2*a*). Three bands are seen for the howler monkey: two heteroduplex bands and a homoduplex band. The presence of heteroduplexes indicates there are at least two different exon 5 sequences, and thus two different X-chromosome pigment genes. By comparison, a human dichromat has only a single exon 5 sequence and no heteroduplexes (Fig. 2*a*). Sequence ladders for exon 5 from two female and six male howler monkeys are shown in Fig. 2*b*. The sequences for all of the monkeys are very similar, but not identical: one female and one male have five sites with variations, whereas the remaining five males have four such sites. These locations of variation for all of these howler monkeys include the two locations that are correlated with the presence of two well-separated middle-to-long-wavelength cone pigments[8,10,11]. To confirm that the combinations of nucleotides are also the same as in the Old World primates, exon 5 of the X-linked pigment genes amplified from genomic DNA from two male howler monkeys (males 3 and 4) was cloned into the plasmid vector pCR2.1 (Invitrogen), and individual clones were sequenced. The sequences fell into two classes with regard to the four locations of variation shown in Fig. 2*b*. In one class of clones, the nucleotides at the four positions are: adenine at position 1,319; guanine at 1,322; thymine at 1,324; and guanine at 1,347. The other class of clones had guanine at position 1,319; thymine at 1,322; adenine at 1,324; and adenine at 1,347. The two classes of clones thus encode the same amino-acid combinations found in the human middle- and long-wavelength cone pigments. For male 3, three of five independent clones corresponded to the middle-wavelength pigment gene sequence, and two corresponded to the long-wavelength pigment gene sequence. For male 4, three of four independent clones correspond to long-wavelength pigment sequence, the other to middle-wavelength pigment gene sequence. The trichromatic colour vision of the howler monkey

TABLE 1 Nucleotide variations in exon 5 sequences for X-chromosome pigment genes

Nucleotide position	Howler monkeys	Old World monkeys	Other New World monkeys	Amino-acid substitution? (position)
1,295	G and A	G	G or A	No
1,314	A	T and A	A	No
1,317	A	C and T	G, C, A or T	Yes (275)
1,319	G and A	G and C	G	No
1,322	T and G	A and G	A or G	No
1,324	T and A	T and A	A or T	Yes (277)
1,329	G	T and G	A or G	Yes (279)
1,343	C	T and C	C	No
1,347	G and A	G and A	G or A	Yes (285)
1,410	C	T	C	Yes (302)

Nucleotide positions are shown where there were variations between howler monkeys, between the two pigment genes of two species of Old World monkey (*Cercopithecus diana* and *C. talapoin*)[22], or between howler monkey and Old World monkeys. Exon 5 sequences were examined for the following New World monkeys: squirrel monkey (*Saimiri sciureus*)[8], saddle-backed tamarin (*Saguinous fuscicollis*)[8], common marmoset (*Callithrix jacchus*)[23], owl monkey (*Aotus trivirgatus*)[24], and spider monkey (*Ateles fusciceps*)[24].

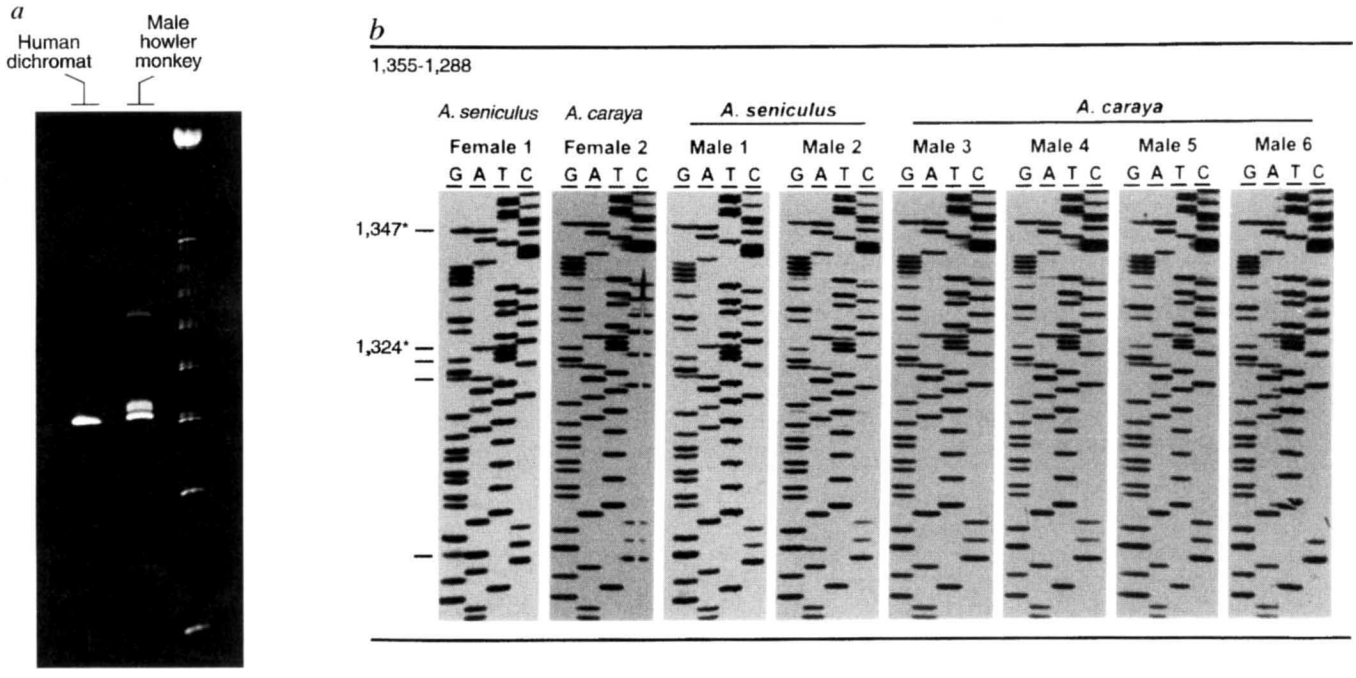

FIG. 2 *a*, Heteroduplex analysis of exon 5 of the X-linked pigment genes. Homoduplexes each contain both strands form a long-wavelength pigment gene, or both strands from a middle-wavelength pigment gene. The mismatches in the heteroduplexes alter their mobility relative to the homoduplexes. *b*, Sequencing autoradiograms spanning exon 5 nucleotides 1,355–1,288 for two female and six male howler monkeys. The numbering system is as ref. 17. Locations of variation are indicated by horizontal lines to the left; the two regions marked with asterisks specify amino-acid differences associated with the presence of two types of cone pigments that are spectrally well separated in the middle to long wavelengths.

METHODS. DNA was isolated from blood and used in the PCR to amplify exon 5. The primers and PCR conditions are described elsewhere[13]. In *a*, amplified DNA was run on a 5% neutral polyacrylamide gel. The nucleotide sequences of PCR products were determined by cycle sequencing (Perkin Elmer).

implied by the electrophysiological results has a genetic basis like that of catarrhine primates. In these catarrhines, trichromacy is based on middle- and long-wavelength pigments with spectral peaks of about 530 and 562 nm (ref. 16). Predictions of sensitivity based on those pigments accounts well for the spectral sensitivity of the howler monkey (Fig. 1).

Trichromatic colour vision occurs in several other groups of animals, but primates are the only mammals to be trichromatic[1]. The high sequence homologies of the X-chromosome pigment genes indicate that a gene duplication is required to convert the typical mammalian pattern (only a single X-chromosome pigment gene) to the multiple pigment genes of the Old World primates[17]. The discovery of widespread polymorphic colour vision among New World monkeys suggests that the evolution of routine trichromatic colour vision might involve an intermediate stage in which routine dichromacy is converted to polymorphic colour vision[18]. Alternatively, the colour-vision polymorphism of platyrrhine monkeys might be considered a degenerate form of catarrhine trichromacy[19]. Regarding howler monkeys, the difference between these two explanations depends on whether the trichromacy of howler monkeys arose from a polymorphic past, or whether it reflects the maintenance of an ancestral trichromacy. Two arguments support the former possibility. First, it is the more parsominious way to explain the present picture: the phylogenetic relationships[7] of the New World monkeys indicate that an ancestral trichromacy must have been independently lost in at least three lineages, whereas the evolution of trichromacy from a polymorphic base would have required change in only one line. Second, consider the 10 nucleotide positions in exon 5 that vary within two species of Old World monkey, within howler monkeys, or between Old World monkeys and howler monkeys. For each, the nucleotides and variants are given for five other species of New World monkeys in Table 1. For all comparisons there is no single case in which the sequence of the howler monkey is like the sequence of Old World monkeys but unlike that of other New World monkeys. For instance, there are positions (1,314 and 1,343) that vary in Old World monkeys but not in any of the New World monkeys; there is a location where all New World monkeys vary (1,295) but the Old World monkeys do not; and at a location where none of the species show variation (1,410), the howler monkey sequence is like that of other New World monkeys but unlike that of the Old World monkeys. Both arguments thus suggest that howler monkeys achieved their trichromacy as a result of evolutionary changes that occurred subsequent to the platyrrhine radiation, and that the capacity is constructed from a polymorphic past. Note that the only positions where all Old World and New World species show variation (1,324 and 1,347) are those yielding the amino-acid changes that are responsible for much of the difference in spectral positioning of middle- and long-wavelength cone pigments[8]. It has been suggested that the similarities at these locations across species represent convergent evolution[20].

It is not yet clear why howler monkeys are routinely trichromatic but other New World monkeys are not. The balanced polymorphic colour vision of the latter species means that groups of these monkeys include dichromatic and trichromatic individuals. It has been suggested that this arrangement is maintained because it provides a net group advantage in the detection of resources[3]. If that is the case, a detailed comparison of the visual ecology of the howler monkey with that of some of these other New World monkeys could prove informative. Alternatively, there might be a more mechanical explanation: perhaps the opsin gene duplication required for routine trichromacy is a low-probability event, achieved during the evolution of howler monkeys, but not as yet in other platyrrhine lines. In this case the constraint of a single X-chromosome pigment gene restricts a superior capacity for colour vision, trichromacy, to females. Female trichromats have higher fitness than dichromats, which in turn leads to stability in the polymorphism through heterozygous advantage[21]. □

Received 15 January; accepted 21 May 1996.

1. Jacobs, G. H. *Biol. Rev.* **68,** 413–471 (1993).
2. Jacobs, G. H. & Neitz, J. *Proc. natn. Acad. Sci. U.S.A.* **84,** 2545–2549 (1987).
3. Tovee, M. J., Bowmaker, J. K. & Mollon, J. D. *Vision Res.* **32,** 867–878 (1992).
4. Jacobs, G. H., Neitz, J. & Neitz, M. *Vision Res.* **33,** 269–274 (1993).
5. Jacobs, G. H. & Deegan, J. F. *Invest. Ophthalmol. Vis. Sci.* **34,** 749 (1993).
6. Jacobs, G. H. & Deegan, J. F. *Am. J. Primatol.* **33,** 217 (1994).
7. Schneider, H. *et al. Molec. Phylogenet. Evol.* **2,** 225–242 (1993).
8. Neitz, M., Neitz, J. & Jacobs, G. H. *Science* **252,** 971–974 (1991).
9. Ibbotson, R. E., Hunt, D. M., Bowmaker, J. K. & Mollon, J. D. *Proc. R. Soc. Lond.* B **247,** 145–154 (1992).
10. Chan, T., Lee, M. & Sakmar, T. P. *J. biol. Chem.* **267,** 9478–9480 (1992).
11. Merbs, S. L. & Nathans, J. *Photochem. Photobiol.* **56,** 869–881 (1992).
12. Williams, A. J., Hunt, D. M., Bowmaker, J. K. & Mollon, J. D. *EMBO J.* **11,** 2039–2045 (1992).
13. Neitz, M. & Neitz, J. *Science* **267,** 1013–1016 (1995).
14. Cotton, R. G. H. *Curr. Opin. Biotechnol.* **3,** 1013–1016 (1995).
15. Nagamine, C. M., Chan, K. & Lau, Y.-F. C. *Am. J. hum. Genet.* **45,** 337–339 (1989).
16. Bowmaker, J. K. in *From Pigments to Peception* (eds Valberg, A. & Lee, B. B.) 1–9 (Plenum, New York, 1991).
17. Nathans, J., Thomas, D. & Hogness, D. S. *Science* **232,** 193–202 (1986).
18. Bowmaker, J. K., Jacobs, G. H. & Mollon, J. D. *Proc. R. Soc. Lond.* B **231,** 383–390 (1987).
19. Mollon, J. D. in *Evolution of the Eye and Visual System* (eds Cronly-Dillon, J. R. & Gregory, R. L.) 306–319 (CRC Press, Boca Raton, FL, 1991).
20. Shyue, S.-K. *et al. Science* **269,** 1265–1267 (1994).
21. Mollon, J. D., Bowmaker, J. K. & Jacobs, G. H. *Proc. R. Soc. Lond.* B **222,** 373–399 (1984).
22. Dulai, K. S., Bowmaker, J. K., Mollon, J. D. & Hunt, D. M. *Vision Res.* **34,** 2483–2491 (1994).
23. Hunt, D. M., Williams, A. J., Bowmaker, J. K. & Mollon, J. D. *Vision Res.* **33,** 147–154 (1993).
24. Hagstrom, S. A. *et al. Invest. Ophthalmol. Vis. Sci.* **34,** 809 (1993).
25. Neitz, J. & Jacobs, G. H. *J. opt. Soc. Am.* A **1,** 1175–1180 (1984).
26. Jacobs, G. H., Neitz, J. & Krogh, K. *J. opt. Soc. Am.* A **13,** 641–648 (1996).

ACKNOWLEDGEMENTS. We thank C. Sweet, S. Evans, R. W. Cooper, T. Pope and S. Nikota for cooperation and assistance; C. Lauer for technical assistance; and J. A. Endler for comments. This work was supported by grants from the NSF, the NIH, and Research to Prevent Blindness.

CORRESPONDENCE and requests for materials should be addressed to G.H.J. (e-mail: jacobs@psych.ucsb.edu).

A PET study of the neural systems of stuttering

Peter T. Fox*, Roger J. Ingham†, Janis C. Ingham†, Traci B. Hirsch*, J. Hunter Downs*, Charles Martin*, Paul Jerabek*, Thomas Glass* & Jack L. Lancaster*

* Research Imaging Center and Department of Radiology, University of Texas Health Science Center at San Antonio, Texas 78284, USA
† Department of Speech and Hearing Sciences, University of California, Santa Barbara, California 93106, USA

THE cause of stuttering is unknown[1]. Failure to develop left-hemispheric dominance for speech is a long-standing theory[1] although others implicate the motor system more broadly[2], often postulating hyperactivity of the right (language non-dominant) cerebral hemisphere[3]. As knowledge of motor circuitry has advanced[4], theories of stuttering have become more anatomically specific, postulating hyperactivity of premotor cortex, either directly[5] or through connectivity with the thalamus and basal ganglia[6]. Alternative theories target the auditory[7] and speech production[8,9] systems. By contrasting stuttering with fluent speech using positron emission tomography combined with chorus reading to induce fluency, we found support for each of these hypotheses. Stuttering induced widespread overactivations of the motor system in both cerebrum and cerebellum, with right cerebral dominance. Stuttered reading lacked left-lateralized activations of the auditory system, which are thought to support the self-monitoring of speech, and selectively deactivated a frontal–temporal system implicated in speech production. Induced fluency decreased or eliminated the overactivity in most motor areas, and largely reversed the auditory-system underactivations and the deactivation of the speech production system. Thus stuttering is a disorder affecting the multiple neural systems used for speaking.

Stuttering can often be transiently alleviated by a variety of interventions, collectively termed fluency inductions[10]. These are