founder population should no longer be attractive to the ancestral males as they have lost the primary (unsaturated) pheromone components of the ancestral species. The derived males could still be attracted to the pheromone of the ancestral females, but the differential number of chromosomes could have contributed to reduced fitness of hybrids and as a consequence, the evolution of the asymmetrical reproductive isolation into complete premating reproductive isolation.

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Polymorphism of the long-wavelength cone in normal human colour vision

Jay Neitz & Gerald H. Jacobs

Department of Psychology, University of California, Santa Barbara, California 93106, USA

Colour vision is based on the presence of multiple classes of cone each of which contains a different type of photopigment[1]. Colour matching tests have long revealed that the normal human has three cone types. Results from these tests have also been used to provide estimates of cone spectral sensitivities[2]. There are significant variations in colour matches made by individuals whose colour vision is classified as normal[3–6]. Some of this is due to individual differences in preretinal absorption and photopigment density, but some is also believed to arise because there is variation in the spectral positioning of the cone pigments among those who have normal colour vision. We have used a sensitive colour matching test to examine the magnitude and nature of this individual variation and here report evidence for the existence of two different long-wavelength cone mechanisms in normal humans. The different patterns of colour matches made by male and female subjects indicate these two mechanisms are inherited as an X-chromosome linked trait.

To determine colour matches we used an adaptation of a standard diagnostic test for colour vision, the Rayleigh match[7]. In such matches the subject is required to adjust the proportions of red and green lights until their mixture appears identical to that of a standard yellow light and the mixture and comparison fields usually appear as two halves of a small spot in the central part of the visual field. In this experiment the mixture and comparison lights were presented in Maxwellian view as a large, bright, annular field and were alternated cyclically. Both the large field and the temporal alternation of mixture and comparison lights substantially improve colour discrimination over that obtained in the conventional arrangement[8]. To determine colour matches, the experimenter presented some mixture of red and green light and the subject attempted to match the appearance of the mixture and comparison lights by adjusting the brightness of the comparison light. After adjusting the brightness to satisfaction, the subject was asked to say whether the mixture appeared identical to the comparison light or whether it was redder or greener. This procedure was repeated for a range of red/green mixtures until it was determined which mixtures appeared just reliably redder or greener[7]. The interval between these two points defines the matching range; the centre of this range was taken as the match midpoint.

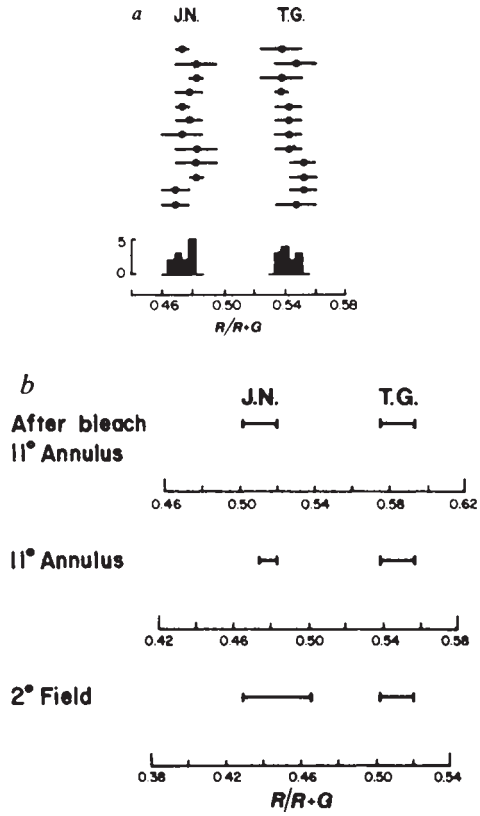

Fig. 1 Rayleigh matches of two male subjects (J.N. and T.G.) with normal colour vision. The stimuli were produced from a three-channel optical system; they appeared in maxwellian view through an exit pupil having a diameter of 0.7 mm. The mixture lights were 546 nm and 690 nm and the comparison light was 600 nm (each light had a half bandwidth of 10 nm). Each light derived from a separate channel and thus, once the beams were combined, each could be brought to an identical focal point. The mixture and comparison lights were alternated cyclically such that the comparison light appeared for 500 ms, followed by a 100 ms period with no stimulus and then the mixture appeared for 1.9 s. The mixture and comparison lights each produced a retinal illuminance of about 1,000 trolands. Overhead fluorescent lights in the laboratory yielded an additional retinal illuminance of about 500 trolands. *a*, Large field Rayleigh matches of J.N. (left) and T.G. (right) obtained over a period of 12 weeks. The stimuli appeared as an annulus with an outer diameter of 11° and an inner diameter of 3°. Filled circles show the match midpoints; bars indicate the matching ranges. The histograms at the bottom are the distributions of match midpoints. *b*, Rayleigh matches obtained under three stimulus conditions. Bars show the matching ranges of J.N. and T.G. obtained: after a full bleach (top), under the conditions for Fig. 1*a* (middle), and with a 2° foveally-viewed field (bottom).

Table 1 The distribution of Rayleigh matches made by females compared with the distribution expected from matches made by males

Requires:	Expected frequency	Observed frequency
Less red	28	28
Intermediate	50	52
More red	22	20

The prediction is based on the assumption that in individuals with normal colour vision the gene(s) located on the X-chromosome that specify the long wavelength mechanism has (have) two identities. If the frequencies of the two groups of males are a and b, then the predicted frequencies of the three groups of females are given by a^2, $2ab$ and b^2. To obtain the observed frequencies, the distribution of female matches in Fig. 1 was divided into three groups at the dips that occur at 0.485 and 0.52.

Application of this matching procedure quickly revealed the presence of individuals who made matches well within the range defined for normal colour vision, but reliably different one from another. That is illustrated in Fig. 1*a* which shows the match midpoints and matching ranges obtained from each of two male subjects who were repeatedly tested over a period of 12 weeks. The matching ranges for these two subjects are small, but they are similar to those obtained from other subjects tested in this experiment. It can be seen that although the differences in the colour matches made by these two subjects are not large, they are consistently different. That fact is indicated in summary form by the histogram of match midpoints at the bottom of Fig. 1*a*, which also provides an indication of the small error of measurement in this test situation.

In order to conclude that the differences between the two subjects of Fig. 1*a* reflect variation in the spectral positioning of their cone pigments, three alternative explanations must be ruled out. (1) The difference is not due to differences in ocular transmissivity. This possibility seemed unlikely because over the wavelengths examined there is little differential absorption by pigments of the lens and macula[9]. To test it directly we measured scotopic spectral sensitivity in these two individuals, and then used these results to derive ocular absorption using the method described by Norren and Vos[10]. The resulting difference in ocular absorption so measured was less than 1% of what would be required to produce the difference in colour matches of the two subjects. (2) The colour matching difference is not due to differences in photopigment density of the two subjects. To test this possibility we measured matches for these same two subjects after they had been exposed to a white light sufficiently intense to produce a full bleach of their photopigments[11]. The resulting matches are shown in Fig. 1*b*. If the difference in the colour matches made by these subjects were due to differences in the density of their photopigments, their matches should become more similar following the bleach. Although, as expected[3], the matches change following the bleach, the matches made by these two subjects do not become more similar. (3) The difference in colour matching of these two subjects is not due to some differences that arise from the use of the large test field. For instance, it has been suggested that photoreceptor orientation has a role in determining the effective optical density of the photopigments and that such effects might be pronounced for large test fields like those used in this experiment[12,13]. To examine this, colour matches were determined using the same test procedure, but for foveally-viewed stimuli subtending only 2°. The result (Fig. 1*b*) is that, again, the two subjects continue to show clear differences in their colour matches. It may be concluded that the difference in colour matching of these two subjects is due to a variation in the spectral positioning of their cone pigments.

Colour matches were obtained from a sample of 200 subjects having normal colour vision. Variations in colour matching are

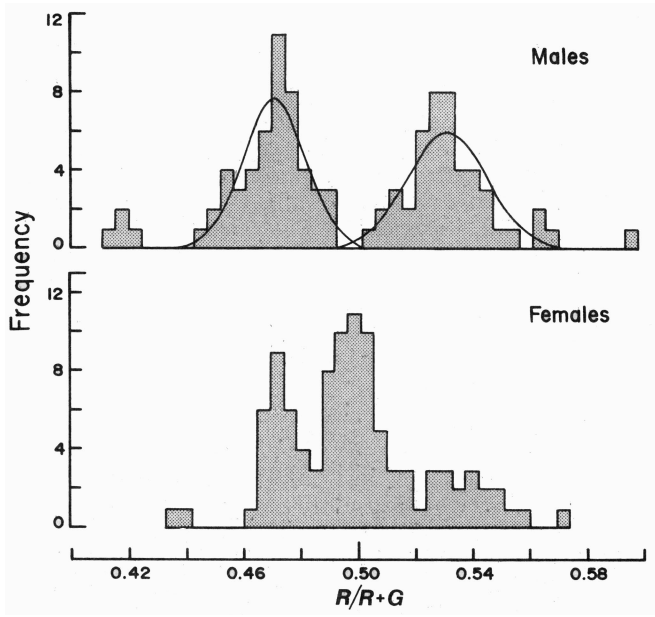

Fig. 2 Distribution of Rayleigh match midpoints for individuals with normal colour vision. The stimulus conditions were identical to those described for Fig. 1*a*. The R and G values used to compute $R/R+G$ are the energies of the primaries multiplied by constants so that the average match midpoint across all subjects is equal to 0.50. The actual energy values at $R/R+G=0.50$ are 3.21 μW for the red light and 1.79×10^{-2} μW for the green light. *Top*, the distribution of midpoints obtained for 100 males. The curves outline the normal distributions expected given the mean and standard deviations for each group. The actual energy values determined for the mean match equation for the two groups are as follows: group to the left $R=3.38$, $G=1.65\times10^{-2}$, $Y=6.56\times10^{-2}$; group to the right $R=3.06$, $G=1.91\times10^{-2}$, $Y=6.81\times10^{-2}$ (all values in μW). *Bottom*, the distribution of midpoints obtained for 100 females.

associated with differences in ocular pigmentation, the latter covarying with both age and degree of skin pigmentation[9]. To minimize the effects of this source of variability the subjects tested were all young (mean age = 20.6 years) Caucasians. An attempt was made to exclude heterozygous carriers of colour anomaly by rejecting from the sample any females who reported that their families contained colour defective individuals[14].

Frequency histograms of match midpoints are shown separately for male and female subjects in Fig. 2. The distribution of matches gathered from the males is very clearly bimodal; the two subjects of Fig. 1 are individual representatives of these two groups. Assuming that, like the subjects of Fig. 1, the matches made by the subjects of Fig. 2 are also based on variation in the spectral positioning of cones, the modal nature of this distribution indicates that the variation in the underlying photopigments must be discrete. From quantal values of the three components in the mean match equation for each of these two groups, the spectral position of peak absorption (λ_{max}) for two putative cone mechanisms underlying this behaviour can be computed by solving simultaneously the colour matching equations and the polynomial expressions for wavelength dependent nomograms. These computed spectral positions depend on several assumptions. The assumptions made were: first, that these mechanisms have a shape defined by the wavelength-dependent visual pigment nomogram appropriate for A_1-based pigments having a spectral peak between 530 and 610 nm (ref. 15), second, the extreme long wavelength slopes of the two mechanism follow, respectively, the slopes of the middle and long wavelength cone sensitivity curves measured by Nunn *et al.*[16], third, the photopigments have a specific absorbance of

0.015 μm^{-1} (1) and the photoreceptors in the parafoveal region from 3° to 11° have an average length of 20 μm (ref. 17), giving them an axial absorbance at their λ_{max} of 0.3, and fourth, that corrections for lens absorption given in the literature[18,19] for young humans are appropriate for the present sample. Given these assumptions, for the group of males requiring slightly more red light to complete the match (at the right in Fig. 2) this computation yields spectral peaks of 530 nm for the middle wavelength cone mechanism and 556 nm for the long wavelength cone mechanism. For males requiring slightly less red light the computation yields values of 530 nm and 559 nm for the two mechanisms. There is thus variability in the estimated location of the long wavelength cone mechanism for these two groups, but no corresponding variability in the location of the middle wavelength cone mechanism.

The distribution of matches obtained from females (Fig. 2, bottom) is significantly different from that of the males ($\chi^2 = 77.2$, d.f. = 7, $P < 0.001$). The match most frequently made by female subjects occurs where no male matches. This difference between male and female matches suggests that the variation is X-chromosome linked. The idea is that among normals the gene(s) specifying the spectrum of the long-wavelength cone mechanism has (have) two identities. Males receive either of these and accordingly fall into two groups. Homozygous females would also fall into either of these same two distributions. Assuming the occurrence of X-chromosome inactivation[20], females who are heterozygous for this trait would have both types of long wavelength cone mechanism. They thus might be expected to make intermediate colour matches. If this idea is correct, then the distribution of matches made by females is predictable from the distribution of male matches. Table 1 shows this comparison. The distribution of female matches is in close accord with the prediction.

The significant variation in colour matching we find among individuals with normal colour vision is consistent with earlier reports[3-6]. The present experiment, however, reveals an unexpected distribution for this variation that leads us to hypothesize that the variation is produced by an X-chromosome linked polymorphism of the long-wavelength sensitive mechanism. Although the trichromacy of normal human colour vision has been established beyond question, the specification of the receptor mechanisms on which the trichromacy is based is not yet complete. This experiment suggests that the long-wavelength cone mechanism assumes at least three distinct identities in different normal human trichromats.

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Cholinergic neuropil of the striatum observes striosomal boundaries

A. M. Graybiel*, R. W. Baughman† & F. Eckenstein†

* Department of Brain and Cognitive Sciences, Whitaker College of Health Sciences, Technology and Management, Massachusetts Institute of Technology, 45 Carleton Street, Cambridge, Massachusetts 02139, USA
† Department of Neurobiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115, USA

Acetylcholine and dopamine are key neurotransmitters in the extrapyramidal motor system, where they are thought to lie in a 'functional balance' brought about by interactions between the terminals of the dopamine-containing nigrostriatal tract and the cholinergic interneurones of the striatum[1-3]. The precise nature of these interactions is not understood, however, nor is it clear how they influence the functioning of striatal systems containing other neurotransmitters. A new clue to understanding such interplay among transmitter-coded systems in the striatum has come from the finding that many of them, including nigrostriatal afferents, follow a macroscopic ordering in which neural elements are concentrated either in or out of the striatal tissue compartments called striosomes[4-8]. We here report that the cholinergic neuropil of the striatum is also compartmentalized: fibres expressing immunoreactivity to antibodies raised against choline acetyltransferase (ChAT) are sparse in striosomes and are dense in the extrastriosomal matrix. These findings suggest (1) that the interactions between acetylcholine and other neurotransmitters in the striatum are spatially constrained, (2) that cholinergic modulation of striatal function predomintes in the extrastriosomal matrix, and (3) that extrapyramidal pathways originating in the matrix, including transthalamic pathways to the frontal lobes, may in particular reflect this cholinergic influence. Such a differential organization of striatal cholinergic circuitry could help to account for the selective therapeutic efficacy of anticholinergic drugs in the treatment of extrapyramidal disorders.

The cholinergic functions of the striatum are thought to depend on the actions of intrinsic cholinergic interneurones[5,9-11] which correspond to the rare 'giant' cells scattered among the much more common medium-sized striatal-projection neurones[5,12,13]. The cholinergic perikarya express high levels of ChAT and acetylcholinesterase (AChE) and their distribution in the striatum has been extensively studied by histochemical and immunohistochemical methods[7,12,13]. It has been more difficult to analyse the cholinergic neuropil of the striatum and to be sure of its origins. Staining of the neuropil for AChE activity is readily achieved, but not all the AChE in the striatum is contained in the cholinergic interneurones[14]. Expression of ChAT-immunoreactivity by striatal fibres should define them as cholinergic, but few antisera raised against ChAT have yet permitted reliable immunolocalization of striatal fibre processes[12,13]. Recently, intense ChAT-immunostaining of the striatal neuropil was found in the rat[13] and the ferret[15]. Some patchiness of staining was noted in the ferret, but the ChAT-positive neuropil was found to be homogeneous in the rat except for broad regional gradients. Taken together with the lack of distinct clustering of cholinergic cell bodies[12,14], this suggested[14] that the cholinergic interneuronal system of the striatum might lack the compartmental ordering characterizing most other systems in this region.

To explore this possibility we used protocols optimizing immunostaining of striatal fibres with antibodies raised in rat against porcine ChAT[16,17]. We studied the distribution of ChAT-positive neuropil in the striatum of two macaque monkeys and six cats and compared ChAT-immunostaining with AChE histochemical staining in adjoining sections to identify the AChE-poor compartments (striosomes) of the striatum. To test whether