Worlds of difference

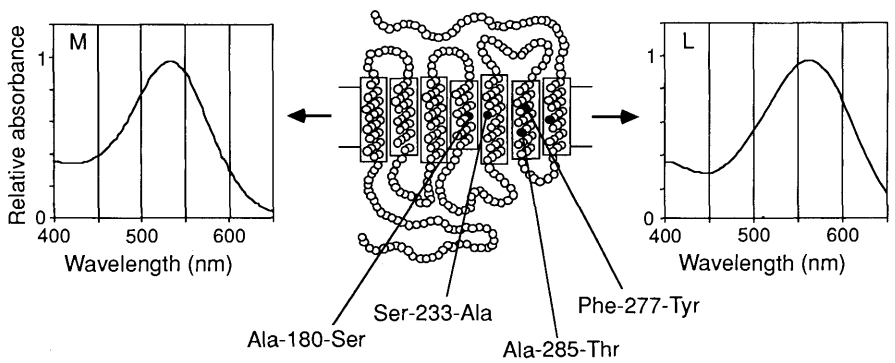
John Mollon

YOU and I may pass through our lives in different perceptual worlds. Although we both may enjoy colour vision that is nominally normal, coloured objects that look alike to you may look distinctly different to me, and those that look different to you may look identical to me. And this small but irreducible discrepancy in our sensations can be traced to a single nucleotide difference in our X chromosomes. That is the implication of two papers published on pages 433[1] and 431[2] of this issue.

It was in these columns in 1881 that John William Strutt, second Baron Rayleigh, introduced the colour match that has ever since served to classify normal and abnormal vision: the observer is asked to find the ratio of red to green light that matches a monochromatic orange[3]. More recently, *Nature* has twice (and independently) carried reports that the distribution of such 'Rayleigh matches' in a colour-normal population is bimodal[4,5]. And those two papers may have been anticipated by an earlier study of over 2,500 Yugoslavs, where a provocative bimodality was reported without comment[6]. Certainly everyone who has measured Rayleigh matches — including Lord Rayleigh — agrees that normal observers differ in their responses (see for example refs 7–9). But what is the physiological basis for these variations in perception?

Our daytime vision depends on light-sensitive molecules embedded in the concertina-like membranes of the cone cells of the retina[10]. Each of these photopigment molecules consists of a large protein that has been bound to retinal, a derivative of vitamin A. The protein component has the 'heptahelical' structure characteristic of all G protein-coupled receptor molecules (see my previous News and Views article[11]). Small variations in the amino-acid sequence of the protein (or 'opsin') yield pigments with peak sensitivity in different parts of the spectrum.

There are usually taken to be three normal human cone pigments, with peak sensitivities in the violet, the green and the yellow–green regions of the spectrum. But in 1983 a microspectrophotometric study of retinae from human patients suggested that there were two alternative types of long-wave cone, which were associated with different psychophysical sensitivities to red light[12]. And when, in 1986, Jeremy Nathans and his colleagues first published nucleotide sequences for the opsin genes, they noted that the normal long-wave gene was polymorphic: slightly different amino-acid sequences could be inferred for the corresponding pigment according to whether the gene was derived from Nathans' own genomic DNA or from a complementary DNA library synthesized from messenger RNA from eyes obtained at autopsy[13]. One of those polymorphic sites corresponded to number 180 in the amino-acid sequence. More recently, phenotypic and genotypic correlations in New World monkeys have strongly indicated that this site is one of those that control the wavelength at which the pigment has its peak sensitivity[14,15].



The visual photopigments are members of the class of heptahelical receptors, each consisting of a palisade of seven membrane-spanning helices. In this figure, the filled circle in the seventh helix indicates the lysine residue at which the molecule is bound to 11-*cis*-retinal, the chromophore. The other filled circles indicate four amino-acid sites that probably control the spectral tuning of the long- and middle-wave cone pigments. For the four sites, the alternative amino acids are shown at the bottom of the figure: in each case, the amino acid to the left is associated with a pigment shifted to middle wavelengths and the amino acid to the right is associated with a red-shifted pigment. The graph on the left shows the absorbance spectrum of a normal human middle-wave cone, that on the right the spectrum of a normal long-wave cone. Absorbance data are from ref. 12.

So far, however, in judging which DNA sequence gives a photopigment of a given spectral sensitivity, we have been restricted to the indirect evidence from correlating genotypes with the phenotypes of monkeys or of colour-normal and colour-deficient men. Now two groups have used tissue culture to express genes for the opsins of the three normal human cones. Oprian *et al.*[16] started with chemically synthesized artificial genes, whereas Merbs and Nathans[1] (page 433 of this issue) began with cDNA clones isolated from human retinae. In both cases, when the gene products were combined with retinal and were purified, they proved to have absorbance spectra resembling those recorded earlier from human cones by microspectrophotometry and electrophysiology[12,18]. An added twist of Merbs and Nathans' work is that they have reconstructed two versions of the long-wave pigment, differing at site 180. As suspected, the two gene products have different absorbance spectra: the pigment with alanine at site 180 exhibits a peak sensitivity at 552.4 nm, whereas that with serine at site 180 has a peak at 556.7 nm.

This result is prettily complemented by the second paper[2] in this issue (page 431). Winderickx and colleagues, in Seattle, have measured the Rayleigh matches of 50 colour-normal males and have analysed DNA from each subject. Using the polymerase chain reaction (PCR) to amplify the relevant part of the long-wave gene, they find that 62 per cent of subjects exhibit the code for serine at site 180 and 38 per cent the code for alanine. As would be predicted from the primate work and from the new absorbance curves of Merbs and Nathans, the subjects with serine at 180 exhibit a higher sensitivity to red light. Qualitatively similar results were described by Neitz *et al.* at a meeting in January[17], although those authors used PCR primers that concurrently amplified the corresponding parts of long- and middle-wave genes: they report a strong, but more graded relationship between the Rayleigh match and the ratio of the two alternative nucleotides that determine whether alanine or serine occurs at site 180.

The significance of these discoveries for psychologists cannot be exaggerated. Here is a case where a difference of a single nucleotide places people in distinct phenomenal worlds and where we know almost all the steps in the causal chain from gene to molecule to neural signals; only the final steps from cortical activity to sensation elude us. It is the first such case in psychology. It cannot be the last.

This is not to say that the genetics of colour vision are all tied up. Here are four examples of unsettled issues.

First, Neitz *et al.*[14] last year put for-

ward an attractively simple theory of the spectral tuning of cone pigments, suggesting that the difference between the human long- and middle-wave pigments depended on three amino-acid substitutions only (at sites 180, 277 and 285) and that these substitutions were additive in their effects on the wavelength of peak sensitivity. A British group (which includes myself) has questioned this theory, proposing that at least one further site (233) is involved, possibly in a nonadditive fashion[15,18]. The evidence is that all ten primate species so far investigated exhibit a non-hydroxyl-bearing residue at site 233 of the long-wave (> 560 nm) pigment, whereas, in all eight cases where the species exhibits a pigment peaking below 540 nm, that pigment has a hydroxyl-bearing residue at site 233. So it is instructive that Winderickx *et al.*[2] have identified two atypical subjects who had hydroxyl-bearing amino acids at site 233 of their *long-wave* pigment: both were less red-sensitive than would be expected from the amino acid they exhibit at site 180.

Second, the Seattle group maintains, as Nathans and colleagues have always done, that an individual male has only one long-wave gene (though perhaps several middle-wave genes)[2]. Neitz and colleagues, on the other hand, hold that more than one long-wave gene may be present, and expressed[17]: so many men are 'pseudo-heterozygotes' for the Ser/Ala substitution at site 180. Their reasoning is that the spread in Rayleigh matches is less than primate data would predict if the variance came mainly from a site-180 polymorphism in a single long-wave pigment. Two alternative explanations would be that the Ala-180 version of the pigment has a higher effective optical density, and thus its relative sensitivity in the red is enhanced, or that the magnitude of the spectral shift due to site 180 depends on other, concurrent, substitutions.

Third, Mathew Alpern has for 15 years held that anomalous trichromats achieve their residual red–green discrimination through the presence of either two versions of the normal long-wave pigment or two versions of the middle-wave pigment[7]. Does his hypothesis draw support from the normal polymorphism now demonstrated?

Finally, given the Ser/Ala polymorphism in the male population, there ought to be women who are heterozygous for this substitution and carry different alleles on their two X chromosomes. Because of random X-chromosome inactivation, only one of the two long-wave pigments will be expressed in any individual cone. In female platyrrhine monkeys, the two corresponding types of cone are able to sustain a colour-opponent signal[20]. Does this mean that a heterozygous woman can be tetrachromatic, experiencing an extra dimension of hue that must forever be forbidden to her male conspecifics? □

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