Mixing genes and mixing colours

Recent work on the molecular genetics and biochemistry of the human cone photopigments provides a basis for understanding variations in colour vision in human populations.

Monkeys are to yellow and orange fruit what *Hymenoptera* are to flowers: there exist species of tropical fruiting tree that are dispersed predominantly by primates. About thirty million years ago, it seems, such trees conspired with Old World monkeys to offer chromatic signals that were visible against the dappled and variegated greens of foliage. Among the mammals, it is only primates that enjoy the ability to distinguish reds, oranges and yellows from one another and from green, and almost certainly this ability co-evolved with the signals presented by tropical trees [1]. The enrichment of primate colour vision was achieved by the duplication of a gene on the X chromosome [2]. The resulting cluster of highly similar genes proves, in our own species, a fertile source of recombinant mischief. A new paper by Merbs and Nathans examines the hybrid proteins that may result [3].

Our colour vision, and indeed all vertebrate daytime vision, depends on light-sensitive molecules embedded in the enfolded membranes of retinal cones [4] (Fig. 1). These photopigment molecules consist of a protein (opsin) bound to 11-*cis*-retinal, a derivative of vitamin A, which acts as the chromophore. All the opsins are members of the super-family of G protein-coupled receptors, which have seven transmembrane segments joined by extra-cellular and intra-cellular loops [5]. It is variations in the amino-acid sequence of the transmembrane segments that lend different spectral sensitivities to the photopigment. And colour vision is achieved by neural comparison of the quantum catches in cones that express different opsins.

Most non-primate mammals have a limited form of colour vision, similar to that preserved in those men (2% of our population) we call 'dichromats'. This ancient and genetically stable system of colour vision allows the discrimination of 'cold' colours from 'warm', and depends on comparing the quantum catch in a sparse population of short-wavelength cones with the quantum catch of a more numerous, single type of cone having peak absorption in the region 510–570 nm.

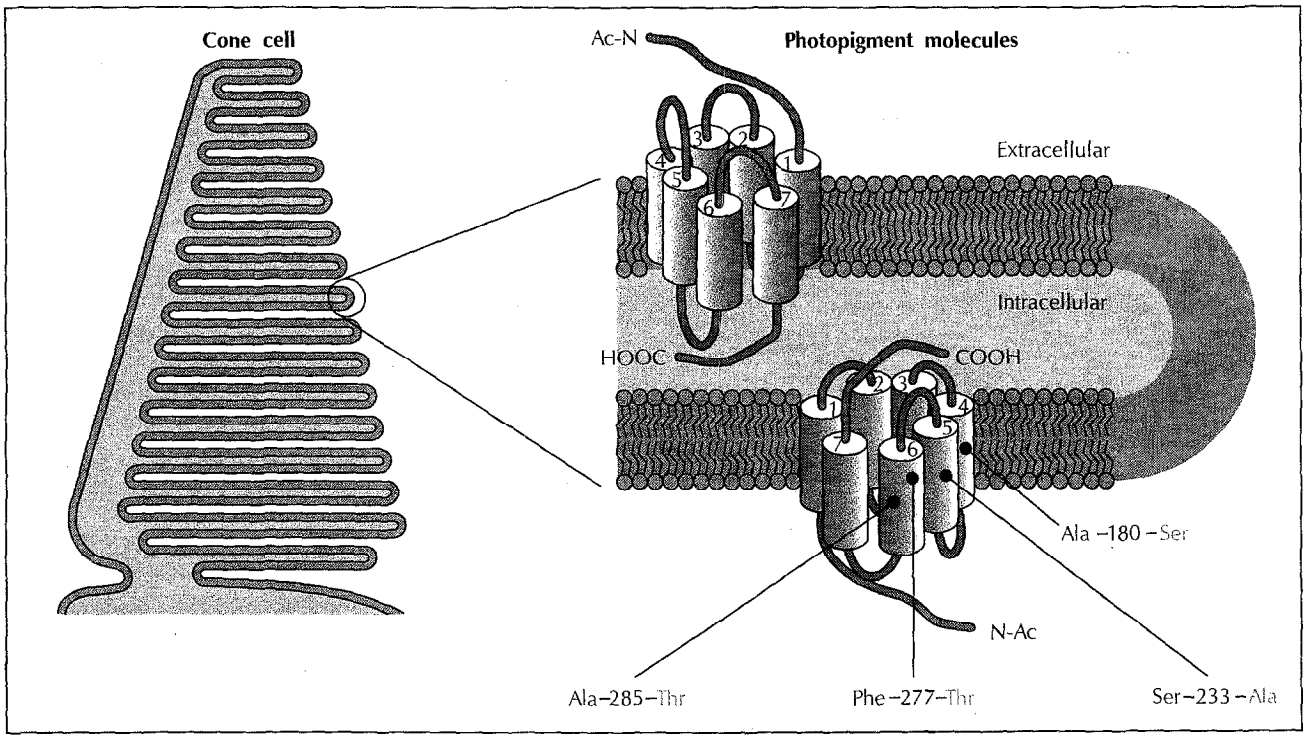

Fig. 1. The enfolded membranes of retinal cone cells are packed with photopigment molecules, consisting of 11-*cis*-retinal bound to an opsin protein. The difference between the middle-wave and long-wave photopigments depends on the identity of a small number of amino acids within the transmembrane segments of the opsin. Four of the most significant sites are indicated: for these four sites, 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.

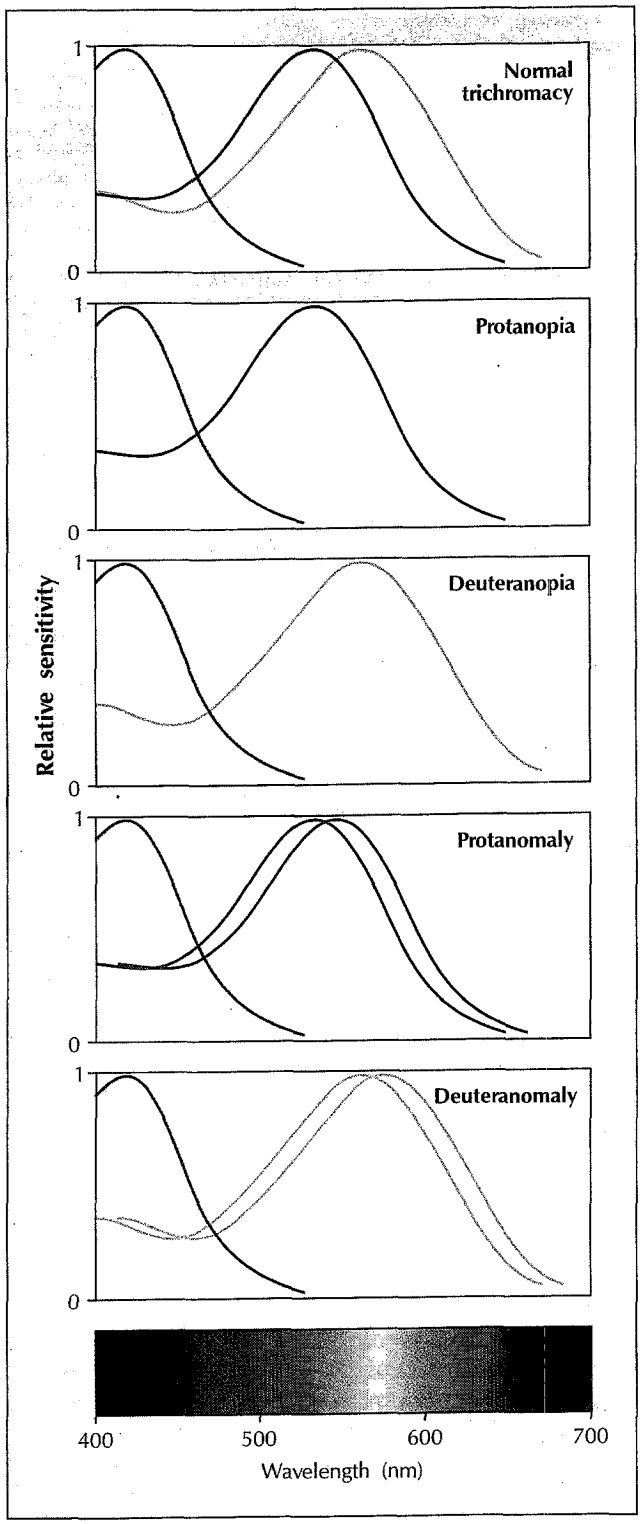

Fig. 2. Sensitivity curves for the photopigments thought to be present in normal colour vision (top) and in the four most common types of hereditary colour deficiency. Protanopes and deuteranopes are dichromats, having lost the long-wave and middle-wave pigments, respectively. In anomalous trichromacy (bottom two panels), residual discrimination in the red–green range is thought to depend on two pigments with reduced spectral separation. Still at issue is whether the two middle-wave pigments found in protanomaly and the two long-wave pigments found in deuteranomaly are simply some of the polymorphic forms found in the normal population.

The enhancement of colour vision in the Old World primates arose from the duplication and modification of the gene on the X chromosome that coded for the ancestral pigment in the 510–570 nm range. In man, the two resulting genes remain very similar, and code for photopigments with peak sensitivity near 530 nm (middle-wave) and 560 nm (long-wave) [6] (Fig. 2). By comparing the quantum catches in these two pigments, the normal retina distinguishes violets from blues, and reds, yellows, and oranges from greens and from each other. But the homology of the genes encoding the long-wave and middle-wave pigments, and their continuing juxtaposition on the X chromosome, are thought to leave them prone to misalignment at meiosis. If crossing over then occurs within the array of opsin genes, unequal recombination will result (Fig. 3). If the breakpoint occurs between genes, then an entire gene may be lost from one chromosome, and a boy who inherits that X chromosome will be a dichromat. If the breakpoint occurs within misaligned long-wave and middle-wave genes, then hybrid genes will be formed, natural chimeras which code in part for the sequence of the long-wave opsin and in part for the sequence of the middle-wave opsin.

In 1986 Jeremy Nathans and his collaborators [7] observed such hybrid genes in cases of anomalous trichromacy. This form of colour-deficiency is milder than dichromacy and is more common, affecting 6% of males in European populations. Whereas a dichromat can match all colours with two primaries, the anomalous trichromat requires three variables in a colour-matching experiment. However, the matches that he makes are different from those of the colour-normal population, and anomalous trichromats are traditionally classifed by the Rayleigh equation — the ratio of red and green light required to match a monochromatic orange: those who need more red are called protanomalous and those who need more green are termed deuteranomalous. Psychophysical studies of colour matching have suggested that the protanomalous observer combines a normal middle-wave pigment with a second pigment that is intermediate between the normal long-wave and middle-wave pigments, and that the deuteranomalous observer similarly combines a normal long-wave pigment with an intermediate pigment [8] (Fig. 2).

What is remarkable is that hybrid genes, consisting of part of a long-wave pigment gene and part of a middle-wave pigment gene, do code for photopigments with intermediate spectral sensitivity. Merbs and Nathans [3] have constructed hybrid genes from cDNA clones for the normal middle-wave and long-wave photopigments and have expressed them in cultured mammalian kidney cells. The gene products – the artificial opsins — were then combined with 11-*cis*-retinal and the cell membranes were solubilized in detergent. The absorption spectra of the resulting pigments were estimated by subtracting the absorption at each wavelength before and after bleaching.

The X chromosome opsin genes consist of six exons, and Merbs and Nathans constructed hybrids made up from a whole number of exons from one gene and the rest from the other. The major determinant of the wavelength of maximal sensitivity (λ_{max}) of the hybrid pigments was

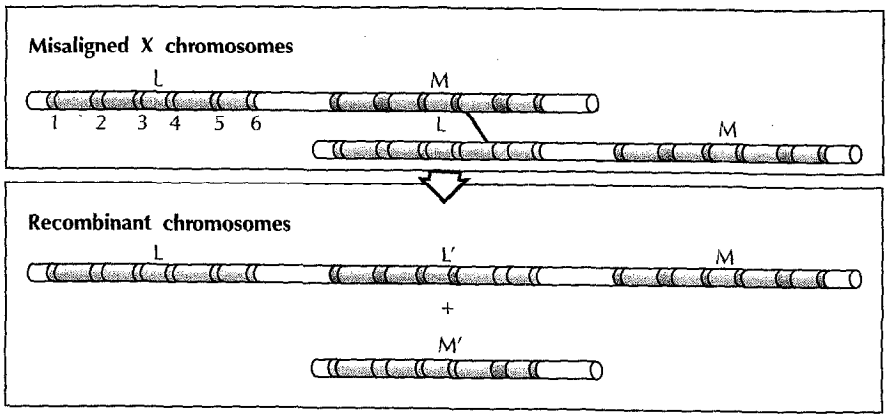

Fig. 3. Nathans' hypothesis of how hybrid opsin genes might be produced by unequal crossing-over. The top panel shows X chromosomes misaligned at meiosis in such a way that the middle-wave pigment gene of one chromosome is apposed to the long-wave pigment gene of the other. Crossing-over (red line) in the intron between exons 4 and 5 generates one chromosome that carries normal long-wave and middle-wave pigment genes plus a hybrid gene (L') and a second chromosome that carries only a hybrid gene (M').

whether exon 5 was drawn from the middle-wave or the long-wave pigment gene. This finding was already expected from studies of the polymorphic opsin genes in platyrrhine monkeys of different phenotypes [9,10] and from a study of a dichromat who had a single gene drawing exons 1–3 from a long-wave pigment gene and exons 4 and 5 from a middle-wave pigment gene [11]. However, Merbs and Nathans find that the effect of the two critical sites in exon 5 (corresponding to residues 277 and 285 in the opsin) depends on the identities of amino acids elsewhere in the hybrid pigment: the shift in λ_{max} varies between 15 nm and 21 nm. Exons 2, 3 and 4 produce shifts that are smaller (up to 4 nm) and which similarly depend on the identity of the rest of the molecule.

Merbs and Nathans are thus led to identify a range of 'middle-wave' hybrids (with exon 5 drawn from the normal middle-wave pigment gene) which exhibit λ_{max} values between 530 nm and 536 nm, and a range of 'long-wave' hybrids (with exon 5 from the normal long-wave pigment gene) which exhibit λ_{max} values between 545 nm and 557 nm. If these hybrids are all in fact available as natural pigments, one classical hypothesis is definitely excluded — the hypothesis that there is a single anomalous pigment common to both protanomaly and deuteranomaly. But also apparently contradicted is a theory of anomaly put forward last year by Jay and Maureen Neitz [12]. The latter authors argued that deuteranomalous colour vision depends on two common variants of the normal long-wave pigment, which differ according to whether alanine or serine occurs at site 180, and which differ by about 6 nm in λ_{max}; similarly, they suggest, protanomalous vision depends on two variants of the normal middle-wave pigment, which likewise differ at site 180.

In the theory of Neitz and Neitz, the separation of the residual pigments is the same in all anomalous observers. So some further explanation is then needed for the incontestable fact that anomalous men vary greatly in the acuteness of their colour discrimination in the red–green range, some being indistinguishable from normal and some being almost as poor as dichromats [13]. On the other hand, this variation falls naturally out of Merbs and Nathans' account: the delicacy of colour discrimination will depend on the spectral separation between the hybrid pigment and the residual normal pigment.

Many questions certainly remain. Are the λ_{max} values of Merbs and Nathans' hybrid pigments the same as those exhibited by the pigment when embedded in the cone membrane, or must we consider Kundt's rule, that λ_{max} depends on the refractive index of the solution? Is λ_{max} *in vivo* altered by the ionic environment of the cone, as reported for non-mammalian long-wave cones [14]? Does natural recombination ever unyoke the two critical sites in exon 5 (corresponding to residues 277 and 285), to give a pigment approximately midway between the normal middle-wave and long-wave pigments? And, most fundamentally, have the distinctions between the normal and hybrid genes and between long-wave and middle-wave pigment genes become merely metaphysical? In other words, is there anything that labels a gene as long-wave or middle-wave other than the identity of those nucleotides that determine spectral sensitivity? If there isn't, then the positions of Merbs and Nathans and of Neitz and Neitz become very close: all that is at issue is the extent of the variance within the middle-wave and the long-wave genes of the colour-normal population — the extent to which the 'hybrids' are simply members of the family of pigments found in normal observers. It is already known that hybrid genes are very frequent in some populations — much more frequent than would be expected from the incidence of anomalous trichromacy [15].

The opsin gene cluster seems bedevilled by recombination that is protean in its variety. But is it all mischief? Is the high incidence of colour deficiency in man simply the unhappy consequence of unequal crossing over? E.B. Ford, who himself gave 'balanced polymorphism' its formal definition, suggested that colour-deficient observers may sometimes enjoy an advantage in penetrating the camouflage of the natural world and that this advantage might maintain minority types of colour vision within our population. We now know that polymorphism of colour vision is in fact the norm among platyrrhine (New World) monkeys [16]. And a recent study by M. Morgan, A. Adam and myself [17] shows experimentally for the first time that colour-deficient human observers are sometimes at an advantage: in the presence of random variation in colour, dichromats were better than normals in detecting variations in texture. Colour deficiency may have hidden compensations.

References

1. MOLLON JD: **Uses and evolutionary origins of primate colour vision.** In *Vision and Visual Dysfunction, Volume 2.* Edited by Cronly-Dillon JR, Gregory RL. London:Macmillan; 1991:306–319.

2. NATHANS J, THOMAS D, HOGNESS DS: **Molecular genetics of human color vision: the genes encoding blue, green, and red pigments.** *Science* 1986, **232**:193–202.
3. MERBS SL, NATHANS J: **Absorption spectra of the hybrid pigments responsible for anomalous color vision.** *Science* 1992, **258**:464–466.
4. BOWMAKER JK: **The evolution of vertebrate visual pigments and photoreceptors.** In *Vision and Visual Dysfunction, Volume 2, Evolution of the Eye and Visual System.* Edited by Cronly-Dillon JR, Gregory RL. London: Macmillan; 1991:63–81.
5. APPLEBURY ML: **Molecular determinants of visual pigment function.** *Curr Biol* 1991, **1**:263–269.
6. DARTNALL HJA, BOWMAKER JK, MOLLON JD: **Human visual pigments: microspectrophotometric results from the eyes of seven persons.** *Proc R Soc Lond* [Biol] 1983, **220**:115–130.
7. NATHANS J, PIANTANIDA TP, EDDY RL, SHOWS TB, HOGNESS DS: **Molecular genetics of inherited variation in human color vision.** *Science* 1986, **232**:203–210.
8. DEMARCO P, POKORNY J, SMITH VC: **Full-spectrum cone sensitivity functions for X-chromosome-linked anomalous trichromats.** *J Opt Soc Am* 1992, **9**:1465–1475.
9. NEITZ M, NEITZ J, JACOBS GH: **Spectral tuning of pigments underlying red-green color vision.** *Science* 1992, **252**:971–974.
10. WILLIAMS AJ, HUNT DM, BOWMAKER JK, MOLLON JD: **The polymorphic photopigments of the marmoset: spectral tuning and genetic basis.** *EMBO J* 1992, **11**:2039–2045.
11. NEITZ J, NEITZ M, JACOBS GH: **Analysis of fusion gene and encoded photopigment of colour-blind humans.** *Nature* 1989, **342**:679–682.
12. NEITZ J, NEITZ M: **Do people with anomalous color vision have anomalous cones.** *Inv Ophth Vis Sci* [suppl] 1992, **33**:754.
13. KOLLNER H: **Uber Ubergänge zwischen normalem Farbensinn und angeborener Rotgrünblindheit und über die Möglichkeit ihrer quantitativen Bestimmung.** *Archiv für Augenheilkunde* 1915, **58**:302–335.
14. KNOWLES A: **The chloride effect in chicken red cone receptors.** *Vis Res* 1980, **20**:475–485.
15. JORGENSEN AL, DEEB SS, MOTULSKY AG: **Molecular genetics of X-chromosome-linked color vision among populations of African and Japanese ancestry: High frequency of a shortened red pigment gene among Afro-Americans.** *Proc Natl Acad Sci USA* 1990, **87**:6512–6516.
16. JACOBS GH: **The distribution and nature of colour vision among mammals.** *Biol Rev,* in press.
17. MORGAN MJ, ADAM A, MOLLON JD: **Dichromats detect colour-camouflaged objects that are not detected by trichromats.** *Proc R Soc Lond [Biol]* 1992, **248**:291–295.

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