PERGAMON

Vision Research 38 (1998) 3371–3376

Vision Research

Relating color discrimination to photopigment genes in deutan observers

S.K. Shevell [a,*], J.C. He [a], P. Kainz [b], J. Neitz [b], M. Neitz [b]

[a] *Visual Sciences Center, University of Chicago, 939 East 57th Street, Chicago, IL 60637, USA*
[b] *Medical College of Wisconsin, Milwaukee, WI 53226, USA*

Received 10 July 1997; received in revised form 18 November 1997

Abstract

Deutan observers are a heterogeneous group, varying nearly continuously from deuteranomalous trichromats with fine chromatic discrimination in the red/green range to deuteranopes who have none. We sought to relate chromatic discriminative ability among deutans measured psychophysically (phenotypes) to observers' separation between long-wave visual pigments inferred from visual pigment genes (genotypes). If middle-wave pigment genes are assumed not to be expressed in these deutan observers there is a clear relation between phenotype and genotype. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Anomalous trichromacy; Color matching; Rayleigh matching; Cone photopigments; Photopigment variation

1. Introduction

Inherited color-vision deficiency is common, affecting 8–9% of men of European descent [1,2]. More than half of these men are deuteranomalous trichromats: they lack a functional middle-wave (M) cone photopigment but maintain some degree of chromatic discrimination in the red-green range. Their chromatic discrimination is mediated by two cone pigments with peak sensitivities at slightly different wavelengths near the peak of the normal long-wave (L) pigment. A typical estimate of the separation between the two deuteranomalous pigments is 6 nm (peak sensitivities of 560 and 566 nm) compared to color-normal M and L pigments which are estimated to be separated by 23 nm [3].

Estimates of 'typical' pigments, however, ignore a basic aspect of deuteranomalous trichromacy: heterogeneity within the population of deuteranomals [4–6]. Pigment separation of 6 nm is a useful generalization but does not apply to each deuteranomalous trichromat. Deuteranomalous cone pigments vary among observers in both wavelength of peak sensitivity and optical density [7]. We attempt here to relate individual differences in chromatic discrimination within the red-green range (deutan color-vision phenotypes) to characteristics of each observer's cone pigment genes (genotypes). Chromatic discrimination is quantified using high-radiance Rayleigh matches chosen to reduce individual differences in optical density. Photopigment genes are analyzed by examining the products of long polymerase chain reaction (PCR) to predict the separation between the peak wavelengths of an observer's photopigments. Chromatic discrimination and photopigment separation are linked by the spectral proximity hypothesis which implies better discrimination with greater separation [8,9].

This research complements two recent studies. Shevell and He [7] focused on deuteranomals with excellent chromatic discrimination because precise measurements were needed to infer individual differences in pigments' wavelength of peak sensitivity. The present study includes observers with a broader range of discriminative ability. Neitz et al., using a different sample of deutans, related pigment genes to performance on a pseudoisochromatic plate test [10]. The present study uses a more rigorous color vision test that also minimizes the potentially confounding effect of pigment optical density.

* Corresponding author. Fax: +1 773 7024442; e-mail: shevell@uchicago.edu.

PII: S0042-6989(97)00434-3

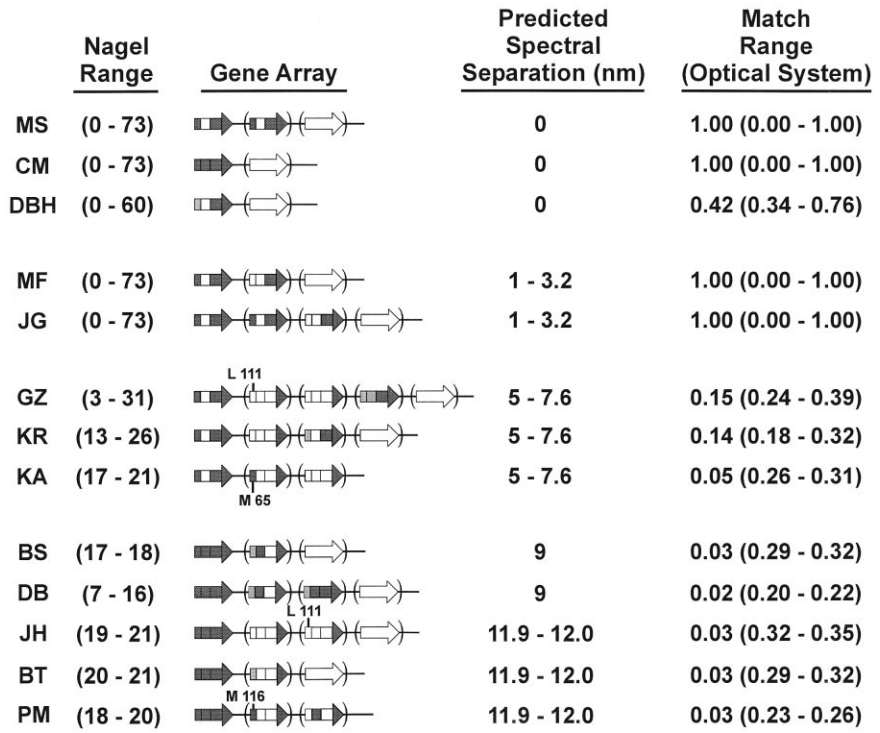

Fig. 1. The Rayleigh-match range determined with a standard Nagel-type anomaloscope (expressed in Nagel units), the gene array, the predicted spectral separation based on the gene array, and the measured Rayleigh match range with a 4.6 log td bleach, for each of 13 deutan observers (see text).

2. Methods

2.1. Phenotypes

2.1.1. Observers

The color vision of each observer was classified by Rayleigh matching [11]. Initially, observers were screened using a standard Nagel-type anomaloscope (Neitz OT model). Thirteen observers who completed subsequent psychophysical measurements and who provided blood samples for genetic testing were classified as deutans by the standard Nagel match (see 'Nagel range' in Fig. 1): four deuteranopes (dichromats) and nine deuteranomalous trichromats (one extreme and eight simple deuteranomals; [12]). None of these observers reported a history of eye disease.

2.1.2. Stimuli

Chromatic discrimination was measured by Rayleigh matching using a Maxwellian view optical system. The light in each channel of the optical system was from a broadband tungsten-halogen source and passed through a three-cavity interference filter with a half-bandwidth of 9–11 nm. A 2° circular bipartite field was composed of a mixture of 547 + 660 nm light in one hemifield, and 589 nm light in the other hemifield. The proportion of 660 nm light in the mixture field could be varied at constant luminance by the experimenter; the observer adjusted the radiance of the 589 nm light via a joystick. A 2 mm artificial pupil was mounted at the image plane of the final lens, and a chin rest was used to maintain a stable head position.

An additional channel of the optical system could provide a 4° bleaching field centered on the matching area. Light from a tungsten-halogen lamp passed through a 500 nm cut-on filter (Tiffen 12) included to protect short-wave (S) cones from intense stimulation. The bleaching light after passing through the cut-on filter had CIE_{1931} chromaticity coordinates $(x, y) = (0.52, 0.47)$ and a maximal level of 6.47 log td.

2.1.3. Procedure

An intense bleaching light desaturates a superimposed bipartite field, which can make matching difficult and imprecise. Thus when the bleaching light was used the bipartite matching field was presented in alternation with the bleaching stimulus. Initially the bleaching light was presented continuously for 3 min. A temporal alternation pattern followed, with the bleaching light presented for 10 s followed by the bipartite matching field for 0.5 s. When the matching field went off, the bleaching light came on again for 10 s, and then the matching field for 0.5 s, and so on.

Rayleigh matches using the optical system were made initially with only a steady bipartite field at 1.8 log td (no bleaching light). The proportion of 660 nm light in the admixture was controlled by the experimenter; the subject adjusted the radiance of the 589 nm hemifield to

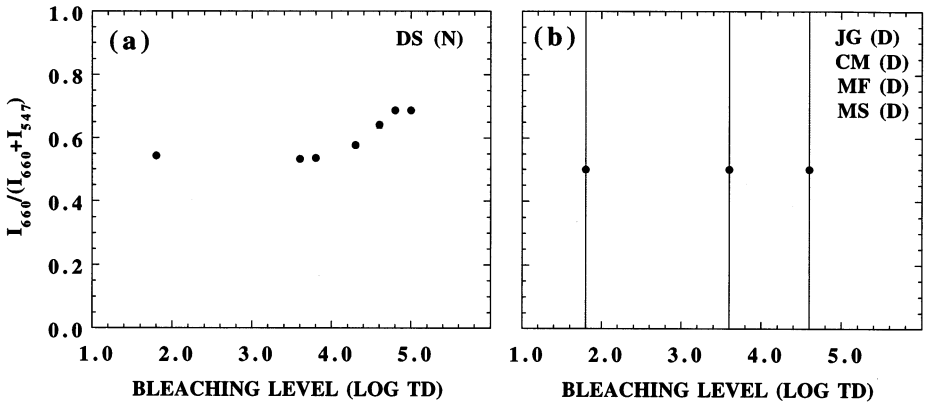

Fig. 2. Proportion of 660 nm light in the Rayleigh mixture field (ordinate) at different bleaching levels (abscissa) for (a) a normal trichromat and (b) four deuteranopes (results for all four are the same). The match mid-point is indicated by a circle; matching range is shown by error bars when the range is larger than the plotted point. Note that the point shown at 3.8 log td [panel (a)] is actually a measurement at 3.6 log td using the alternation procedure, offset for clarity. All measurements above 4 log td used the alternation procedure (see text). Rayleigh units are in deutan mode. Prior to these measurements, the color vision of each observer was classified using a standard Nagel-type anomaloscope (N, normal; D, deuteranope).

determine whether a match could be found. The critical measurement is the range of mixture proportions perceived to match 589 nm. This was determined using the Linksz procedure [13]. Next, the bipartite field was raised to 3.6 log td to determine the matching range at that level. Subsequently, Rayleigh matches were measured using the bleaching light and the alternation procedure. The bleaching light was first presented at 3.6 log td, which provides a direct comparison between the alternation procedure and the steady-presentation paradigm (no separate bleaching light) for color matches at the same light level. The bleaching light was then raised successively to 4.3, 4.6, and (for some observers) 5.0 log td, with the alternating mixture field held at 3.6 log td. All measurements reported below are based on at least two repetitions of the match in separate sessions on different days. A more thorough description of the apparatus, calibration and procedure (including validation of it) are given by Shevell and He [7].

2.2. Genotypes

The L and M pigment genes in each array were determined as described previously by Neitz and Neitz [14].

Long-distance PCR was done specifically to amplify the first gene in the array separately from the downstream L pigment genes. The PCR primers and conditions were those described in Neitz et al.[10]. Exons 2, 3, 4 and 5 of the first gene, and exons 2, 3 and 4 of the downstream L pigment genes, were individually amplified in PCR and used directly for DNA sequencing as described by Neitz et al.[15].

3. Results

3.1. Phenotypes

The Rayleigh matches measured at multiple stimulus levels, and with a bleaching field up to 5 log td, provide a more comprehensive assessment of an observer's chromatic discrimination than results from plate tests or from Rayleigh matching at only the usual light level near 1.8 log td. A 4.6 log td bleaching light causes over 65% of the photopigment to become transparent, and therefore substantially reduces effective optical density [16,17]. Individual differences in deutan pigment optical density may alter the usual Rayleigh-match range [9] so chromatic discrimination measured with a strong bleach—when all observers have reduced, relatively low optical density—should be more closely related to photopigment separation inferred from genetics than results from plate tests or the usual Rayleigh match.

3.1.1. Control experiments with color-normal and dichromatic observers

Rayleigh matches with bleaching for color-normal observers and for deuteranopes (dichromats) follow well known patterns [18–21]. For normals, the reduction in optical density due to bleaching requires a greater proportion of 660 nm light in the mixture field. Typical results from a normal trichromat are shown in Fig. 2(a). The plotted circles show the midpoint of each match range; error bars would normally show the range of mixture proportions perceived to match 589 nm but the ranges for this normal are smaller than the circles. Note that bleaching affects the match midpoint but range remains narrow at all bleaching levels (smaller

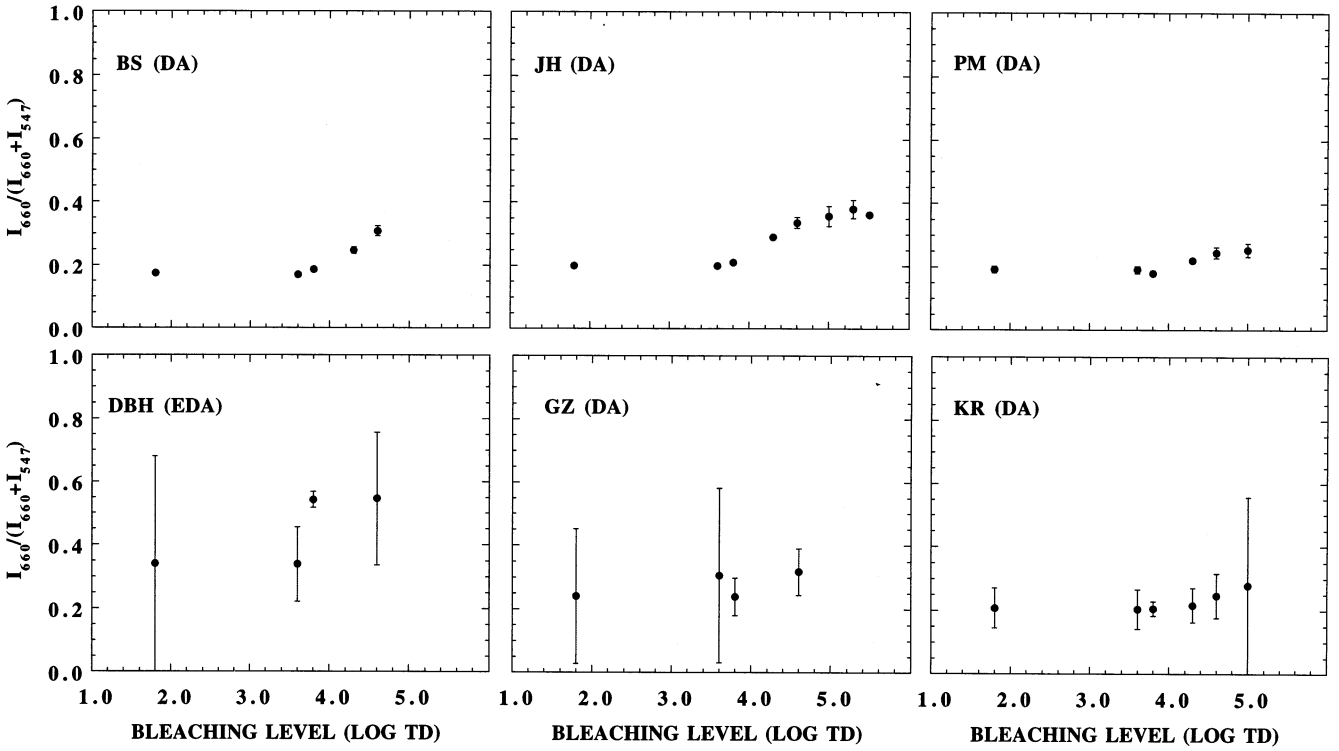

Fig. 3. As Fig. 2 but for six deuteranomalous trichromats. Each panel shows results for a different observer. The top panels are for observers who maintain fine discrimination at all bleaching levels; bottom panels are for observers with less good discrimination. Prior to these measurements, the color vision of each observer was classified using a standard Nagel-type anomaloscope (DA, (simple) deuteranomalous trichromat; EDA, extreme deuteranomalous trichromat).

than the plotted symbol). Dichromats, on the other hand, can match any proportion of 660 nm light in the mixture field to 589 nm regardless of bleaching level [Fig. 2(b)] because they have only one cone pigment with significant sensitivity above 545 nm.

3.1.2. Deuteranomalous trichromats

Measurements from the nine deuteranomalous trichromats separated them into two clear groups: (i) six deuteranomals maintained fine chromatic discrimination at all bleaching levels, with match ranges approaching color-normal values (results from three of these observers are shown in the upper panels of Fig. 3); and (ii) three deuteranomals had matching ranges that clearly were larger than the ranges of the others (lower panels, Fig. 3). The matching range with the 4.6 log td bleaching field, which bleaches over 65% of pigment, is used below to compare chromatic discrimination to genotype. This range for each observer is shown in the right column of Fig. 1. Note that use of the match at 4.6 log td is not critical; the matches in each bottom panel of Fig. 3, while not dichromatic, clearly show weaker discrimination than the matches for the other deuteranomals (cf. top panels in Fig. 3).

3.2. Genotypes

The structure of the X-linked pigment gene array in each subject was deduced from the DNA sequence of the first gene and the downstream L pigment genes, and from the number and ratio of L and M genes estimated for each array. In Fig. 1, L and M genes are depicted by arrows, with the arrowhead corresponding to exon 5 and the tail of the arrow corresponding to exon 2. Exon 5 specifies amino acid substitutions that produce the spectral difference between L and M pigments [22–24]. Exons 2–4 encode amino acid differences responsible for small spectral shifts that produce subtypes of L or subtypes of M pigments (for review see Neitz and Neitz [25]). Exons 1 and 6 are identical between L and M genes.

In the arrows representing exons 2–5 of the L or M genes (Fig. 1), the arrowhead representing exon 5 is white for M genes or black for L genes. The remaining parts of the arrow represent exons 2, 3 and 4 in segments from left to right. Exons 2 and 4 together encode six dimorphic amino acid positions. Exons shown as either white or black indicate that the combination of amino acids is either most typical of M

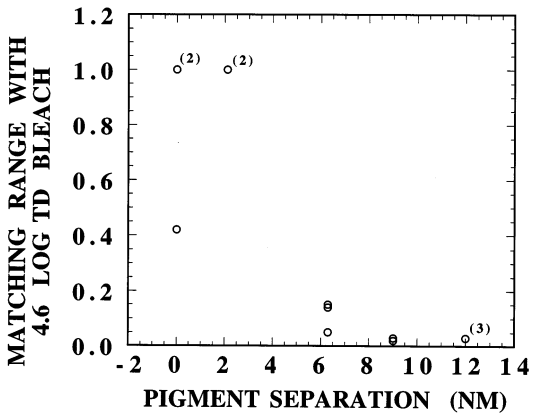

Fig. 4. Each observer's Rayleigh matching range with the 4.6 log td bleaching field (ordinate) is plotted against his predicted pigment separation from genetic analysis (abscissa). Some points represent superimposed values for two or three observers, as indicated in the plot by '(2)' or '(3)'. The Spearman rank-order correlation between phenotype and genotype is -0.88 ($P < 0.001$).

pigments or of L pigments, respectively. In some cases, the sequence for exon 2 is not typical of L or of M. In these instances the shading indicates whether the majority of the exon-2-encoded dimorphic positions is more typical of L or of M genes, with the one that is atypical indicated by the amino acid position number and the letter L or M to show whether the amino acid at that position is more typical of L or M pigment. Also, in some other cases, we were unable to determine the sequence of exon 2; this is indicated by light gray. Exon 3 encodes five dimorphic amino acid positions, and the combinations of these cannot be categorized as typically M or L. Exon 3, therefore, is white or black to indicate whether the amino acid encoded at position 180 is alanine or serine, respectively.

The identity of the first gene in each array is known from the experiments but the order of the downstream genes is not so their shown order is arbitrary.

A significant feature of these gene arrays is the M pigment genes found in most of them (completely white arrows in Fig. 1) even though, as deutans, these observers are missing functional M cones. In the analysis that follows we ignore the M-pigment genes of these color defectives who are classified as deutans by standard Rayleigh criteria [12]. We calculate pigment separation between only L pigments.

We use the spectral peaks determined in vitro for the pigments encoded by the chimeric L genes in each array [22,23] to predict from each observer's genes the spectral separation between his L pigments. In the 'predicted spectral separation' column of Fig. 1, the values to the left are from [23] and those to the right are from [22]. Only one number is given if both studies gave the same value.

The spectral separation between L pigments of an individual is taken as the largest spectral separation possible from the known gene sequences. These values would not change if the unknown exon 2 sequences were available.

4. Discussion

The relation between deutan phenotypes and genotypes is apparent by plotting for each observer the Rayleigh-match range with the 4.6 log td bleaching field (right column in Fig. 1) against the separation between the peak wavelengths of the cone pigments predicted from genetics (adjacent column in Fig. 1). When alternative genetic models imply slightly different pigment separations the average separation is used in the plot. There is a clear correlation (Fig. 4), with match range decreasing rapidly with greater genetically-predicted pigment separation: the five observers with the poorest chromatic discrimination have predicted separation of 0–3 nm; the 5 observers with the best discrimination have predicted separation of 9–12 nm; and 3 observers with intermediate discrimination have predicted separation of 5–8 nm. The Spearman rank-order correlation for the 13 observers is -0.88 ($P < 0.001$).

While the correlation between deutan phenotypes and genotypes is strong, this result does not imply that color deficiency can be predicted from genetic analysis alone. This study concerns deutan observers; that is, the correlation in Fig. 4 is conditional on testing a sample of individuals known to have a deutan color defect. As described above, the genetic results from most of these 13 observers reveal a normal-appearing M pigment gene (white arrowheads, Fig. 1), though their abnormal color vision implies that this gene does not result in a functional M-cone response. Given these M pigment genes, the cause of color vision deficiency for most of these deutan observers remains a mystery.

Acknowledgements

Supported in part by PHS grants EY-04802 (Shevell), EY-09303, EY-09620, and EY-01931 (Neitz), and by Research to Prevent Blindness via unrestricted grants to Ophthalmology at MCW and at UC and the James S. Adams Scholar Award to M. Neitz.

References

[1] Wright WD. Researches on Normal and Defective Color Vision. London: Henry Kimpton, 1946.
[2] Pokorny J, Smith VC, Verriest G. Congenital color defects. In: Pokorny J, Smith VC, Verriest G, Pinckers AJLG, editors. Congenital and Acquired Color Vision Defects. New York: Grune and Stratton, 1979:183–241.
[3] DeMarco P, Pokorny J, Smith VC. Full-spectrum cone sensitivity functions for X-chromosome-linked anomalous trichromats. J Opt Soc Am A 1992;9:1465–76.

[4] Nelson JH. Anomalous trichromatism and its relation to normal trichromatism. Proc Phys Soc (London) 1938;50:661–97.
[5] Willis MP, Farnsworth D. Comparative evaluation of anomaloscopes. Medical Research Laboratory Report. No 190, Bureau of Medicine and Surgery, Navy Department, Washington, DC, 1952.
[6] Schmidt I. Some problems related to testing color vision with the Nagel anomaloscope. J Opt Soc Am 1955;45:514–22.
[7] Shevell SK, He JC. The visual photopigments of simple deuteranomalous trichromats inferred from color matching. Vis Res 1997;37:1115–27.
[8] Regan B, Reffin JP, Mollon JD. Luminance noise and the rapid determination of discrimination ellipses in color deficiency. Vis Res 1994;34:1279–99.
[9] He JC, Shevell SK. Variation in color matching and discrimination among deuteranomalous trichromats: theoretical implications of small differences in photopigments. Vis Res 1995;35:2579–88.
[10] Neitz J, Neitz M, Kainz PM. Visual pigment gene structure and the severity of color vision defects. Science 1996;274:801–4.
[11] Lord Rayleigh. Experiments on color. Nature 1881;25:64–6.
[12] Franceschetti A. Die Bedeutung der Einstellungsbreite am Anomaloskop fuer die Diagnose der eizelnen Typen der Farbensinnstoerungen, nebst Bemerkungen ueber ihren Vererbungsmodus. Schweiz med Wochenschr 1928;58:1273–9.
[13] Linksz A. An Essay on Color Vision and Clinical Color-Vision Tests. New York: Grune and Stratton, 1964.
[14] Neitz M, Neitz J. Numbers and ratios of visual pigment genes for normal red-green color vision. Science 1995;267:1013–6.
[15] Neitz M, Neitz J, Grishok A. Polymorphism in the number of genes encoding long-wavelength sensitive cone pigments among males with normal color vision. Vis Res 1995;35:2395–407.
[16] Rushton WAH. Kinetics of cone pigments measured objectively on the living human fovea. Ann New York Acad Sci 1958;74:291–304.
[17] Coolen ACC, van Norren D. Kinetics of human cone photopigments explained with a Rushton-Henry model. Biol Cybernet 1988;58:123–8.
[18] Wright WD. The breakdown of a color match with high intensities of adaptation. J Physiol 1936;87:23–33.
[19] Brindley GS. The effects on color vision of adaptation to very bright lights. J Physiol 1953;122:332–50.
[20] Alpern M. Lack of uniformity in color matching. J Physiol 1979;288:85–105.
[21] Burns SA, Elsner AE. Color match at high illuminances: the color match area effect of photopigment bleaching. J Opt Soc Am A 1985;2:698–704.
[22] Asenjo AB, Rim J, Oprian DD. Molecular determinants of human red/green color discrimination. Neuron 1994;12:1131–8.
[23] Merbs SL, Nathans J. Absorption spectra of the hybrid pigments responsible for anomalous color vision. Science 1992;258:464–6.
[24] Neitz M, Neitz J, Jacobs GH. Spectral tuning of pigments underlying red-green color vision. Science 1991;252:971–4.
[25] Neitz M, Neitz J. Molecular genetics and the biological basis of color vision. In: Backhaus W, Kliegl R, Werner JS, editors. Color Vision. De Gruyter: Berlin/New York 1998:101–19.