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Severity of color vision defects: electroretinographic (ERG), molecular and behavioral studies

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Abstract

Earlier research on phenotype/genotype relationships in color vision has shown imperfect predictability of color matching from the photopigment spectral sensitivities inferred from molecular genetic analysis. We previously observed that not all of the genes of the X-chromosome linked photopigment gene locus are expressed in the retina. Since sequence analysis of DNA does not necessarily reveal which of the genes are expressed into photopigments, we used ERG spectral sensitivities and adaptation measurements to assess expressed photopigment complement. Many deuteranomalous subjects had L, M, and L–M hybrid genes. The ERG results showed that M pigment is not present in measurable quantities in deutan subjects. Using these results to determine gene expression improved the correlations between inferred pigment separation and color matching. Furthermore, we found a subject who had normal L and M genes and normal proximal promoter sequences, yet he had a single photopigment (M) by ERG and tested as a protanope. These results demonstrate the utility of ERG measurements in studies of molecular genetics of color vision deficiencies, and further support the conclusion that not all genes are expressed in color deficient subjects. In particular, deuteranomaly requires a presently unknown mechanism of selective expression which excludes normal M genes and allows expression of L–M hybrid genes in one cone type, and the normal L in another.

Keywords: Color vision; Genetics; Flicker ERG; Photopigments; Rayleigh matches

1. Introduction

Inherited differences in the number and sequence of the X-linked photopigment genes underlie the common red/green color vision deficiencies and anomalies (e.g. [1,25]) as well as some less obvious variations in red/green color vision that occur among individuals with normal color vision (e.g. [2,3]). Humans may possess one or more pigment genes on a given X chromosome. The presence of a single X-linked pigment in addition to a normal *S*-cone pigment typically results in dichromacy (protanopia or deuteranopia) whereas the presence of two or more X-linked pigments that are spectrally separated typically results in trichromacy. Normal trichromacy requires that two of these X-linked pigments have a spectral separation of about 20–30 nm with peaks around 530 and 560 nm. Individuals having pigments with spectral separations between 1 and 10 nm typically possess anomalous trichromatic color vision (protanomaly or deuteranomaly). It has been suggested that spectral positioning of the photopigment is a major determinant of red/green color discrimination (e.g. [4,5]) and that analysis of the photopigment genes should allow one to predict color discrimination among normal and anomalous observers (e.g. [6]).

The relationship between X-linked genotype and red/green color phenotype is not completely understood. For example, normal and deuteranomalous individuals may possess seemingly identical genotypes, such as a normal L, one or more normal M, and M–L hybrid genes (e.g. [7]). It has been suggested (e.g. [7]) that in

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some cases not all genes in the array are expressed. A person who expresses the normal L and M genes would then be predicted to be color normal while a person who expresses the normal L and a M–L hybrid gene would be deuteranomalous. This idea is supported by the results of Winderickx et al. [3] and Yamaguchi et al. [8], who found by analysis of mRNA and DNA from donor eyes, that some individuals possess genes that are not expressed.

Because the mechanisms of expression of the M, L and M–L pigment genes in the array are poorly understood, it would be desirable to add a direct assessment of the expressed photopigment complement. In the present study we use ERG techniques to provide an independent measure of the expressed photopigments, and compare these measures with genotype and color vision phenotype assessed with standard molecular analysis and color matching, respectively.

ERG techniques have been employed successfully to determine the spectral positioning of the photopigments in many species, including humans (reviewed in [9]). In a variant of this technique, measurements of relative spectral sensitivities under varying conditions of chromatic adaptation can be used as a test for the presence of more than one photopigment in the long to middle wavelength region (cf. [10]). In brief, an unchanging spectral sensitivity curve across various adaptation wavelengths indicates the presence of a single photopigment, while variation of spectral sensitivity with changes in adaptation wavelength indicates the presence of more than one photopigment. This technique has been used successfully to distinguish between dichromats, normals, and anomalous trichromats in both monkeys and humans. The present investigation extends these techniques to distinguishing within groups of normals and color deficient individuals.

The goals of the present study were: (1) to use the ERG technique to estimate the presence and separation of photopigments in human observers and (2) to see to what extent the ERG results can be used to improve predictions of color matching made from molecular analysis.

2. Methods

2.1. General

The study was part of a larger investigation that included dichromats, anomalous trichromats, and color-normal subjects, and was carried out in a double-blind manner. Rayleigh matches and ERG measurements were conducted in the laboratory of M.C., while molecular analysis was conducted in the laboratory of S.D. The results were kept separate until all measurements were completed. Retinal illuminances were calculated from measurements made with a United Detector Technology radiometer, a Gamma spectral radiometer and a Spectrascan 650 (Photo Research) spectral radiometer.

2.2. Subjects

Experimental subjects were chosen from a larger group of color-normal and color-deficient subjects recruited through poster advertisements, word of mouth and a subject pool of psychology students. The subjects selected for the present report were 28 male subjects with more than one X-linked pigment gene. Results from single-gene subjects are reported separately [11]. Informed consent was obtained from all subjects and the experimental protocol was approved by the University of Washington Human Subjects Committee. Subject ages ranged from 18 to 45 years.

2.3. Psychophysics

Small-field (2°) color matches were performed using a modified Nagel anomaloscope [7]. A computer controlled LED anomaloscope [12,13] was used to measure large-field color matches. The large field was composed of an annulus (3° inside diameter, 9° outside diameter), and mixture and comparison fields were presented in temporal alternation. Large-field matches were run in deutan mode for normal and deutan subjects and in protan mode for protan subjects. Maximum retinal illuminances for the red and green LEDs in deutan (D) and protan (P) mode respectively were: (D: red = 576 td, green = 908 td; P: red = 626 td, green = 67 td).

Subjects who tested as anomalous trichromats ($N = 17$) on either large- or small-field matches were retested under conditions designed to reduce possible rod contributions. These subjects were preadapted for 10 s to a large (64° broad-band, white adapting light (1.26×10^5 td; color temp, 3100 K). Rayleigh discriminations were then tested between 3 and 8 min following the offset of preadaptation. In those cases, match widths reported here are for the rod-bleached conditions.

2.4. Molecular analysis

Molecular genetic methods have been described previously [14,8]. Briefly, the gross structure of the genes encoding the L and M cone opsins as well as the sequences of the coding and promoter regions were determined using quantitative PCR amplification followed by SSCP analysis. To determine the presence of Ser or Ala at position 180, exon 3 was PCR amplified and the products digested with the restriction enzyme *Fnu*4HI. The predicted absorption maxima of the resultant photopigments were inferred from published data on photopigments expressed in vitro [15].

2.5. Flicker electroretinography

Flicker ERGs were recorded using a procedure similar to that of Neitz and Jacobs [16]. Briefly, a train of flickering pulses (35 Hz) of a monochromatic test light was interleaved with a train of pulses (35 Hz) from a fixed reference light (white or monochromatic). The intensity of the test light was stepped in small increments to determine the point at which the two lights produced ERG signals of equal amplitude and phase (the equation point). The inverse of the test intensity at the equation point was taken as the measure of relative sensitivity.

2.5.1. Spectral sensitivity

Monochromatic test lights (10 nm half-bandwidth) were flickered against a broad band white reference light (2300 td; CIE $x = 0.450$, $y = 0.444$). With this technique, the relative sensitivity to individual wavelengths across the spectrum can be measured to an accuracy of about ± 0.02 log units [9]. Sensitivity values were corrected for lens absorption [17] and fit to standard photopigment absorption curves [18] on a log wavenumber axis [19,20].

To distinguish subjects with both L and M pigments from subjects with L, or M pigments alone, the data were fit to single pigment nomograms as well as to a summative combination of two nomograms. In the two-cone fitting procedure, the λ_{max} of one cone was restricted to fall in the M pigment range (525–538 nm) while the λ_{max} of the other pigment was restricted to lie within the L pigment range (550–568 nm). The λ_{max} within the restricted ranges and the relative sensitivities of the two pigments were free to vary. The relative amounts of L and M pigment (symbolized by L/(L + M) below) required in these fits were used to infer which genes were expressed (see Section 2.6).

2.5.2. Chromatic adaptation

Chromatic adaptation increases the power of the ERG technique for distinguishing a single pigment from two pigments with small differences in λ_{max}. This experiment estimated photopigment spectral separation by testing for univariance as in the measurement of a heterochromatic threshold reduction factor (HTRF; [10]). The relative spectral sensitivity of a 640 nm test light versus a fixed 520 nm reference light was measured in the presence of 640 nm versus 520 nm adapting lights. The luminance of the adaptation and reference lights was varied to optimize signals for the different classes of observers (520 reference light: 8000 td for normals, 13000 td for deutans, and 2500 td for protans; 520 nm adaptation light: 7000 td for normals, 15000 td for deutans, and 2500 td for protans; 640 nm adaptation light: 14000 td for all subjects). The amount of change in the equation point with chromatic adaptation was used as an indicator of the spectral separation. If only a single pigment is present, the equation point should remain unchanged. If two separately adaptable photopigments are present, the equation point should change; the more widely spaced the pigments, the greater should be the shift in equation point.

The choice of the intensity of the reference light has little influence on the adaptation effect, merely shifting both equation points concordantly. The choice of the intensity of the 520 nm adapting light also has very little influence on the spectral sensitivity function because of the great overlap of spectral sensitivities on the short wavelength side of photopigment spectral sensitivity curves and the dominance of the longer wavelength cone in unadapted flicker photometric spectral sensitivities (e.g. Vλ). However, since there is a possibility of small differences in effects with the different adaptation intensities, correlations were computed separately for protans, deutans and normals, as well as for the combined group.

2.6. Analysis

When comparing color matching with molecular analysis, inferred pigment separation was computed both: (a) with no assumptions regarding expression and using the largest possible photopigment separation predicted from the genes; and (b) applying the ERG spectral sensitivity results and the following rules: (1) If the value of L/(L + M) is greater than 0.75 then no middle-wavelength pigments are expressed; (2) If the value of L/L + M is less than 0.25 then no long-wavelength pigments are expressed; (3) Assume the maximum possible pigment separation from the remaining genes.

3. Results and discussion

3.1. Classification by ERG spectral sensitivity

The values of L/(L + M) obtained from fitting the ERG spectral sensitivity functions can be seen in Table 1. These values fell into 3 groups (0.07–0.19; 0.53–0.68; 0.79–1.0) that reliably classify phenotype (protan, normal and deutan, respectively) when applying the rules described above. Thus, the ERG spectral sensitivity data provide independent support for assuming that L and M genes are not expressed in protans and deutans, respectively.

3.2. Color matching versus molecular analysis

Color match results are also shown in Table 1. Six subjects had small-field match widths at the instrument maximum (73 Nagel units) and were thus classified as

Table 1
Results of color matching, ERG, and molecular analysis

Subject	Color matching			ERG		Molecular analysis				
	Diag	Width		Adaption effect	Spectra (L/(L+M)	Inferred pigment separation	Prom (G:R)	Exon 3	Exon 4 (G:R)	EXON 5 (G:R)
		SF	LF							
3551	P**	73	0.036	−0.018	0.105	0	1.2, 1.4, 1.7	A	No red	No red
3616	P	73	0.950	0.029	0.136	0	1.1, 1.3	A	No red	No red
3636	P	73	0.975			0	1.3	A	No red	No red
*3491	P**	73	0.980	0.013	0.045	(0) 24	1.1, 1.1, 1.3	A and S	1.1, 1.0, 1.1	0.92
3727	PA	4	0.030	0.092	0.071	0	1.0, 0.85	A	No red	No red
3634	PA	14	0.035	0.057	0.188	2	1.3, 1.2	A and S	No red	No red
3607	PA	14	0.145	0.069	0.071	2	2.3	A and S	No red	No red
3638	PA	14	0.030	0.080	0.160	4	3.7, 4.9	A	3.7	No red
3626	PA	2	0.005	0.114	0.170	6	1.8	A and S	2.0	No red
3553	PA	3	0.015	0.052	0.184	6	2.1, 2.5, 2.3	A and S	1.6, 1.8	No red
3614	PA	5	0.055	0.088	0.177	6	1.2, 1.1	A and S	0.93	No red
3682	D**	73	0.302	−0.032	0.790	(1) 24	1.9, 2.3	A	0.73	1.3
3703	D	73	0.260			24?	2.2, 1.9	A	0.40, 0.52	0.34, 0.33
3609	DA	4	0.066	−0.040	0.796	1	1.1	A	No green	No green
3707	DA	9	0.027	0.070	0.877	5	0.92, 0.70	A	0.80, 0.95	No green
3676	DA	5	0.030	0.092	0.826	(4) 24	3.9, 3.1	A	1.0	2.4
3678	DA		0.010			24?	4.6	A	0.91	1.9
3731	DA	8	0.050	0.090	0.807	(1) 24	3.0, 3.0	A	0.95, 0.89	0.77, 0.84
3555	DA	16	0.040	0.081	0.995	(5) 29	2.7, 1.8, 2.3	S	0.50, 0.43, 0.44	1.9
3625	DA		0.075			24?	4.7, 4.6, 3.2	A and S	1.8	0.7
3561	N	1	0.009	0.209	0.676	31	1.0, 0.95	A and S	1.2	1.0, 1.4
3732	N	1.5	0.005	0.231	0.528	31	0.85	A and S	0.97, 0.89	1.2
3490	N	0.5	0.001	0.232	0.559	31	1.9, 1.8	A and S	1.8, 2.0, 2.2	2.3, 2.7
3568	N	1.5	0.001	0.210	0.582	31	1.7	A and S	2.3	2.0, 2.5, 2.2
3643	N	2	0.075	0.271	0.535	31	2.6, 2.7, 2.3	A and S	2.1	1.9
3639	N	1	0.058	0.130	0.671	24	2.6, 2.8, 2.1	A	4.1	1.9
3733	N	0.5	0.005	0.337	0.569	24	1.9, 1.3	A	1.8, 1.8, 2.2	2.2, 2.4
3545	N	1	0.031	0.259	0.537	22	3.2, 1.9	A and S	No red	2.3

Blank fields indicate that measurements were not made. Small-field color match diagnosis (column 2) is indicated by D (deuteranope), DA (deuteranomalous), P (protanope) and PA (protanomalous). SF and LF indicate small- and large-field color match widths respectively (columns 3 and 4). Magnitude of ERG adaptation effect (column 5) and the relative amounts of L and M pigments required to best fit ERG spectral sensitivity data (column 6) are shown. Pigment separations (column 7) are inferred from the data of Asenjo et al. [15]. Parenthetical values indicate separations corrected with ERG spectral sensitivity data (see Section 2.6). Question marks indicate values that remain ambiguous because ERG data could not be obtained. Molecular genetic results (columns 8–11) are presented using the original nomenclature of Nathans et al.[1]. The presence of serine (S) or alanine (A) at position 180 in exon 3 is given (column 9). The ratios of green to red promoter, exon 4 and exon 5 sequences as quantified by PCR amplification followed by SSCP analysis are indicated (columns 8, 10 and 11 respectively). Multiple entries represent results of independent determinations. Note that a 'red' exon 5 produces a long-wavelength pigment while a 'green' exon 5 produces a middle-wavelength pigment.
** Subjects that tested as anomalous w/o rod bleach.

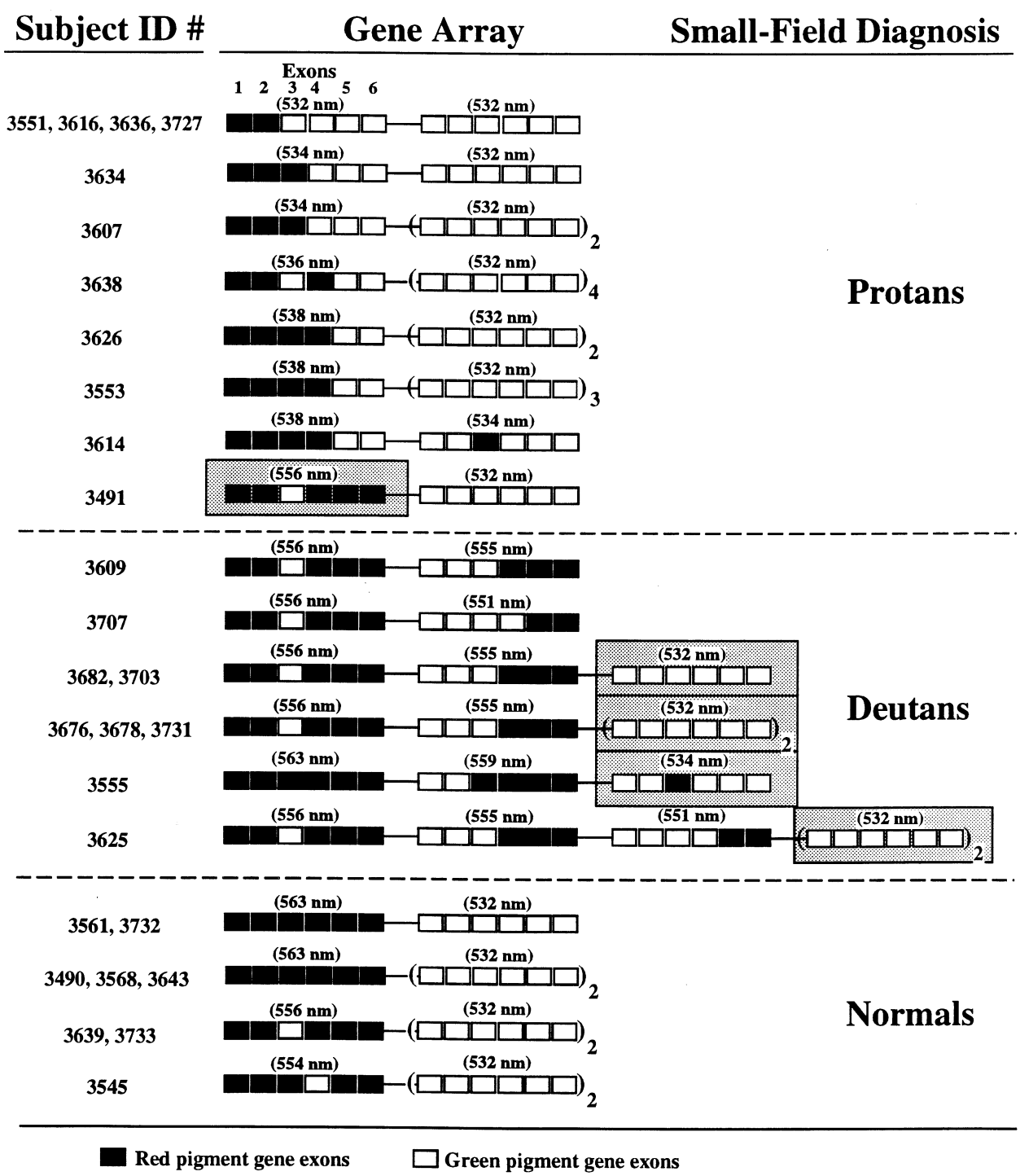

Fig. 1. Photopigment gene arrays and inferred absorption maxima. Small rectangles represent the six exons of the prototype red (filled) and green (open) pigment genes [1]. The absorption maxima inferred [15] from the sequence of these genes are given in parentheses above the exons. Multiple genes of the same sequence are indicated with subscripts. Genes that apparently are not expressed as functional pigments are enclosed by shaded boxes. Small-field diagnoses are also indicated.

either protanopes (P; $N=4$) or deuteranopes (D; $N=2$). Three of the protanopes had zero pigment separation which predicted their dichromacy. However, the fourth protanope (# 3491, single asterisk) had normal L and M photopigments by molecular analysis. This subject will be discussed in more detail below. The remaining subjects, tested as simple anomalous or normal on the small-field match. The data from which the gene structure was determined are also given in Table 1.

Fig. 1 shows the structure of the genes and the inferred absorption maxima of the encoded pigments.

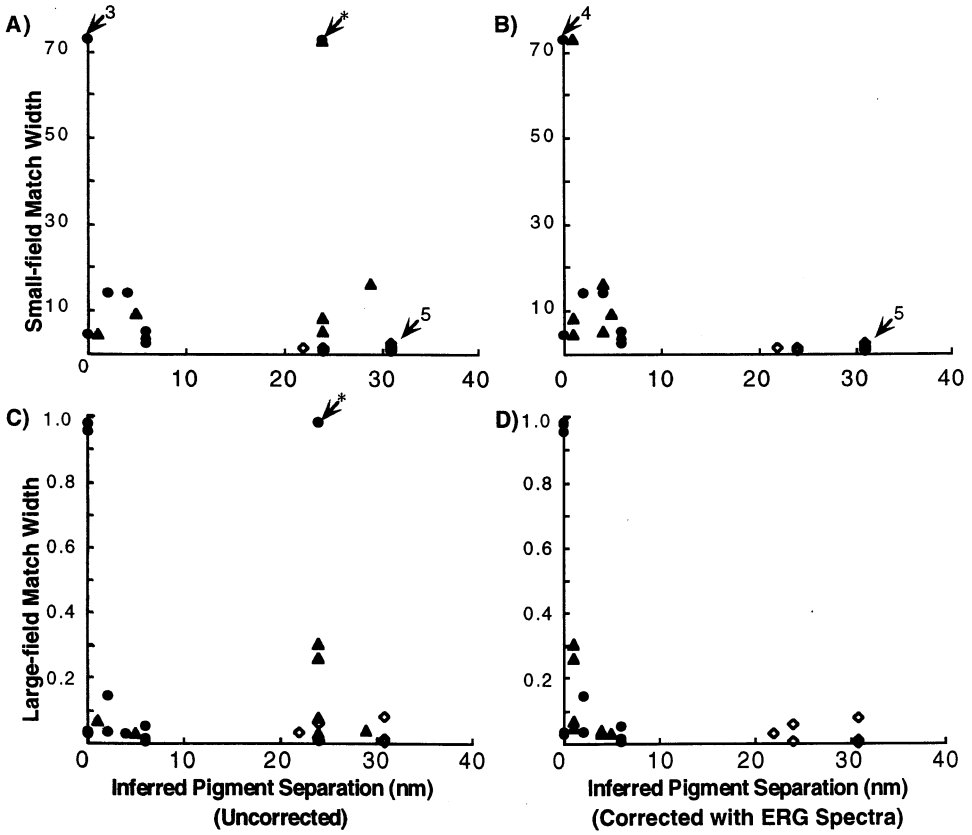

Fig. 2. Match width plotted as a function of inferred photopigment spectral separation. (A) Small-field match width in Nagel units plotted against pigment separation inferred from molecular analysis without correction with ERG spectral sensitivity (circles—protans; triangle—deutan; open diamonds—normal). An asterisk with an arrow indicates subject # 3491. Numbers with arrows indicate the number of superimposed data points. (B) Small-field match width plotted as a function of inferred photopigment spectral separation using corrections from ERG spectral sensitivities. (C) Large-field match width in LED anomaloscope units plotted against inferred photopigment spectral separation without ERG corrections. (D) Large-field match width plotted against inferred photopigment spectral separation using ERG corrections.

Shaded boxes enclose genes that are unlikely to be expressed by ERG analysis.

Fig. 2 shows comparisons of inferred pigment separation and color match width. The small-field match widths plotted against inferred pigment separation computed without using the ERG spectral sensitivity data are shown in Fig. 2A. Non-parametric correlations [21] computed without correcting the molecular genetics data with the ERG spectral sensitivity data (Fig. 2C) were weak for the entire group ($\gamma = -0.436$) and for the protans ($\gamma = -0.450$), and in the wrong direction for the deutans ($\gamma = 0.467$).

Inferred pigment separations corrected with the ERG spectral sensitivity data are shown in Fig. 2B. The effect of this correction is to shift the data for four deutan observers and one protan observer (# 3491) towards smaller pigment separations. Small-field match widths for the group correlated well ($\gamma = -0.734$) with corrected pigment separation, while individually, correlation for the protans was strong ($\gamma = -0.795$) and for the deutans was much weaker ($\gamma = 0.091$). It should be remembered here and elsewhere that correlations for the individual groups should be interpreted with caution because of small sample sizes and limited ranges. Interestingly, the subjects with inferred pigment separations between 1 and 6 nm showed individual differences in match widths from normal (2 Nagel units) to 16 Nagel units.

Large-field match widths plotted against inferred pigment separations are shown in Fig. 2C (uncorrected) and 2d (corrected with ERG). As before, correction moves 4 deutans and 1 protan towards smaller pigment separations. Interestingly, the 4 protans (5, corrected) with pigment separations of zero showed large individual differences in match width spanning from normal (0.030) to almost full range (0.980). Similarly, the deutans with 1 nm inferred pigment separations showed match widths varying from 0.027 to 0.302. This result is consistent with observations made on subjects with a single X-linked pigment gene [11]. As in the small-field match, subject # 3491 (single asterisk) with normal pigment genes, tested as a severe color deficient. Using the uncorrected pigment separations, the large field matches for the group correlated poorly with pigment separation ($\gamma = -0.295$) as did those for the protans ($\gamma = 0.227$) and the deutans ($\gamma = 0.048$). The correla-

tions were improved for the entire group using the corrected pigment separation data ($\gamma = -0.568$), for the protans ($\gamma = -0.650$) and for the deutans ($\gamma = -1.00$). For the entire group, the correlations for the large-field matches were relatively moderate, probably reflecting a strong 'floor effect' i.e. most subjects had small match widths.

The comparison of small- and large-field match widths with inferred pigment separation suggests that subjects who have pigment separations 2 nm or greater are able to make reasonable trichromatic matches. However pigment separation does not predict small-field match widths for deuteranomalous subjects who have pigment separations ranging from 1 to 5 nm. This result is consistent with previous suggestions that other factors such as optical density may play a role in determining color vision for anomalous subjects and even for subjects with a single X-linked gene (e.g. [22,23,11]).

3.3. ERG adaptation effect versus color matching

Fig. 3 shows comparison of match widths with the magnitude of ERG adaptation effect. Small-field match widths plotted against ERG adaptation effect are shown in Fig. 3A. Five subjects showed an adaptation effect of less than 0.05 log units. Four of these subjects had match widths indicating dichromacy while one subject behaved as an anomalous trichromat with good discrimination. All other anomalous trichromatic subjects showed ERG adaptation effects above 0.05 but below the range of normals (0.13–0.34). The small-field matches for the entire group correlated well with magnitude of ERG adaptation ($\gamma = -0.705$). Individually, correlation for the protans was strong ($\gamma = -0.744$) while again for the deutans was much poorer ($\gamma = -0.200$). The genetically normal, but phenotypically protanopic subject (#3491, asterisk) showed near zero adaptation effect, consistent with dichromacy.

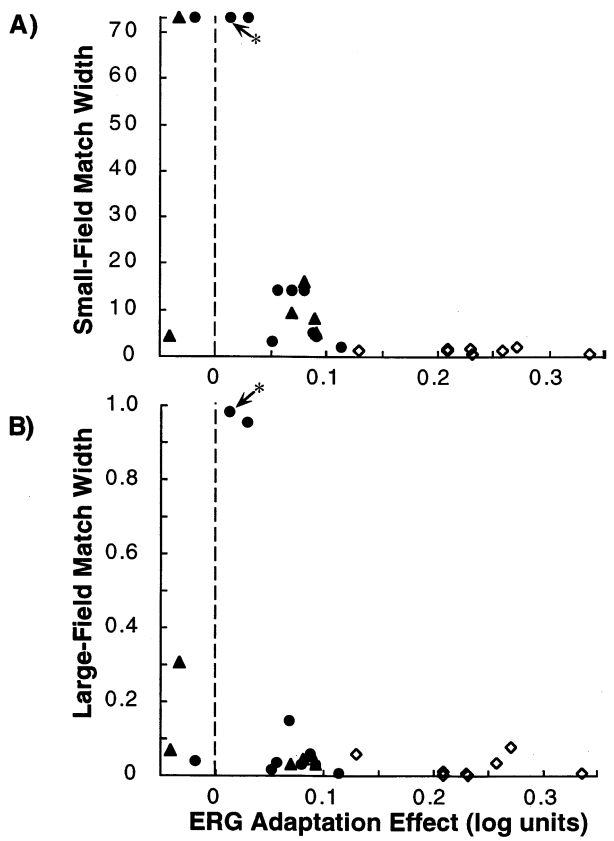

Fig. 3. Match width plotted as a function of ERG chromatic adaptation effect. (A) Small-field match width plotted as a function of ERG chromatic adaptation effect in log units. The dotted vertical line indicates zero adaptation effect. (B) Large-field match width plotted as a function of ERG chromatic adaptation effect in log units.

Large-field match widths plotted against magnitude of ERG adaptation effects are shown in Fig. 3B. With large-fields, only two of the five subjects with adaptation effects less than 0.05 showed match widths consistent with extreme color anomaly. One of these is subject #3491. Two other subjects showed match widths near normal on the large field. Ten of the 11 anomalous subjects with ERG adaptation effects between 0.05 and 0.12 also showed near normal match widths on the large field. Overall the large-field match widths showed a moderate correlation with magnitude of ERG adaptation ($\gamma = -0.413$). Individually, correlation for the protans was also moderate ($\gamma = -0.455$) as was that for the deutans ($\gamma = -0.333$).

The comparison of ERG adaptation effect with match width demonstrated that all subjects who showed evidence for more than one L or M photopigment (adaptation effect > 0.05) have relatively good red/green color discrimination. However some subjects with no evidence for a second L or M pigment (one subject on the small-field match and several subjects on the large-field match), nonetheless demonstrated red/green color discrimination. These results suggest that like the residual color discrimination of obligate dichromats [11], color matching in these subjects may be based upon other cues such as retinal inhomogeneities or photopigment optical density differences. Another possible explanation could be that the techniques employed may not have the accuracy to distinguish reliably among the anomalous subjects at this fine a scale. As has been suggested previously [6,11], other measures of color vision such as wavelength discrimination (e.g. [24]) or performance on pseudoisochromatic tests (e.g. [6]) may provide a more realistic measure of color discrimination than does color match width.

3.4. ERG versus inferred pigment separation

Fig. 4 shows how inferred pigment separation agrees with the magnitude of the ERG adaptation effect. Uncorrected data are shown in Fig. 4a. The correlation

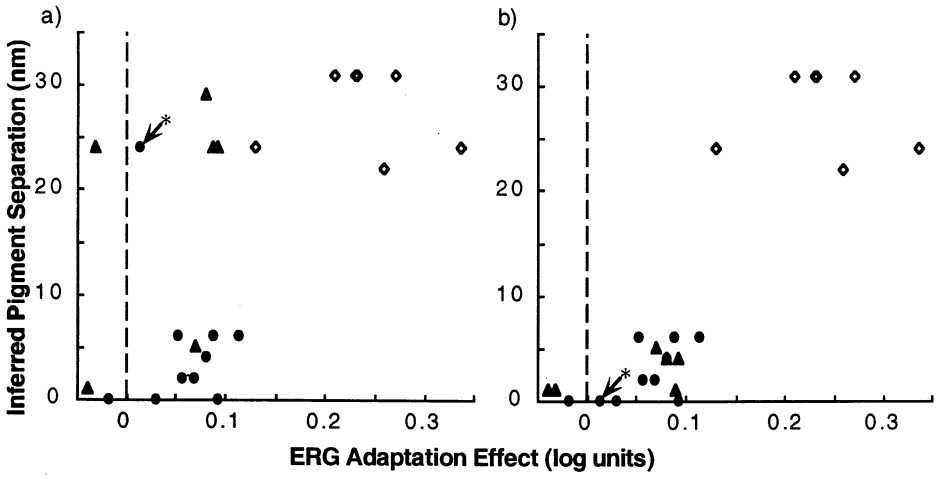

Fig. 4. (a) Inferred pigment separation without corrections from ERG spectra plotted as a function of ERG chromatic adaptation effect. (b) Inferred pigment separation using corrections from ERG spectra plotted as a function of ERG adaptation effect.

for the entire group ($\gamma = 0.523$) and for the deutans ($\gamma = 0.500$) was moderate, while that for the protans ($\gamma = 0.158$) was weak. After correction with ERG spectral sensitivity data (Fig. 4b) there was good general agreement, correlation for the entire group was stronger ($\gamma = 0.653$). Within groups, the correlations were moderate for the protans ($\gamma = 0.543$) and weaker for the deutans ($\gamma = 0.273$). The correlation of adaptation effect with pigment separation within the group of normals was also very weak and slightly negative (-0.176). These results suggest that resolution of the differences within the groups is beyond the capabilities of present techniques or that other yet unexplained factors may be contributing to the weaker correlations.

Seven of the nine deutan subjects had, in addition to normal L and M–L hybrid genes, one or more normal M photopigment genes (see Fig. 1 and Table 1). Color matching and ERG spectral sensitivity results suggest that these normal M pigment genes must not be expressed in significant quantities. The small ERG adaptation effects exhibited by these subjects are consistent with this notion and confirm our earlier observations that not all photopigment genes in the X-linked array are expressed [3,8]. To explain this selective gene expression, we proposed a model in which only the first two genes in the array are expressed sufficiently to influence color vision. According to this model, the normal M pigment genes of deuteranomals are hypothesized to occupy distal positions (Fig. 1) and are not expressed. In sum, the presence of similar genes arrays in normals and deuteranomalous individuals suggests that although required, the presence of hybrid genes is not sufficient to produce deuteranomaly. An additional mechanism directing expression is also required.

3.5. Subject # 3491

Fig. 5 shows an unadapted ERG spectral sensitivity obtained from subject # 3491. The solid line through the data is the best fitting visual pigment curve, with a λ_{max} of 530 nm. In agreement with color matching, and measures of ERG adaptation effect, the flicker ERG spectral sensitivity reveals a single M pigment (mean squared error = 0.00086). The fit is not improved with the addition of any amount of the predicted L pigment. Peripheral blood samples were subsequently drawn on two additional occasions and DNA analyzed separately each time to check for errors in molecular analysis. All three analyses showed the presence of one normal L

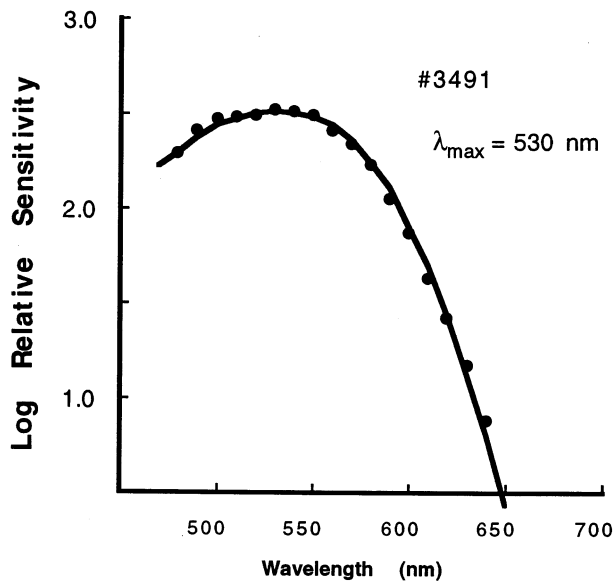

Fig. 5. Unadapted ERG spectral sensitivity obtained from protanopic subject # 3491. The solid line is the best fitting single visual pigment curve ($\lambda_{max} = 530$ nm).

and one normal M gene. In addition, the coding regions, the intron/exon junctions, the 3′ and 5′ untranslated regions, and the proximal (200 bps) promoters were sequenced and determined to be normal.

Unlike the deutans described above, lack of pigment gene expression is unusual for an individual with exactly two apparently normal pigment genes, particularly a protanope. Because the hypothesis that only the first two genes in the array are expressed in appreciable quantities is not tenable for this protanope, we suggest that lack of a functional L-pigment gene may be due to a mutation either in the introns or distal flanking regions that were not sequenced.

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