RE-ASSESSING PERCEPTUAL DIAGNOSTICS FOR OBSERVERS WITH DIVERSE RETINAL PHOTOPIGMENT GENOTYPES

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Introduction

For many years, reports have surfaced of atypical performance on standardized color-deficiency tests among female carriers of color-vision anomalies (presumed, on the basis of pedigree, to be heterozygous for the genetic alteration manifesting as the anomaly in their male relatives). As discussed by Cohn, Emmerich and Carlson (1989) heterozygous females fail to be detected by the use of an anomaloscope, although there are reported shifts in their anomaloscope color matches (Schmidt 1955; Crone 1959; Pickford 1959; Krill & Beutler 1964; Feig & Ropers 1978) as well as shifts using flicker photometry (Crone 1959; Yasuma, Tokuda & Ichikawa 1984). Heterozygous females were also found to exhibit higher absolute thresholds to small spots of red light (Krill & Beutler 1964, 1965). Unlike normal controls, these heterozygotes exhibit a failure of *additivity* of trichromatic color matches after exposure to a light bleaching of the rod system (Nagy, MacLeod, Heyneman & Eisner 1981). Thus, in some existing research, albeit much of it before 1984, subtle deviations from trichromacy were found using heterozygote participants. Still, these subtle deviations are generally considered to be examples of the large individual differences possible in color perception, and are not interpreted as deviations from functional trichromacy.

Some investigators have conjectured that such individuals, whose retinal cone-cell mosaics contain four photopigments, might experience a dimension of perceptual experience denied to trichromat individuals (Jordan & Mollon 1993; Mollon 1995; Deeb & Motulsky 1996). A model exists among some New World primates, where polymorphism of one of the two cone opsin genes they possess supports trichromatic vision among heterozygous females, although homozygous females and hemizygous females are dichromatic (Shyue,

Boissinot, Schneider, Sampaio, Schneider, Abee, Williams, Hewett-Emmett, Sperling, Cowing, Dulai, Hunt & Li 1998). However, the above conjecture about human observers is not popular among most color vision researchers.

Over the last decade, this research direction was invigorated by progress in molecular genetics: in particular, the discovery that variant alleles exist for the L and M photo-opsins, even within the non-deficient population, associated with shifts in the spectral sensitivity functions. The shifts are smaller (~5 nm) than those causing color-vision deficiencies, but not negligible, given that the peak sensitivities of the L- and M-opsins differ by only ~35 nm. This creates a prospect of alternative phenomenal worlds (Mollon 1992), with divergent color processing according to these alleles. Moreover, two polymorphisms (substitution of alanine for serine at codon 180 of the L-opsin, or *vice versa* at codon 180 of the M-opsin) appear to be sufficiently common – at least, in Caucasian populations – for heterozygosity in females to approach the norm rather than the exception. At the level of distinct photoreceptor classes, four and even five classes are possible in some groups; this adds salience to the suggestion that human color vision is capable of extension beyond trichromacy at a *functional* level.

The standard instruments for assessing color vision in applied settings – pseudoisochromatic plates (PICs) and hue sorting tests – are primarily tests of color consensus. Does the subject use color terminology in a manner sufficiently similar to population norms for effective communication, in situations where color is critical? Historically, occupational and vocational concerns have been paramount (for example, resistor coding, train signals, printing, hue-matching in industry and decoration). Such tests serve as valuable screening procedures for detecting and classifying dichromat and anomalous-trichromat observers, whose color perception is deficient in certain ways (a pattern of confusions characteristic of reduced discrimination along particular axes in color space). Such individuals also exhibit impoverished color-naming behavior, recognizing fewer color bands within the spectral hues. However, such tests were not designed with the possibility in mind of observers whose color discrimination is good, but operates in a non-standard way. They are not necessarily appropriate for detecting deviations that might occur if the neural trivariant property of human color vision were extended to include more than the usual three color processing channels – a deviation perhaps made possible in individuals possessing more than three classes of retinal cones. As discussed at the end of this paper, there may be methodological reasons why color perception differences apparently experienced by some female heterozygotes are not widely demonstrated using standard psychophysical methods of color

vision assessment.

Based on the controversy described above, this article investigates four issues concerning widely-used standardized tools for color vision assessment:

1. Do existing color vision assessment methods permit the detection of non-deficient deviations, or extensions, of trichromacy (if such extensions exist)?
2. Do existing methods sometimes mis-diagnose observers as deficient who otherwise have superior color vision abilities?
3. Are the patterns of confusions found in the Farnsworth-Munsell 100 Hue test (hereafter: F-M 100) predictable from individual observer's photopigment opsin genotype?
4. Do the F-M 100 results for genotypes capable of expressing four or more retinal cone classes clarify the nature of the color perception difference experience (if any), and do such F-M 100 results provide insights into the debate raised by others (namely, Mollon, 1992; Jordan & Mollon 1993; Deeb & Motulsky 1996) concerning the potential for extended dimensionality in human color perception?

In the Discussion we suggest directions for updating existing color vision assessment methods to identify and classify color perception differences found correlated with photopigment opsin genotypes.

The genes for retinal photopigments

Recent research into the molecular genetics of retinal photopigments enables an understanding of photopigment sensitivity as well as the genetic basis for individual differences in color perception. Studies show that variation at the level of the genotype corresponds to shifts in the absorption spectra of expressed retinal pigments (Asenjo, Rim & Oprian 1994, Merbs & Nathans 1992a, 1992b, 1993) that produce concomitant shifts in spectral sensitivity (Neitz, Neitz & Jacobs 1991, 1995; Winderickx, Lindsey, Sanocki, Teller, Motulsky & Deeb 1992).

The genes for medium-wavelength sensitive (M) and long-wavelength sensitive (L) retinal photopigments are located on the X chromosome, in a head-to-tail array, with the L gene first. The genetic sequences for these two photopigments are almost identical (Asenjo et al. 1994; Sharpe, Stockman, Jaegle, Knau, Klausen, Reitner & Nathans 1998; Neitz & Neitz 1998). The DNA sequence homology or identity for the two genes is 98%. Although the amino acid sequences of the M- and L-pigments are thus almost identical, studies have shown that photopigment sensitivity to medium or long wave light

is determined entirely by substitutions of seven amino acids occurring at codons 116, 180, 230, 233, 277, 285 and 309 of each gene (Asenjo et al. 1994).

Individual variability in color perception is associated with genetic variability at one of these critical amino acids, codon 180 in exon 3 of the L- and M-opsin gene. In both genes, the amino acid at codon 180 has been shown to be polymorphic. In the Caucasian population, approximately 60% and 40% of males will have the amino acids serine and alanine respectively at codon 180 in their single L-photopigment gene. The average λ_{max} for red light is 557 nm for the 60% majority, but, in the minority, it is 552 nm: their red-light spectral sensitivity is shifted closer to the λ_{max} for green light, which is 532 nm. Thus, this amino acid substitution, or polymorphism, gives rise to differences in spectral sensitivity to light, and thus, to individual variation in color vision. The corresponding substitution in the M gene – of serine for alanine at codon 180 – is present in about 9% of Caucasian males, but appears to have a smaller effect on spectral sensitivity to green light (Sharpe et al. 1998).

Complicating further the analysis of the relationship between genotype and perceptual behavior is the location of the M- and L-genes on the X chromosome. By virtue of two X chromosomes, females have two arrays of M- and L-genes, whereas males, with only one X chromosome, are limited to a single array. As a result, the genetic variability in the M- and L-photopigment gene combination is potentially greater for females than for males: the number of possible M-opsin and L-opsin genotype combinations at codon 180 is ten and four respectively. For this reason, one might expect to find greater variability in perceptual behavior in females.

Here we use modern molecular methods to determine an individual's M- and L-photopigment opsin genotype, especially codon 180 polymorphisms, and examine its relationship to color vision behavior. The genotyping method employed has been described elsewhere, and will only be reviewed here as necessary for explanation of subjects' genotype classifications (Wasserman, Szeszel & Jameson 2001). Briefly, the method makes use of a long-range polymerase chain reaction technique (LR PCR) to generate gene-specific PCR products, DNA sequencing to confirm this gene specificity and then PCR and restriction digest to determine M and L codon 180 genotypes. Results from the use of this method demonstrate a correlation with perceptual behavior and give significant insight into mechanisms contributing to the variability in perceptual behavior (Wasserman et al. 2001).

Note, first, that a female who is putatively homozygous for the codon-180 polymorphism may, in fact, be heterozygous for protanopy or deuteranopy (if the L- or M-opsin gene respectively is missing from one X chromosome).

Conversely, more than one copy of either of these genes may occur in a single X-chromosome array. However, PCR analyses can detect the presence of both alleles of the L- or M-opsin in a female, without guaranteeing that they lie on separate arrays. The possibility must also be considered that both lie on the same X chromosome. So, if in a given female, the PCR detects the presence of both L-180 alleles and a normal M-180 gene, then one possible configuration is that both X chromosomes possess multiple L-opsin variants in the first two positions of the array. According to one theory, however, only the first two genes in the array are expressed, regardless of the opsins they encode; the M-opsin genes would not be expressed (being third or further downstream on the array). The individual would be thus heterozygous for deuteranopy. Another version of this theory states that, in the case of two L-opsin genes present on one X chromosome, only the first will be expressed, that is, the individual is *effectively* homozygous for the L-opsin, despite the PCR result indicating two L-180 variants. Similar complications exist for interpreting PCR detection of M-180 polymorphisms. However, the notion that L-gene variants are expressed with a greater probability than M-gene variants seems to be a popular idea. Until the mechanisms underlying these issues are resolved (see Carrel & Willard 2005), these alternatives to expression of heterozygosity must be borne in mind.

A final possibility is that, when the PCR detects (for instance) the alanine allele of the L-opsin, it is actually responding to a chimeric M-opsin gene (into which exon 3 of the L-opsin has been grafted by meiotic mishaps). The parallel possibility applies to the serine allele of the M-opsin. Thus, an individual who is putatively heterozygous for the polymorphism may, in fact, be heterozygous for protanomaly or deuteranomaly. Though rare in the population overall, these conditions may be encountered in a portion of the subjects' data discussed here, since some were recruited by an advert emphasizing a family history of color deficiency.

For these reasons, not all females genetically identified as heterozygotes possessing M- and L-opsin gene polymorphisms necessarily express more than three retinal cone classes in their phenotype. In a few cases, such heterozygous females could be phenotypically anomalous trichromat or deficient, some might be normal trichromat, whereas others, through the right combination of genes and expression events, might phenotypically express four or five classes of cones in their retinae. This issue is worth noting in the discussion of color vision assessment methods because it implies that, even under the assumption that neural trichromacy is a fixed feature of the system, color perception diagnoses might be expected to be more variable, or to be differently distributed

for a group of female heterozygotes compared to a group of females with non-heterozygous genotypes. Thus, issues of functional tetrachromacy aside, the question of whether standard tests are capable of differentiating two such groups on purely perceptual grounds is a question of interest for evaluating the utility of such tests in genotype / phenotype investigations.

Color-vision assessment and photopigment genotyping

Subjects

With permission of the University of California, San Diego (UCSD) Human Subjects Committee, informed consent was obtained from 39 female and 26 male UCSD undergraduates for participation in this study. (Data for five of the male subjects sampled are not included in the present study because insufficient DNA was available for genotyping at the time of the implementation of the LR PCR method). Three milliliters of venous blood from each student was collected into EDTA vacutainer tubes by a trained phlebotomist. Subjects were solicited through either the Psychology Department Human Subjects pool, or by posted solicitations for experimental participation for either cash payment or for course extra-credit. To address specific empirical hypotheses, some female subjects were obtained through solicitations designed to maximize the yield of participants that were carriers or expressors of color vision deficiencies or anomalies. Following DNA extraction, a long-range polymerase chain reaction method was used to specify the presence of codon 180 polymorphisms on Exon 3 of the L- and M-opsin genes. The method used is described in Wasserman et al. (2001). Results of the genotype classification for 60 subjects are presented in Tables 1 and 2.

Stimuli and procedure

All subjects were assessed using (1) a chromatic banding task (Jameson, Highnote & Wasserman 2001), (2) standardized color vision assessment, and (3) photopigment opsin genotyping. In task (1) all subjects indicated where they saw distinct bands of color within a chromatic spectrum produced with a diffraction grating (Task 1 data from one subject was discarded due to non-compliance with the established protocol). Details of this chromatic banding task are provided elsewhere (Jameson et al. 2001). Next, all subjects were tested with the Ishihara PIC plates. In addition, the F-M 100 Hue test was administered to the subjects, using standard illumination and instructions. Finally, opsin genotyping was conducted, as described above, and in Wasserman et al. (2001). Experimenters and subjects were uninformed

regarding retinal genotyping of the subjects assessed.

The F-M 100 belongs to a family of sorting and matching tests in which small pigment-coated stimuli ('caps') are arranged in linear sequence, so that most-similar caps are adjacent, together forming a color gradient (Farnsworth 1943). It uses eighty-five caps, of moderate lightness and saturation (Munsell Value 5, Chroma 5). They form a complete hue circle but, for convenience, are presented in four quadrants, to be sorted separately. Analysis consists of considering each cap's immediate neighbors in the sequence: departures from their *numerical* sequence (the 'correct' arrangement) are converted to an error score. For instance, a simple transposition of two caps (for example, 1, 2, 4, 3, 5, 6…) means that four caps (2, 3, 4 and 5) are each adjacent to one cap that is two steps away in the sequence numbering, rather than a single step, and each accrues a score of one. Scores are generally plotted in polar coordinates (Figure 4).

The F-M 100 rationale is that the possibility of sequencing errors increases with any reduction in color discrimination. Reduced blue / yellow discrimination, for instance, as in tritanopia, makes confusions likely among red caps (which are distinguished only by tinges of blue or yellow) and among green caps. Since the difference between adjacent caps is small – near the threshold of perception – even a normal observer will commonly make a few minor errors such as transpositions. The total error score to be expected from a normal observer is dependent on age (Verriest, Van Laethem & Uvijls 1982, provide norms and standard deviations), but, according to one rough guideline, a total of less than twenty indicates superior color discrimination, while more than one hundred warrants further testing of the subject (on its own, the F-M 100 is not sufficient for diagnostic purposes). Because of floor effects, superior discrimination over *part* of the spectral range will not be localized in the same way as decreased discrimination, if it is detected at all.

Data analysis method

Results from the chromatic-banding task (Jameson et al. 2001) are summarized here by a simple descriptive measure of individual perceptual behavior: 'median number of perceived colors'. This is tantamount to the median number of different chromatic percepts a given observer detects in a series of judgments for diffracted spectrum stimulus. Here, when group measures of individual data are reported, they are given as group means of median numbers of individually perceived colors. In essence, Jameson and colleagues found that subjects possessing polymorphous photopigment opsin genotypes significantly identified more chromatic appearances in diffracted

spectra compared to subjects with non-polymorphous genotypes (see Jameson et al. 2001). Comparisons are made here between subjects' F-M 100 performance and the chromatic banding results reported in the Jameson et al. (2001) study. Total F-M 100 error scores were computed for each subject, as described above.

Underlying the development of the F-M 100 is a geometrical model: that the dissimilarities among colors perceived by a congenitally color-deficient individual correspond to a personal color space that is a compressed version of the color space perceived by normal trichromats (Farnsworth 1943). The direction and extent of compression indicate, respectively, the class and severity of the deficiency. Combined with the mathematical techniques of individual-differences MDS, this insight can be used to extract parameters of color-space compression from individuals' ratings of color dissimilarity (Paramei, Bimler & Cavonius 2000). However, MDS is not restricted to explicit *values* of dissimilarity. The algorithms can also handle *comparisons* between dissimilarities. Since F-M 100 sequences can be reduced to comparisons (to arrange two caps as neighbors is to assert that they are more similar to each other than to the other caps with which they were not arranged), they are amenable to this form of analysis. In this case, the emphasis is on the parameters for each subject, rather than on the 'map' of the stimuli also produced by MDS. Indeed, a *constrained* form of MDS was used: the coordinates of the F-M 100 points in CIELUV color space were provided (for details see Bimler, Kirkland & Jacobs 2000).

There followed another, more exploratory, application of MDS, involving comparisons between subjects rather than stimuli. A displacement was found for each cap (the absolute value of the difference between its positions in the correct F-M 100 and in an actual sequence). These were treated as the coordinates of a single point in an 85-dimensional space, and Euclidean distances among points were calculated, resulting in a 39-by-39 matrix of distances among female subjects, and a 21-by-21 matrix among males. The task of MDS in this case was to arrange subject points in a lower-dimensional space so that inter-point distances reconstructed these matrices as accurately as possible.

Results

Though results for males will be used to illustrate particular points, the emphasis on this report is on the female subjects. Their data (grouped by genotype) are summarized in Table 1. Here Z values are the square root of the F-M 100 total error, converted into the number of standard deviations away

from age-specific norms (Verriest et al. 1982). Table 2 presents only opsin genotype information for male subjects, without going into detail about their test scores. Note, two males were found with both serine (Ser) and alanine (Ala) amino acid residues on the L-opsin gene at codon 180 in exon 3 (Table 2, row 4). Two additional males showed both Ser and Ala at M-180 in exon 3 (Table 2, row 5). And one additional male showed both Ser and Ala responses to both L-

ID	FM100	Z	Ishihara	Bands
Heterozygous females				
L-180-Ser/Ala	M-180-Ser/Ala			14 cases
27	84	1.68	2	13.5
28	20	-1.13	0	8
34	112	.96	0	9
36	20	-1.13	0	16.5
37	32	-.02	0	14.5
43	20	-.59	0	8
44	28	-.19	0	9
52	52	.73	1	9
58	64	.72	0	7
61	56	.87	1	8.5
67	32	-.51	0	7
70	16	-.93	0	14.5
85	132	2.54	0	12
91	84	.38	1	—
L-180-Ser/Ala	M-180-Ser			1 case
51	16	-.82	0	6
L-180-Ser	M-180-Ser/Ala			7 cases
08	32	-.36	0	13
10	80	1.57	0	11
16	56	.45	0	14
49	92	1.55	0	6
63	32	-.02	0	7
75	32	-.51	0	8
87*	20	-.59	0	11

ID	FM100	Z	Ishihara	Bands
Homozygous females				
L-180-Ser/Ser	M-180-Ser/Ser			10 cases
14	192	3.78	2	6
23	44	.00	0	10.5
38	24	-.38	2	6
46	108	2.27	1	8
68	28	-.19	1	12.5
73	12	-1.66	0	7
76	56	.87	2	7
77	60	.58	0	8.5
86	72	.97	2	8
89	40	.31	—	6
L-180-Ala/Ala	M-180-Ala/Ala			7 cases
22	120	1.46	3	6
25	60	.99	1	6
32	36	-.33	0	10
54	20	-.59	0	7
62	36	.15	1	6
69	32	-.36	0	12
71	52	.73	2	6.5

* 'L-180-Ser / Ala' indicates Codon 180 amino acid residues present for both serine (Ser) and alanine (Ala) of the L-cone photopigment opsin gene. 'M-180' denotes M-cone opsins detected. The frequencies in Table 1 do not arise from a random sample and should not be taken to reflect population genotype frequencies.

Table 1: *Frequencies of genotypes from 39 female participants evaluated using the new LR PCR method*

L-180-Ala	M-180-Ala	9 cases
L-180-Ser	M-180-Ser	6
L-180-Ser	M-180-Ala	1
L-180-Ser+Ala	M-180-Ala	2
L-180-Ser	M-180-Ser+Ala	2
L-180-Ser+Ala	M-180-Ser+Ala	1

* 'L-180-Ser+Ala' indicates Codon 180 amino acid residues present for both serine (Ser) and alanine (Ala) of the L-cone photopigment opsin gene. 'M-180' denotes M-cone opsins detected. The frequencies in Table 2 do not arise from a random sample and should not be taken to reflect population genotype frequencies.

Table 2: *Frequencies of genotypes from 21 male participants evaluated using the new LR PCR method.*

180 and M-180 in exon 3 (Table 2, row 6). Of these five, only the two males with both residues at M-180 made few F-M 100 errors: 20 and 28. These genotype cases reflect complexities produced by hybrid opsin genes that encode chimerical photopigments.

Ethical reasons made it necessary to test subject 87 (heterozygous at M-180) twice on the F-M 100. The first test result showed signs of tritanomaly, raising concerns about the state of her visual health. Fortunately, her retest results were normal and locate her within the central normative clusters of Figures 1 and 3. Retaining her initial results would locate her as an outlier, without affecting the conclusions.

Next, the subjects' F-M 100 sequences were quantified with the individual-differences MDS method described by Bimler et al. (2000). Involving a Maximum-Likelihood algorithm, this yields a pair of color-space compression parameters that best account for a subject's sorting decisions: an axis and an extent of compression, θ and r. A third parameter, the 'discriminance' β, is not used here. Here $-90° \leq \theta \leq 90°$ (where 0° corresponds to the Red-Green axis of the CIELUV color plane), and $0 \leq r \leq 1$ (where 1 corresponds to a dichromat's color plane, collapsed to a line). The results for thirty-eight female subjects are plotted in polar coordinates as Figure 1, using 2θ as the angular coordinate, and r as the radial coordinate. Subject 14 (a double homozygote) is omitted as an outlier. As well as erring on two Ishihara plates and discerning only six bands in the spectrum (below the median), her parameters pointed to a protanomalous color deficiency. Also included in Figure 1 are parameters for two males (Subjects 80 and 90) who failed the Ishihara test: one (genotype Ser / Ser)

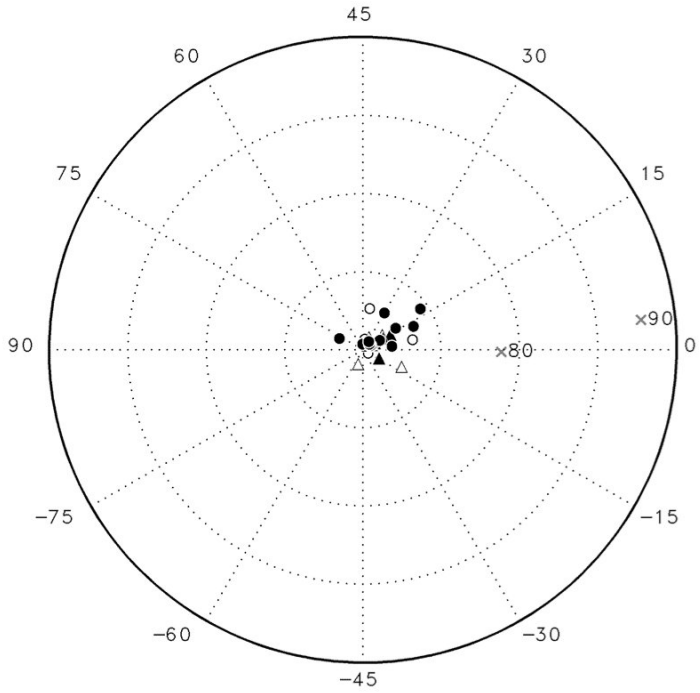

Figure 1: *Color-space compression parameters, plotted in polar coordinates, for female subjects. Results for two color-deficient male subjects (Ss 80 and 90) are also presented for comparison. Female genotype classes are represented by different symbols in the central cluster of the compression parameter plot.*

seems to be severely deuteranomalous, and the other (Ala / Ala) extremely so.

For clarity, the females' compression parameters are grouped by genotype and plotted on an enlarged scale in Figure 2. An interesting feature in panel (a) is the trend for cases to be displaced from the center in a specific direction, corresponding to an axis of compression of about 15° (causing confusions among green-yellow caps in the range 25-30, and among purple caps around 70). The compressions are subtle, but five out of fifteen cases exceed an arbitrary cut-off value of $r > 0.1$ (a sixth, S 34, also shows compression but in a different direction). These are subjects heterozygous for the L-180 polymorphism – all but one are also heterozygous for the M-180 polymorphism (giving the latter group five different classes of opsin gene variants). Similar but smaller compressions can be seen among the seven subjects heterozygous for the M-180 polymorphism only. The 16 homozygotes are spread with more

symmetry; though three exceed the same cut-off, their axes of compression are different (S 22 performs below average in terms of Ishihara errors and color bands).

The impression of a systematic difference is necessarily only suggestive: statistical certainty would require considerably more data. Results of these analyses that show systematic differences in F-M 100 responses suggest improvements in F-M 100 scoring which could extend the diagnostic capabilities of the F-M 100 to identification of S-180-A heterozygotes.

The result of applying non-metric MDS to the matrix of inter-subject dissimilarities is shown as Figure 3. A two-dimensional MDS solution provided a good representation of the matrix, with $Stress_1 = 0.060$. Most of the F-M 100

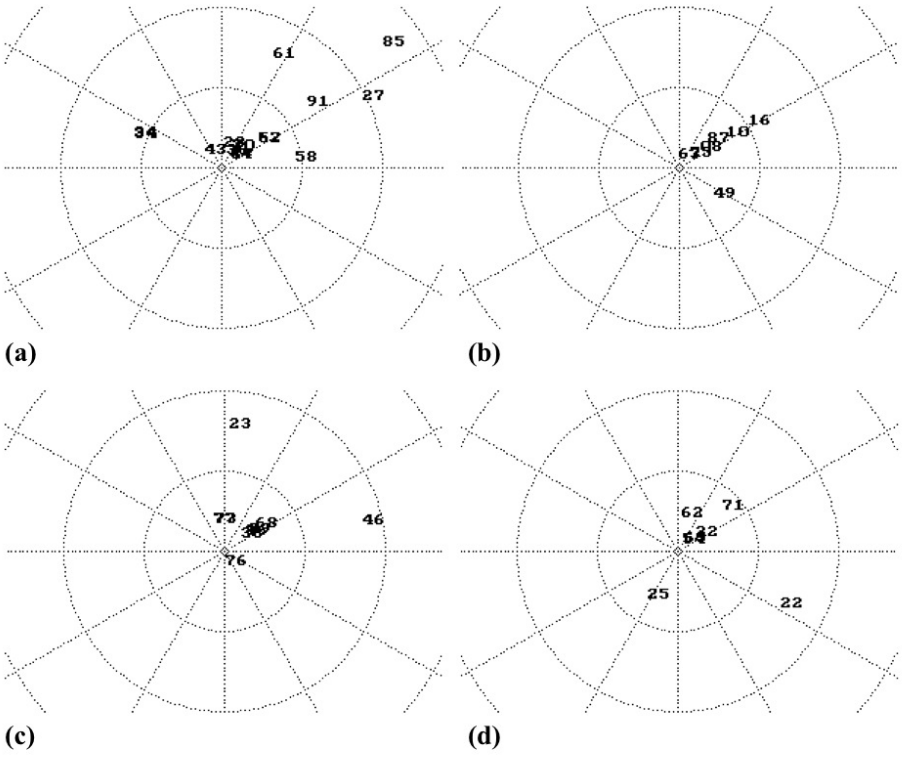

Figure 2: *Color-compression parameter plots for subgroups of genotyped females. Starting at upper-left, panels a, b, c and d depict: (a) 15 females heterozygous for L-180 polymorphism; (b) 7 females heterozygous for M-180 polymorphism; (c) 9 homozygous females (L-180-Ser, M-180-Ser genotype); (d) 7 homozygous females (L-180-Ala, M-180-Ala genotype).*

responses are close to the correct sequence, and consequently close to one another, forming a tight central cluster. The outlier 14 is not visible in the solution and lies some distance over to the left.

The index of inter-subject dissimilarity is crude and global. This way of approaching the data is less constrained than the individual-differences algorithm used above, and makes fewer assumptions about any pattern underlying the errors. Even so, some heterozygotes are again separated from the bulk of the individuals. A straight line can be drawn that discriminates six of fifteen double heterozygotes (Ss 85, 27, 61, 58, 91, and 34, whose point falls off

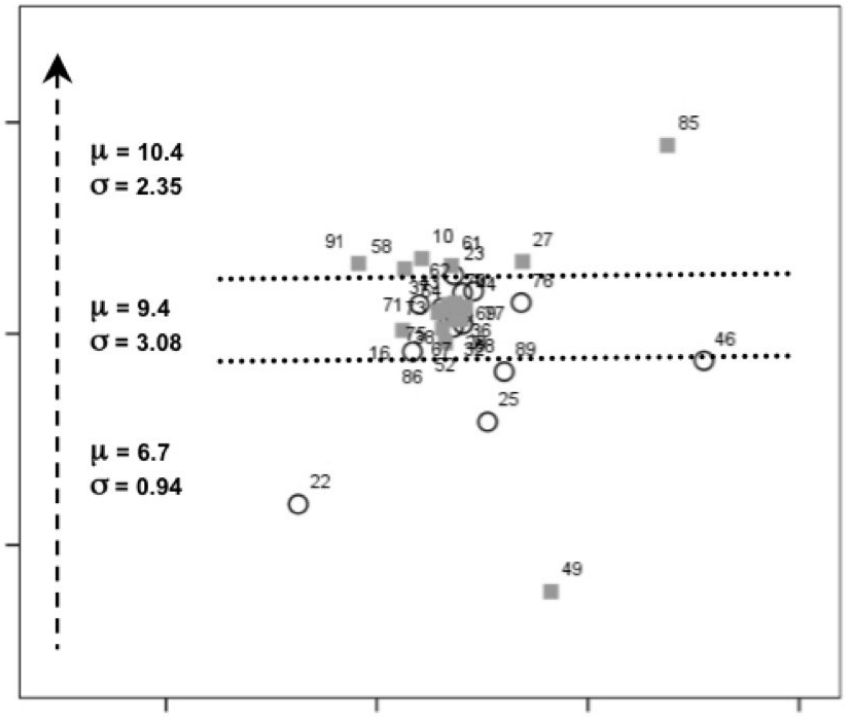

Figure 3: *Two-dimensional dissimilarity scaling of F-M 100 sorting performance for 37 female subjects. The two horizontal lines were drawn* ad hoc *to illustrate the association between F-M 100 performance, genotype, and perceived colors delineated in the Jameson et al. (2001) task. Gray square symbols denote heterozygote females, and unfilled circles denote homozygous females. Plotted numerals correspond to ID numbers in Table 1. The vertical dashed arrow represents increases and decreases in measured banding behaviour. Mean (*μ *) and standard deviation (*σ*) banding values for subgroup partitions are also shown.*

the top of the Figure 3 plot) and one M-180 heterozygote (S 10) from all the homozygotes.

Figure 3's 'top partition' above the top-most horizontal line contains only heterozygotes; the 'middle partition' contains both heterozygotes and homozygotes; and the 'bottom partition', beneath the bottom-most horizontal line, contains homozygotes and one aberrant heterozygote. Gray square symbols indicate subjects who possess at least one codon-180 opsin gene polymorphism (n=21, excluding aberrant heterozygote 34 who is off the top of the plot). Unfilled circle symbols indicate subjects who are codon-180 homozygous (n=16, excluding the aberrant homozygote 14 who is off the left side of the plot). Summary data (means, μ and s.d., σ) for the median-banding measures are presented for each partition. The structure has been rotated to bring a regression-line for the chromatic banding data close to the vertical axis (in fact 5º counter-clockwise off vertical) which is shown as Figure 3's vertical dashed arrow.

Interestingly, the seven heterozygotes departing from the normative grouping of homozygotes and heterozygotes in Figure 3 are not perceptually color-abnormal, as the F-M 100 scaling might appear to suggest. Indeed, these same subjects are also differentiated by their banding behavior in the Jameson et al. (2001) task as *above average*, not deficient, color perceivers. In the top partition of heterozygotes, the average number of median chromatic delineations was 10.4 bands (s.d.=2.35) (banding data was not available for S 91), compared to 7.9 bands (s.d.= 2.12) for homozygotes represented by open circle symbols across the 3 partitions. Five participants plotted below the bottom horizontal partition are homozygous and one is heterozygous for the M-180 opsin. This bottom partition averages a 6.67 (s.d.=0.94) chromatic banding measure. Although Figure 3's horizontal lines form *ad hoc* partitions, they serve to illustrate a monotonic relationship between the number of chromatic bands observed and subject similarity based purely on dissimilarity scaling of F-M 100 data.

The important point conveyed by Figure 3 is that the results from two different and independent tasks converge in showing a difference between the color perception of female subjects with heterozygous and homozygous genotypes. Subject groupings derived by scaling F-M 100 inter-subject dissimilarities are related systematically to chromatic banding behavior (Jameson et al. 2001). While confirming the difference, this raises the question why observers who are excellent color discriminators – the top partition in Figure 3 – should emerge from the F-M 100 as non-normative 'outliers'. While

the F-M 100 appears to detect the variation in these observers' color perception, in some cases, the scoring procedure does not distinguish between *non-normative* deficient and *non-normative* good color perception. The explanation that these aberrant heterozygotes are truly anomalous in the sense of deficiency is further undermined by the absence of color confusions in their everyday color experience, and the fact that the M-180 heterozygote (S 10) was the only one to report familial color deficiency (she reported paternal anomalous trichomacy).

These findings are further supported by comparing Table 1 data with the Jameson et al. (2001) banding results just described. Table 1 includes seven heterozygous females and six homozygous females with Z-values differing from normative F-M 100 performance by one standard deviation or greater (heterozygotes 27, 28, 34, 36, 85, 10, 49, and homozygotes 14, 46, 73, 86, 22, 25). Comparing the banding behavior of these seven heterozygotes and six homozygotes shows the heterozygotes average 10.9 median chromatic bands (s.d.=3.54), compared to the homozygotes' 6.9 (s.d.=1.11) average. A t-test shows this difference to be significant at p=.025 (two-tailed).

However, if outlier heterozygote S 49 is excluded from the heterozygote group, then the difference in banding behavior between the two groups obtains significance at p=.005 (two-tailed) based on the recomputed mean=11.7, s.d.=3.09 for heterozygotes. Also, the perceptual banding behavior of these six heterozygotes is not significantly different from the other heterozygotes who received Z-values indicating normal F-M 100 performance (p=.215, two-tailed).

At a minimum, these results suggest that, with the exception of S 49, the female heterozygotes who 'failed' the F-M 100 have good color perception on a spectral delineation task, their banding behavior does not differ from other heterozygotes whose Z-values indicate they passed the F-M 100 ($-1 < Z < 1$), and their banding behavior significantly differs from homozygotes who similarly 'failed' the F-M 100. A less conservative interpretation of these results is that the F-M 100 can diagnose heterozygotes as false-positive deficient when their color perception is otherwise unimpaired, and their color sense is generally regarded as excellent.

To illustrate how the subjects' F-M 100 responses are interpreted by Farnsworth's (1943) recommended scoring procedure, the error scores per cap are plotted in Figure 4 for two heterozygous observers, polymorphic at S-180-A for both the L and M genes (c. and d.), juxtaposed with the results for a 'classic' protanope (a.) and a normal trichromat (b.). Clearly, compared to normal (b.), the F-M 100 polar coordinate plots for (c.) and (d.) suggest impaired color perception, and resemble more closely the plot for dichromat (a.). Such a magnitude of difference would almost certainly affect everyday color

processing: in dichromats, it is already known to dramatically affect the processing of color in applied circumstances such as color-coded data in information displays.

Despite the patterns in Figure 4 suggesting a 'deficiency' in color discrimination capabilities, individuals (c.) and (d.) were reported by Jameson et al. (2001) as exhibiting above-average color perception performance (they perceived 12 and 9 chromatic spectral bands, respectively). Compare this with the protanope (a), who perceived only 4 chromatic bands in the spectrum, and the 'normal' trichromat (b) who perceived 7 bands. Such discrepancies indirectly support the Jameson et al. (2001) results discussed earlier, in that the F-M 100 standardized method characterizes the color perception of some heterozygous females as *non-normative* compared to homozygous females who are generally characterized as color-vision 'normal'.

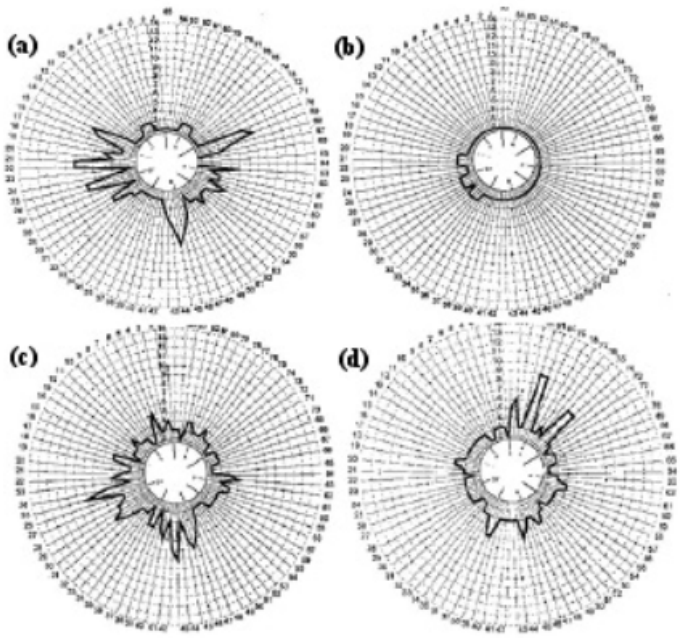

Figure 4: *Examples of Farnsworth-Munsell 100-Hue test results for: (a) a dichromat; (b) a trichromat; and two potential retinal tetrachromats ((c) and (d)).*

Such anomalies arising from color-vision assessment are perhaps not surprising. Standard color-vision tests like the F-M 100 were not designed to assess individuals expressing four photopigment classes, and, for this reason,

they are difficult to evaluate as appropriate measures of the color perception abilities of such individuals. With their vocational focus, such tests were generally calibrated for males, for a combination of reasons – including the lower incidence of overt color deficiency among women, and the effective exclusion of women from many occupations at the time of the tests' design. These details further support the idea that some color vision screening tests may erroneously identify four-pigment females as color-deficient or anomalous.

To sum up, some of the heterozygotes depart from what is considered the trichromatic norm in a systematic way: in some circumstances, they experience color more richly than normal (for example, color bands delineated in spectra), while making color confusions in particular zones of the hue circle. Using standard interpretations, the latter phenomenon could be construed as reduced color discrimination in those zones; or, more directly, as evidence that a different sorting sequence for the stimuli is more natural for some heterozygotes. In the former case, there is a possibility that the reduced discrimination is compensated for by heightened discrimination in other zones (not picked up by the F-M 100, because of floor effects). Such heightened discrimination might dictate a subjective ordering of the F-M 100 samples that is at odds with the 'correct' ordering recommended by the scoring manual. In any case, this kind of discrimination difference does not necessarily translate into perceived dissimilarities when the color differences are larger.

Interestingly, although the present heterozygote subjects never reported experiencing color confusions, their F-M 100 errors were more than one standard deviation above average (age-adjusted Z-scores) for four out of twenty-two cases. Similarly, many errors were made by three out of eighteen homozygous subjects (including the deuteranomalous S 14). The more structured analysis of constrained MDS suggested some degree of polar anomaly ($r > 0.1$) for 32% of heterozygotes and 22% of homozygotes. These data warrant further analyses of the F-M 100 as a diagnostic tool for groups of potential retinal tetrachromats.

Also important, and not unexpected given the additional factors determining opsin gene expression, is that this departure is not *universal* among heterozygotes. That is, among the twenty-two female heterozygotes (on one or both genes), only seven of these were differentiated as perceptually non-normative in the F-M 100 dissimilarity scaling (despite superior color judgment in other tasks). Physiological tests may soon be able to determine if such individuals are expressing more than three photopigment classes (e.g. Roorda & Williams, 1999).

To consider a more mainstream position, it is also conceivable that retinal

tetrachromacy from the codon-180 polymorphism has no effect on color processing, and that the aberrant minority observed in the present sample are actually heterozygous for color-vision deficiency, with chimeric or hybridized opsin genes, and thus, more likely to phenotypically express anomalous trichromacy or deficiency. Going on the F-M 100 scoring alone, this possibility is hard to exclude, although the aberrant minority of heterozygotes have little in common by dissimilarity measures with S 14, interpreted earlier as heterozygous and deficient by virtue of her F-M 100 sorting data. The problem with this explanation, however, is that it does not agree with patterns of results found in Figure 3 which illustrate how the F-M 100 dissimilarity scaling systematically tracks variation in chromatic banding results found using the Jameson et al. (2001) task, nor does it accord with the systematic tendencies in the angular and radial compression parameters shown in Figure 2. Finally, recent independent results by Sayim, Jameson, Alvarado and Szeszel (2005) suggest that female heterozygotes show significant differences in cognitive color processing in ways that accord with the results presented here.

Summary

The assessment of color perception in observers with the potential for four or more classes of retinal photopigments has often been undertaken with diagnostic tools designed to assess color vision under an assumption of trichromatic retinas and neural trichromacy. But what if neural trichromacy was not a constraint present in observers with four or more retinal photopigment classes? Non-human primate evolution provides precedents which serve as illustrative examples of how the human species could be polymorphous for color processing; and some existing research, albeit much of it before 1984, hints at this possibility through findings of subtle deviations from trichromacy in heterozygote participants. Jameson et al. (2001) found that color perception differences are associated with photopigment opsin genotype, and they suggest that some standard color vision assessment methods may not be appropriate for assessing the perception of retinal tetrachromats (that is, female heterozygotes). These new findings are consonant with some results in the existing literature.

As mentioned earlier, the standard instruments for assessing color vision in applied settings – pseudoisochromatic plates (PICs) and hue-sorting tests like the F-M 100 – are tests of color consensus, where consensus has been defined largely by a trichromatic norm. Previous research by Cohn et al. (1989) reports that heterozygotes are not generally detected by PICs. However, when such plates are used under conditions that make the task more difficult (modification of the spectral profile of the illuminant), the performance of the heterozygote is

impaired to a greater degree than that of normal trichromat controls. Although such differences are typically interpreted to suggest perceptual deficiencies in heterozygote observers, such differences need not give rise to functional deficiencies under naturalistic viewing circumstances, and, indeed, retinal tetrachromacy could give rise to above-average capabilities under some circumstances. Such possibilities, and the suggestions inherent in existing findings, raise important questions about the nature of color differences experienced by persons with four or more cone classes and the optimal ways to assess such differences (if they do exist).

The results presented here suggest the somewhat surprising finding that the F-M 100, and its comparatively straightforward procedure, has some utility as a tool for differentiating the perceptions of putative retinal tetrachromats from those presumably with trichromat retinas. The results suggest that, through further investigation, the F-M 100 test and scoring methods could be refined and extended to serve as a useful diagnostic tool for retinal tetrachromat observers who tend toward the pole of normal to above-average color perception. Development of such advances requires further testing and confirmation of phenotypes through physiological assessment in order to develop proper scoring and descriptive interpretations.

Such advances are suggested for the first time by two findings presented here. First, it was found that the F-M 100 stimuli and task are capable of systematically differentiating some heterozygotes (presumably those expressing at least four cone classes) from persons possessing normal trichromat genotypes. The fact that this differentiation was achieved, opens the possibility of refining the test, as appropriate, for classifying such observers. Second, the manner with which the heterozygotes varied in their color sorting and dissimilarity scaling is itself systematic and suggestive regarding which color regions of the test might be adapted to increase diagnostic specificity when classifying non-standard retinal phenotypes. Thus, the present results call for further investigation on this issue, and the modification of the F-M 100 for assessing the color perception of the retinal tetrachromat, as it possibly differs from that of the trichromat.

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