Four cone pigments in women heterozygous for color deficiency

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We find that additivity of trichromatic color matches holds, even in parafoveal vision, for most observers if rods are prevented from seeing the stimuli. Some heterozygous women are exceptions. The failure of the additivity law for these women implies that their eyes contain more than three types of cone with different spectral sensitivities.

It is generally accepted that human color matching is trichromatic (requires three primaries) because there are three types of cone, each containing one visual pigment. A color match between two stimulus fields is thought to be determined at the receptor level where the rate of quantum absorption by each of the three cone pigments must be equated to obtain a color match. Since there are three cone pigments, a set of three primaries is required to match any given light. A fundamental law of color matching, which follows directly from this view, is the law of additivity (known also as Grassmann's third law). Additivity means that, if the same light is added to two stimulus fields that match in color appearance, the resulting mixtures will match in color appearance. The color match is not upset because the addition of the same light to both fields does not upset the equality of quantum absorptions for each of the visual pigments.[1]

The additivity law holds quite well for small visual fields presented to the rod-free foveal area, but it typically fails in the parafovea, in which rods as well as cones may see the stimuli.[2] Recent evidence suggests that the breakdown is due to the involvement of signals from rods (as well as from the usual three types of cone) in the match.[3] In support of this view we find that additivity holds in the parafovea of most observers if rods are prevented from seeing the stimuli. However, the law does not hold for some women who are heterozygous for red–green color deficiency. Although their color vision is relatively normal, these women carry abnormal, as well as normal, genes for color vision. It is now well established that there are two loci on the X chromosome involved in red–green color vision. Each locus is thought to determine the nature of the visual pigment contained in one of the two red–green sensitive cones.[4] Since women have two X chromosomes, they may be heterozygous and carry two different alleles at either of these loci. Recent developments in genetics suggest that when this happens, each cell is influenced predominantly by one allele, with the predominant allele varying from cell to cell,[5] resulting in the production of more than three types of cone photoreceptor cells with different visual pigments. The failure of additivity for some heterozygous women means that, for them, subjectively similar colors may differ in their effects on the cones, and this in turn means that their eyes must contain more than three types of cone. However, because these women accept trichromatic matches, the cones must feed signals into only three neural channels.

In our experiments we asked observers to make Rayleigh matches[6] between two halves of a large annular field presented to the parafovea. A uniform mixture of red (660-nm) and green (546-nm) lights in the upper half of the field was matched to a yellow light (588 nm) in the lower half. The wavelengths were chosen to avoid any significant stimulation of the short-wavelength-sensitive cones. Therefore only two primaries were required to match the 588-nm light. We used the large annular test configuration to obtain matches that were as precise as possible.[7]

In order to prevent the rods from seeing the stimuli, matches were made during the cone plateau period after exposure to a bright bleaching light. During this period cones have nearly completely recovered from the bleach, but rods are still too insensitive to detect the test stimuli.[8] Observers made matches under three experimental conditions: (1) with no background light present, (2) with a concentric blue background light (455 nm) superimposed over both halves of the test field, and (3) with a red background light (670 nm) superimposed over the test field.[9] Under each experimental condition the experimenter determined the set of 660–546-nm-mixture ratios that was judged to be a perfect color match to the 588-nm light.[10] The additivity law predicts that the 660–546-nm mixture that matches the 588-nm light should not be altered by superposition of the backgrounds. If some of the settings that were acceptable as a match with no background present were also acceptable with a background present, we concluded that additivity held for that observer within the limits of his color discrimination. If none of the mixtures that were acceptable with no background present was accepted on the background, we concluded that additivity failed for that observer.

Nineteen men and twenty-one women served as observers. The Rayleigh matches of all 40 in the no-background condition fell within the normal range, but 19 of the 21 women were recruited because they reported some incidence of red–green color deficiency in their families, suggesting that they might

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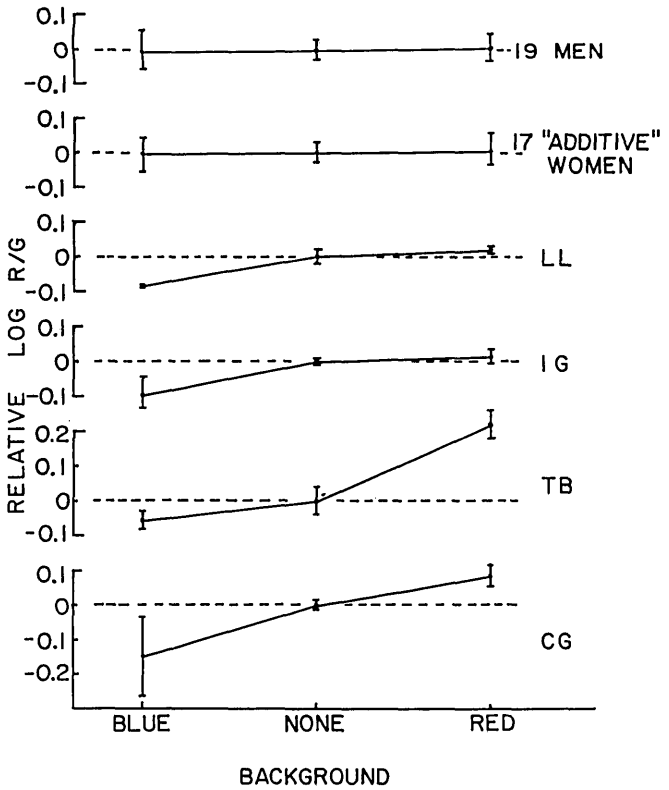

Fig. 1. The log of the ratio of the red and green primaries needed for a match in the three conditions is plotted relative to the log ratio in the no-background condition. The uppermost two curves are the mean midpoints and mean ranges for observers who show no additivity failure. The four lower curves are the individual midpoints and ranges of the women for whom additivity fails.

be heterozygous. Whereas the luminosity functions of heterozygous women sometimes differ from normal, their Rayleigh matches generally fall within normal limits.[11]

Using the criterion described above, we found that additivity held for all 19 men and 17 of the 21 women. Mean results for these two groups are shown in the upper half of Fig. 1. Each vertical line segment indicates the range of mixture ratios acceptable as a perfect color match to the 588-nm light for each condition for each group. The filled circles indicate the mean midpoint of the range of mixtures acceptable as a match. Within each group the mean midpoints for the three experimental conditions do not differ from one another significantly ($p > 0.10$ on a T test for related means). Additivity holds extremely well for both groups, confirming that the failure typically found in the parafovea is due to the involvement of rod signals in the match.[2,3] The additivity of trichromatic matches in cone vision implies that in these observers only three different visual pigments are operative and that there is no appreciable variation in the spectral sensitivity from cone to cone in a given observer's eye.[12]

Individual results from the four heterozygous women for whom additivity fails are shown in the lower half of Fig. 1. Three of these women reported color-deficient fathers, and the fourth, IG, reported a color-deficient brother and maternal grandfather, suggesting that her mother was a carrier. The line segments again indicate the range of mixtures acceptable as a match, and the filled circle indicates the midpoint of the range. For each of these women the range of acceptable mixture ratios with no background present does not overlap the set of acceptable mixture ratios on one or both of the backgrounds, indicating that two fields that match with no background present become distinguishable when the background is added. This result is a clear violation of the additivity law.

In experiments with small foveal fields, we have been unable to demonstrate the failure of additivity for these four women, partly because of reduced discrimination with the smaller field size and partly because the shifts in the match (if any) are fewer with the small field than with the large field. However, unpublished observations on one heterozygote by Alpern indicate a failure of additivity even in foveal observation.[13]

The failure of the additivity law in these women means that their eyes contain more than the usual three cone pigments; presumably they contain the three normal cone pigments plus an anomalous pigment corresponding to the abnormal gene that they carry. Signals generated by the four cone pigments must be fed into only three independent neural channels, however, since there appears to be no evidence to suggest that heterozygous women can reject trichromatic matches.[11] The fact that all the heterozygous women were able to make acceptable Rayleigh matches with only two primaries is further support for this assumption. The trichromatic color matches of these individuals must be determined at the level of the three independent neural channels, not at the receptor level, where the rates of quantal absorption by each of the four pigments will generally be unequal for a trichromatic match. Superposition of the red (660-nm) and blue (455-nm) backgrounds would be expected to upset the color matches of such a visual system, since they would be expected to adapt different types of cones differentially, resulting in a change in the spectral sensitivities of the neural channels at the level at which the match is determined.[13] Changes in the spectral sensitivities of the neural channels would generally disrupt the equality of signals from the two fields that had been matched, resulting in a breakdown of the match.[1]

Nevertheless, it is clear that additivity holds for 15 of 19 women who reported histories of color deficiency in their families. Why does additivity hold for them? Four of the 15 reported information suggesting that their mothers were heterozygous carriers. Each of these four women has a probability of only 0.5 of being heterozygous carriers herself, since she could have inherited either a normal gene or an abnormal gene from her mother with equal probability. However, the other 11 reported that their fathers were red–green deficient, and these 11 should therefore have been heterozygotes. Additivity failure requires the presence of a cone type sufficiently deviant from normal in spectral sensitivity (and sufficiently sensitive) to register a distinction for lights matched from normal cones. Among red–green color deficients, only some, known as simple anomalous, can reject normal matches. Thus it may be that only carriers of simple anomaly have an abnormal cone type that is sufficiently deviant and sensitive enough to result in an additivity breakdown. Heterozygotes for other forms of red–green deficiency, such as dichromacy, might not be expected to show failure of additivity.[15] On this argument it might be expected that the proportion of women who show additivity failure in our sample should be higher since simple anomaly is the most common form of red–green deficiency. Our recruitment procedure, an advertisement in the campus newspaper, re-

quested male color deficients as well as female carriers, and only 4 out of 26 color-deficient males were simple anomals. This low proportion is presumably due to the fact that simple anomals are less often aware of their deficiency. By the same argument, carriers of simple anomaly are less likely to be aware of color deficiencies in relatives and thus less likely to respond to the advertisement.

Our results can be interpreted as evidence that Lyonization, or X inactivation,[5] does occur for the X-chromosome loci that control development of the red–green-sensitive visual pigments. In Lyonization either the maternal or the paternal X chromosome randomly determines the development of a given cell in a heterozygous female, whereas the other chromosome in that cell is inactivated. Lyonization has been demonstrated for several loci on the X chromosome, but there is evidence that it may not occur at all sites.[16] Prevous attempts to determine whether it occurred at the loci for red–green color vision have met with only limited success.[14] Although other interpretations of the additivity failure may be possible,[2] the fact that it occurred only in heterozygous women, coupled with the evidence for Lyonization, strongly suggests that it is due to the presence of at least four cone types.

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REFERENCES

1. For a recent discussion on this view of trichromatic color matching, the additivity laws, and a summary of the evidence, see G. S. Brindley, *Physiology of the Retina and Visual Pathway*, 2nd ed. (Arnold, London, 1970), Chap. 8, pp. 199–223; R. M. Boynton, *Human Color Vision* (Holt, Rinehart, and Winston, New York, 1979), Chap. 5, pp. 97–155.
2. For a discussion of the breakdown of additivity in the parafovea and a summary of recent work on parafoveal color vision see J. D. Moreland, "Peripheral colour vision," in *Handbook of Sensory Physiology*, D. Jameson and L. M. Hurvich, eds. (Springer-Verlag, Berlin, 1972), Vol. VII/4, pp. 517–536.
3. P. W. Trezona, "Additivity in the tetrachromatic colour matching system," Vision Res. **14**, 1291–1303 (1974), has recently developed a method of making unique tetrachromatic color matches in the parafovea, which produces a match for rods as well as for cones. Additivity appears to hold for these matches, suggesting that additivity failures typically found in the parafovea are due to the involvement of rod signals.
4. For discussions of the genetics of color vision and the color vision of heterozygous women, see W. Jaeger, "Genetics of congenital colour deficiencies," in *Handbook of Sensory Physiology*, D. Jameson and L. M. Hurvich, eds. (Springer-Verlag, Berlin, 1972), Vol. VII/4, pp. 625–642; P. J. Waardenburg, in *Genetics and Ophthalmology*, P. J. Waardenburg, A. Franceschetti and D. Klein, eds. (Charles C. Thomas, Springfield, Ill., 1963), Vol. II, p. 1425; H. Kalmus, *Diagnosis and Genetics of Defective Colour Vision* (Pergamon, New York, 1965); T. P. Piantanida, "Polymorphism of human color vision," Am J. Optom. Physiol. Opt. **53**, 647–657 (1976).
5. M. F. Lyon, "Gene action in the X-chromosome of the mouse (*mus musculus L.*)," Nature London **190**, 372 (1961).
6. Rayleigh matches are often used to diagnose red–green color deficiencies. Two aspects of the match are useful in differentiating different types of color vision: the value of the red–green ratio required to match a yellow test light and the width of the range of red–green ratios acceptable as a match. See L. M. Hurvich, "Color vision deficiencies," in *Handbook of Sensory Physiology*, D. Jameson and L. M. Hurvich, eds. (Springer-Verlag, Berlin, 1972), Vol. VII/4, pp. 582–624; R. M. Boynton, *Human Color Vision* (Holt, Rinehart, and Winston, New York, 1979), p. 377.
7. Because of spatial summation, color matches are typically more precise with larger fields. The annular test configuration was used to avoid retinal inhomogeneities from fovea to parafovea that sometimes cause difficulties with large-field color matches. See W. S. Stiles, "The basic data of colour-matching: 18th Thomas Young Oration," *Phys. Soc. Yearbook* (The Physical Society, London), pp. 44–65 (1955). The test annulus had an inner diameter of 40° and an outer diameter of 12°. Matches were made at a retinal illuminance of approximately 20 trolands. The optical system used to deliver stimuli to the observer was conventional in design and is described in A. L. Nagy, "Large-field substitution Rayleigh matches of dichromats," J. Opt. Soc. Am. **70**, 778–784 (1980).
8. The bleaching field, which was 16° in diameter with a retinal illuminance of 6.6 log scotopic trolands, was obtained from a 750-W Kodak projector with a 500-nm interference filter taped over the lens. The observer placed his eye near the front of the lens and looked into the light beam for 10 sec. Two control experiments were done to determine the stimulus presentation interval. Thresholds for the test stimuli were determined after a bleach in order to determine when the cones had recovered from the bleach and when the rods became sensitive enough to determine the threshold. Cones had recovered 3 min after the bleach, but extrapolation of the rod branch of the threshold curve upward above the cone plateau portion of the curve indicated that rods were not able to detect the test stimuli less than 10 min after the bleach. In the second experiment observers adjusted a Rayleigh match soon after the cones had recovered from a bleach and then observed the match until the two halves of the field began to look dissimilar. When rods begin to see the test field, the mixture half of the field becomes much less saturated than the monochromatic half. The breakdown in the match always occurred more than 10 min after the bleach. Therefore we asked observers to make matches between the third and the tenth minute after the bleach. The matches made were stable throughout this interval. Bleaches were repeated to obtain a sufficient number of observations from each observer.
9. The red and blue background fields were 20° in diameter with a retinal illuminance of approximately 20 photopic trolands.
10. The procedure used is similar to one suggested by A. Linkz, *An Essay on Color Vision and Clinical Color-Vision Tests* (Grune and Stratton, New York, 1964), Chap. 15. The experimenter adjusted a particular 546–660-nm-mixture ratio into the upper half of the field and then asked the observer to attempt a match by varying the brightness of the lower half of the field with circular graded neutral filter. The observer then reported whether the match was a perfect color match or whether the upper half of the field was redder or greener than the lower half of the field. This procedure was repeated until the experimenter had determined values of the mixture ratio that could be discriminated reliably from the 588-nm field and values that could not.
11. See R. A. Crone, "Spectral sensitivity in color-defective subjects and heterozygous carriers," Am. J. Ophthalmol. **48**, 231–238 (1959); M. Ikeda, K. Hukami, and M. Urakubo, "Flicker photometry with chromatic adaptation and defective color vision," Am. J. Ophthalmol. **73**, 270–277 (1972) for evidence supporting this statement. However, see also H. L. DeVries, "The fundamental response curves of normal and abnormal dichromatic and trichromatic eyes," Physica **XIV**, 367–380 (1948). DeVries appears to be the first to suggest that heterozygous women may have four cone pigments. He also suggested they might be tetrachromatic, that is, they might reject all trichromatic matches. Our results, however, suggest they are trichromatic like normal observers.
12. Any variability in absorption spectrum among cones of the same type should generate minor additivity failure even in normal observers. With the help of plausible assumptions, additivity data like these can be used to compute a constraint on the extent of such variability. These data permit the conclusion that the three absorption spectra of normal vision are replicated with high precision from cone to cone. If Weber's law is assumed to hold at the level of the cones, an adapting light of (say) long wavelength will bias sensitivity in favor of those individual cones with shorter than average λ_{max}. This will shorten the effective λ_{max} of each

cone type by an amount proportional to both (1) the variance of the distribution of λ_{max} in that cone type (assumed Gaussian) and (2) the slope of the spectral sensitivity curve at the adapting wavelength. The ratio of red to green light required for a match in our apparatus increases by about 0.033 log unit/nm reduction in the λ_{max} of either red- or green-sensitive cones [compare J. Pokorny, V. C. Smith, and I. Katz, "Derivation of the photopigment absorption spectra in anamalous trichromats," J. Opt. Soc. Am. **63**, 232–237 (1973)]. In our blue- and red-adapted conditions, the measured ratios for the 36 nonshifting observers differed by less than 0.01 log unit (90% confidence interval is 0.001–0.016 log unit). It follows that changes in effective λ_{max} because of adaptation did not exceed 0.25 nm. The corresponding upper limit for the standard deviation of λ_{max} for cones of a given type is only 1.6 nm. Unfortunately, the Weber's law assumption has not been tested for our conditions, so further work on this point would be desirable.

13. M. Alpern, Vision Research Laboratory, University of Michigan, Ann Arbor, Mich. 48104, personal communication.
14. For evidence on adaptation within the receptors see D. A. Baylor and A. L. Hodgkin, "Changes in time scale and sensitivity in turtle photoreceptors," J. Physiol. (London) **242**, 729–758 (1974) and R. A. Normann and F. S. Werblin, "Light and dark adaptation of vertebrate rods and cones," J. Gen. Physiol. **63**, 37–61 (1974).
15. This view was confirmed for one heterozygote whose son was a dichromat. In these women one might expect a pattern of retinal mocaicism in which areas of the retina containing both normal cone types alternate with areas in which only one of the normal cone types is detectable. See A. Krill and E. Beutler, "Red-light thresholds in heterozygote carriers of protanopia: genetic implications," Science **149**, 186–188 (1965); P. Grutzner, G. Born, and H. J. Hemminger, "Coloured stimuli within the central visual field of carriers of dichromatism," Mod. Probl. Ophthalmol. **17**, 147–150 (1976); see also a study by B. Wooten and G. Wald, Department of Psychology, Brown University, Providence, R.I. 02912 (in preparation). It is interesting that several of the heterozygous women for whom additivity held complained that the matches were difficult because the test fields appeared to be composed of tiny red and green dots. The patchy appearance could be due to mocaicism. In contrast, none of the male observers made similar complaints.
16. L. J. Shapiro *et al.*, "Non-inactivation of an X-chromosome locus in man," Science **204**, 1224–1226 (1979).