

The L:M cone ratio in males of African descent with normal color vision

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Among Caucasian males with normal color vision, long-wavelength-sensitive (L) cones outnumber middle-wavelength-sensitive (M) cones by nearly three to one, on average, and the L and the M cone opsin genes are arrayed on the X-chromosome with the L opsin gene being closest to an upstream enhancer element termed the locus control region (LCR). Interaction between an opsin gene promoter and the LCR is required to mediate normal opsin gene expression, and the relative proximity of the L opsin gene promoter (4,000 base pairs for L compared to 40,000 base pairs for the M opsin gene promoter) has been proposed to endow the L gene with the advantage in competing for interaction with the LCR, thereby accounting for the nearly 3:1 ratio of L:M cones. This proximal advantage hypothesis predicts that the L:M cone ratio will be similar among populations that share the same X-chromosome opsin gene array organization. Here, we tested this hypothesis by examining a sample of males of African descent and found them to have a significantly different average L:M ratio compared to Caucasian males, even though their X-chromosome opsin gene arrays were indistinguishable from arrays in males of Caucasian descent. How these observations might be reconciled is discussed.

Keywords: cone ratio, cone photopigments, human color vision, cone photoreceptors, variation in cone ratio

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Introduction

The ratio of long-wavelength (L) to middle-wavelength (M) cones is widely variable among Caucasian males, averaging 2.7:1 in men with normal color vision (Carroll, Neitz, & Neitz, 2002; Hofer, Carroll, Neitz, Neitz, & Williams, 2005), and it has been hypothesized that it is determined, at least in part, by the organization of the L and M opsin genes on the X-chromosome (Smallwood, Wang, & Nathans, 2002). The most typical arrangement is an L gene followed by one or more M genes. Transcription of the X-chromosome opsin genes requires a promoter element contained within the 236 base pairs (bp) immediately 5' to the coding sequence of each gene, and an enhancer element, also termed the locus control region

(LCR), contained within 600 bp of DNA that lies between 3.1 and 3.7 kilobase pairs (kb) upstream of the translational start codon of the X-chromosome opsin gene array (Nathans et al., 1989; Wang et al., 1992).

In adult human L and M cone photoreceptors, only one opsin gene is expressed (Hagstrom, Neitz, & Neitz, 2000), and it has been proposed that mutually exclusive expression is mediated by the LCR (Nathans et al., 1989; Smallwood et al., 2002; Wang et al., 1992, 1999). From an evolutionary perspective, it appears likely that human L and M cone photoreceptors represent a single cell type, and the stochastic choice of which gene is expressed, L or M, determines the cone type. The L and M cone opsin genes in humans resulted from a gene duplication that occurred in the Old World primate lineage after the divergence of Old and New World primates (Nathans,

| | Subject ID no. | %L cones | λ_{\max} | %L genes (No. L genes) | %DS genes (No. DS genes) | Country of origin | Size 1st gene | |
|----------|-------------------|-------------|------------------|---------------------------|-----------------------------|----------------------|------------------|---|
| Africans | 330 | 69.51 | 559* | 46.72 (1) | 55.07 (1) | Ethiopia | L | |
| | 334 | 52.32 | 559 | 51.38 (1) | 52.39 (1) | Ghana | S | |
| | 337 | 63.95 | 559 | 49.12 (1) | 52.44 (1) | Senegal | L | |
| | 338 | 69.59 | 559 | 33.92 (1) | 67.03 (2) | Ghana | L | |
| | 339 | 71.94 | 559 | 34.64 (1) | 65.73 (2) | Ghana | L | |
| | 342 | 48.36 | 559* | 34.61 (1) | 69.14 (2) | Ghana | L | |
| | 343 | 57.74 | 559* | 35.32 (1) | 71.43 (2) | Ghana | L | |
| | 344 | 42.37 | 559* | 33.69 (1) | 70.55 (2) | Ghana | L | |
| | 347 | 79.96 | 559 | 41.63 (1) | 50.88 (1) | Ghana | L | |
| | 348 | 51.45 | 559 | 32.63 (1) | 66.24 (2) | Nigeria | S | |
| | 351 | 64.44 | 559* | 49.25 (1) | 51.91 (1) | Kenya | L | |
| | 352 | 70.14 | 559* | 32.08 (1) | 68.35 (2) | Kenya | L | |
| | 353 | 57.63 | 559* | 50.19 (1) | 52.54 (1) | Kenya | L | |
| | African Americans | 354 | 69.13 | 559 | 50.81 (2) | 86.27 (4) | USA | S |
| | | 323 | 84.52 | 555.5 | 51.29 (2) | 75.2 (3) | USA | S |
| 324 | | 81.47 | 559* | 31.71 (1) | 68.26 (2) | USA | L | |
| 325 | | 81.23 | 559 | 49.75 (1) | 53.01 (1) | USA | S | |
| 328 | | 63.63 | 559 | 30.62 (1) | 67.64 (2) | St. Lucia | S | |
| 329 | | 70.26 | 559* | 43.07 (1) | 43.15 (1) | USA | L | |
| 331 | | 65.18 | 559 | 30.37 (1) | 65.82 (2) | USA | L | |
| 332 | | 65.65 | 559 | 48.76 (1) | 54.52 (1) | USA | L | |
| 333 | | 50.41 | 559* | 31.91 (1) | 69.36 (2) | USA | L | |
| 335 | | 70.64 | 559* | 32.63 (1) | 67.74 (2) | USA | L | |
| 336 | | 52.68 | 559* | 33.29 (1) | 66.99 (2) | USA | L | |
| 340 | | 64.24 | 559 | 50.69 (1) | 52.66 (1) | USA | S | |
| 341 | | 68.55 | 559 | 34.68 (1) | 67.11 (1) | USA | S | |
| 349 | | 71.34 | 559* | 29.85 (1) | 64.92 (2) | USA | L | |
| Average | | | 65.12 | | (1) | (1.7) | | |

Table 1. Summary of data for each subject. Subjects in the African category reported that both parents and both sets of grandparents still live in Africa, and subjects in the African American category have one or both parents and grandparents living in the United States. The %L cones was calculated from FP-ERG-derived spectral sensitivity data as previously described (Carroll et al., 2000), using the λ_{\max} for the L cone indicated in the table and using a λ_{\max} of 530 nm for the M cone. In addition, a correction factor was applied to the estimate of the %L cones to account for the 1.5 times greater contribution to the ERG signal for each M cone compared to each L cone (Hofer et al., 2005). The λ_{\max} values used for the L cone spectra in determining the %L cones was determined based on genetic analysis of each subject's L opsin gene, as previously described (Carroll et al., 2000). An asterisk for the λ_{\max} values for L cones indicates those subjects for whom the λ_{\max} was measured in a single gene dichromat who had the identical L cone pigment. The %L genes and percentage of downstream (DS) genes were determined as previously described (Neitz & Neitz, 2001).

M cone spectral sensitivity functions that best fit the FP-ERG-derived luminosity function obtained from each subject (Carroll et al., 2000). The L cone spectra were individualized in that each subject's L opsin gene was sequenced, and the wavelength of maximal sensitivity (λ_{\max}) was inferred from previous studies (Carroll et al., 2000). Summarized in Table 1 are both the estimated L:M cone ratios and the λ_{\max} values used to calculate the ratio for each subject. The distributions of cone ratios for Africans and Caucasians are compared in Figure 2. The ratios for the males of African descent ranged from 42% to 85% L cones, with an average of 65% L cones and a standard deviation of 10.7%. Males of Caucasian descent ranged from 37% to 95% L cones, with an average of 73% L cones and a standard deviation of 11.1%. Thus, the

degree of variance for these two groups was similar; however, the mean ratio for the Africans was significantly lower as compared with the Caucasians ($p = .0015$ Student's t test, and $p = .0013$ Mann–Whitney non-parametric test).

We considered the possibility that differences in the structure of the X-chromosome opsin gene arrays in the African versus Caucasian subjects might account for the difference in mean cone ratio for the two groups. For example, if an M gene occupied the 5'-most position in the arrays from African subjects, this would be predicted to give rise to a lower L:M cone ratio than observed in Caucasians because the M gene would be closer to the LCR. In addition, it was reported that 35% of African Americans have a variant of the L opsin gene that is

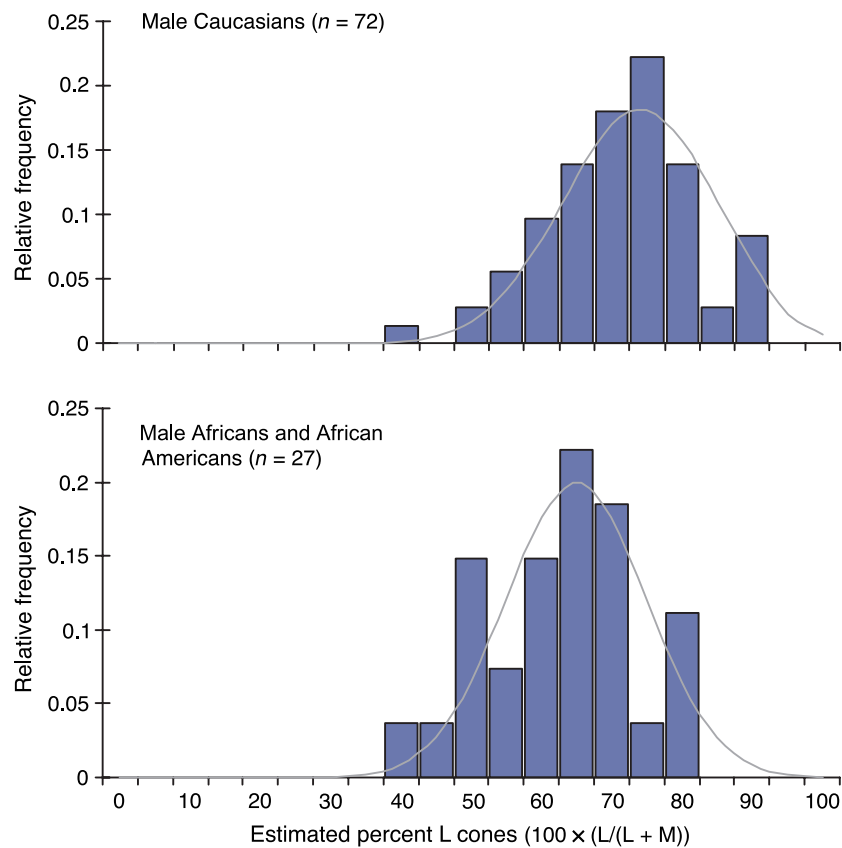


Figure 2. L:M cone ratio in Africans, African Americans, and Caucasians. L:M cone ratio is calculated as the percentage of L plus M cones that are L [%L = $100 \times (L / (L + M))$]. The difference between the two populations is significant ($p = 0.0015$ Student's t test and $p = 0.0013$ Mann–Whitney nonparametric test).

1.9 kb shorter in intron 1 compared to L genes in Caucasians (Jørgensen, Deeb, & Motulsky, 1990). Sequence differences in the long versus short version of the L gene could potentially contribute to differences in cone ratio (Mollon, 1999). In addition, nucleotide sequence differences in the L and M gene promoters have been hypothesized to be potential candidates for influencing cone ratio (Smallwood et al., 2002). To investigate these possibilities, genetic analyses were performed on each subject.

Genomic DNA was used in long distance PCR to amplify the first gene in the array, from which exon 5 was subsequently amplified and analyzed for a polymorphism that distinguishes L from M genes. Exon 5 of the first gene was incubated with restriction endonuclease *RsaI*, which cleaves within exon 5 of L genes but not M genes. For all of the African subjects, exon 5 from the first gene in the array was cleaved by the *RsaI* restriction enzyme (data not shown), indicating that all subjects had an L gene first in the array. The full-length long-distance PCR product containing the first gene in the array was also incubated with restriction enzyme *EcoRI* to determine whether each subject had the long or the short version of the L gene in the first position in the array. An example of this analysis is shown in Figure 3, and the data from this assay are summarized in Table 1. There was no correlation between

L gene length and the ratio of L:M cones ($p > .999$; Mann–Whitney test).

Real-time quantitative PCR estimates of the ratio of L:M genes and the number of genes per array estimated from the ratio of the first:downstream genes are summarized in Table 1. Ratios of L:M genes are expressed as the percentage of L plus M genes that encode L pigment [%L genes = $100 \times L / (L + M)$], and the ratios of first:downstream genes are expressed as the percentage of genes in the array that are downstream of the first gene [%DS = $100 \times DS / (1 + DS)$]. For example, if a subject was estimated to have 50% L genes and 50% downstream genes, this was interpreted as his having an array with one L and one M gene. An estimate of 33% L genes and 66% downstream genes was interpreted as one L and two M genes, and an estimate of 50% L genes and 75% downstream genes was interpreted as two L and two M genes. There was no difference between Africans and Caucasians in the percentage of L genes ($p = .27$, Mann–Whitney test) or in the percentage of downstream genes ($p = .61$, Mann–Whitney test).

A comparison of the sequences of the 236-bp promoter-containing DNA segment upstream of the translational start codon of the L and M genes revealed the absence of nucleotide sequence L variations in the L and the M gene

the L pigment encoded by the gene in the first position in the array for each subject. We previously catalogued the L opsin gene sequences and λ_{\max} values for dichromats who had a single X-chromosome opsin gene (Carroll et al., 2000). Fourteen of the subjects in this study had an L opsin gene that encoded a pigment corresponding to one for which the spectrum had been measured in a dichromat. The remaining 13 subjects differed from catalogued pigments only by polymorphisms that have been previously demonstrated not to influence the λ_{\max} of L pigments. To account for variation in the optical density of the lens, the spectral sensitivity curves used to calculate the L:M cone ratios were determined using an age-specific correction for lens absorption (Pokorny et al., 1987).

It is possible that there is a correlation between high skin pigmentation and increased lens density (cf. Said & Weale, 1959). We considered the possibility that the downward shift in L:M cone ratio in African subjects compared to Caucasian subjects is due to an underestimated lens density for the former. However, increasing the lens density values for a given set of spectral sensitivity data decreases the L:M ratio further. Thus, we conclude that the FP-ERG-derived L:M ratio estimates reflect a real difference in the average L:M ratio between males of African versus Caucasian descent.

According to the model proposed by Nathans and colleagues, a difference in the distance between the LCR and the M opsin gene promoter in Caucasians versus Africans could account for the dissimilarity in L:M cone ratio; however, molecular genetic results ruled out all of the obvious possibilities. Both Africans and Caucasians have arrays in which an L gene is closest to the LCR, so it is not due to a difference in gene order. The absence of a correlation between the L:M cone ratio and the short versus long variant of the L opsin gene rules out the possibility previously suggested by Mollon (1999) that the DNA insert in the long variant has a measurable affect on L:M cone ratio. An alternative hypothesis proposed by Smallwood et al. (2002) that promoter sequence polymorphisms can account for cone ratio variation can be eliminated here by the almost complete absence of promoter sequence differences both between and among groups.

In the model illustrated in Figure 2, the DNA is envisioned to bend to allow the LCR to form a complex with the opsin gene promoter (Smallwood et al., 2002). In the absence of other constraints, the relative probability of spatial coincidence between the LCR and a promoter should decrease in proportion to the volume of the sphere, or in proportion to the cube of the distance from the LCR, so that a cone photoreceptor is 1,000 times more likely to

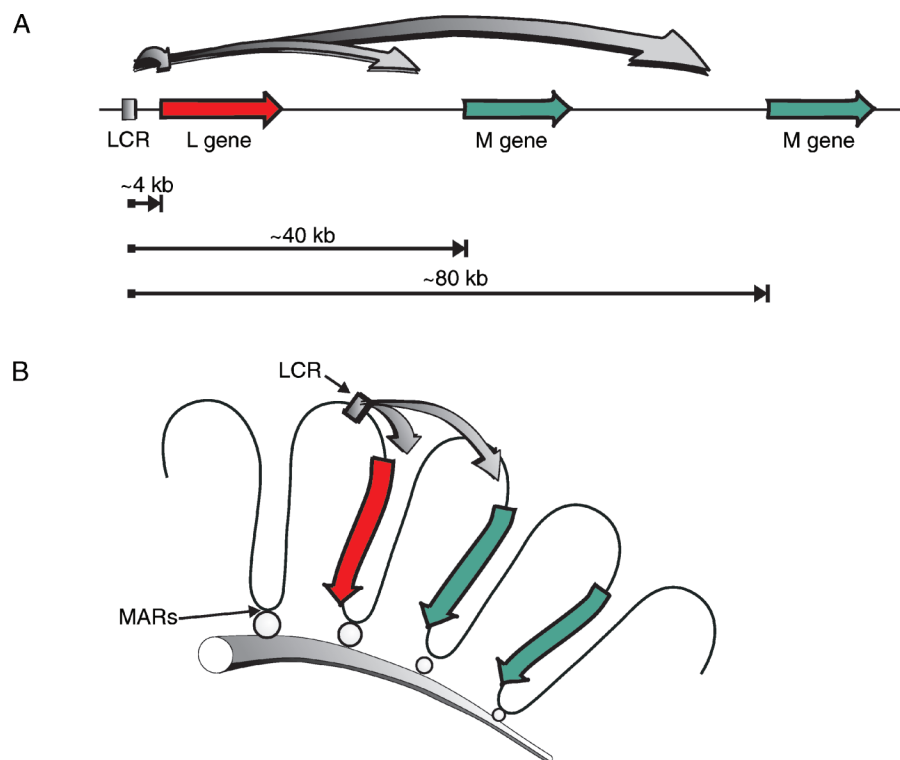


Figure 4. Relative accessibility of the L or M opsin gene promoters to the LCR. (A) The L gene enjoys a huge advantage compared to the M genes if linear distance is the determining factor. The L gene is only 4 kb away from the LCR, whereas the distance between the LCR and an M gene is a multiple of 40 kb. (B) The first and the second genes enjoy a similar degree of accessibility to the LCR if distances are determined by the packaging of DNA into chromatin loop domains. Loops are attached to the nuclear matrix via matrix attachment regions (MARS).

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