Supplemental Text

METHODS

Subjects: Subjects were unrelated males with normal color vision (n=63; average age = 27.3 years) and brothers with normal color vision (n=39, 17 different families; average age = 22.9 years). Color vision was classified based on color matching performance on a Nagel anomaloscope, in addition to other standard color vision tests (AO-HRR, Ishihara plates, Farnsworth Panel D-15, Dvorine Color Plates and the Neitz Test of Color Vision.[1] Informed consent was obtained after explanation of the nature and possible consequences of the study. All experiments followed the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board (IRB) at the Medical College of Wisconsin.

*Genetic Analysis***:** Isolation of genomic DNA from whole blood, amplification and sequence determination of L-pigment genes was done as previously described.[2, 3] Estimation of the number and ratio of L and M pigment genes on the X-chromosome has also been described previously.[4]

ERG Flicker Photometry: Details of the apparatus and procedure used to record the flicker photometric ERG have been described elsewhere.[3, 5, 6] Two beams (identified here as a reference and a test beam) of a three-channel Maxwellian-view optical system were superimposed to illuminate a circular portion of the retina subtending approximately 70°. High-speed electromagnetic shutters were used to alternately present the reference and test lights at 31.25 Hz, with a neutral density wedge used to control the intensity of the test light. The wavelength of the test light was controlled by a Varispec liquid-crystal electronically tunable filter

(Cambridge Research & Instrumentation, Boston, MA). An electrode made from fiber from the DTL Plus ™ was used as the active corneal electrode, and the subject's pupil was dilated with tropicamide 0.5%. Spectral sensitivity was determined by adjusting the intensity of the test light until the ERG signal it produced was equal to that produced by the fixed intensity reference light. This null point was determined at 10 nm increments over a range of 480-680 nm. The average of two complete runs through each wavelength was used to determine a subject's spectral sensitivity function. Final spectral sensitivity values are reported as quantal intensities, and were corrected for lens absorption with an age-dependent lens correction.[7]

RESULTS & DISCUSSION

Distribution of L:M Ratio in the Normal Population: The flicker-photometric ERG has been shown to yield both accurate and reliable estimates of L:M ratio.[3, 8] To obtain an estimate of L:M ratio, a subjects' spectral sensitivity data is best-fit to a weighted sum of an L- and an M-photopigment template. Individual differences in the spectral sensitivity of the L photopigment have been shown to greatly influence estimates of L:M ratio derived from flicker photometry.[9] We have been able to eliminate variability in L-cone spectral sensitivity as a source of error by sequencing each subject's L gene and using an individualized L-cone template to estimate their L:M ratio.[3] The ERG-derived estimates of L:M cone ratio among the 63 unrelated males were distributed normally (per the Kolmogorov-Smirnov normality test) about a mean of 63%L (**Figure 1**), consistent with previous estimates of an average L:M ratio of about 2:1 in men with normal color vision.[10-15]

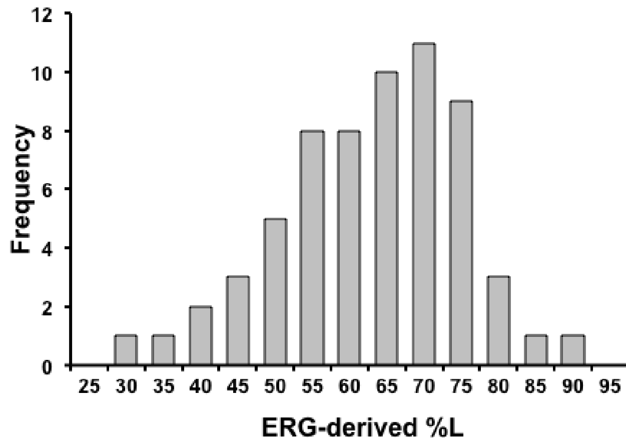

Figure 1 - Distribution of L-cone relative to M-cone contributions to ERG spectral sensitivity for 63 unrelated males with normal color vision. The relative L:M ratios are represented as %L in linear combination with M required to best fit each subject's ERG spectral sensitivity function.

L:M Ratios in Brothers: ERG spectral sensitivity functions were recorded from 30 pairs of brothers (39 different men). Cone ratios were estimated using individualized L-cone spectra deduced from photopigment gene sequence results as above. Shown in **Table 1** are the %L estimates for each brother, the deduced amino acid sequence at each of the polymorphic positions encoded by exons 2, 3 and 4 of L pigment genes and λ_{max} of the L pigment. Brothers who had different L-pigment gene sequences were identified as having different L/M gene arrays. Further analysis of the L/M gene array to determine the pigment gene number and ratio was done in the remaining brothers to verify that they had the same L/M gene arrays.

The brothers who shared the same L/M gene array were found to have very similar L:M ratios as measured with the ERG. The mean difference was 8.9%L, though it ranged between 0.2 – 13.8%L. For comparison, when repeated measurements are made on a single subject, an average variation of about 2 – 3%L is observed.[3] However, brothers with different L/M gene arrays had even larger L:M ratio differences, with a mean difference of 11.4%L (range = 0.9 – 33.6%L). This difference was not quite significant ($p = 0.053$, two-tailed Mann-Whitney test).

Table 1 – L:M Cone Ratio Variability Among Brothers

Family[1]	Subject ID	Age	% L Genes	% Downstream Genes	No. L Genes	No. M Genes	L Gene Sequences[2]: Exon 2 65 111 116	Exon 3 153 171 174 178 180	Exon 4 230 233 236	λ_{max} (nm)	ERG-derived L:M (%L)
1	NMRG015	28	56.04	66.02	1	1	T I S	M V V I S	I A V	559	92.55
1	NMRG043	29	37.36	63.24	1	2	T I S	L V A I S	I A M	559	58.99
2	NMRG203	25	32.42	67.14	1	2	T I S	M V A I A	I A M	555.5	60.19
2	NMRG204	30	50.17	54.58	1	1	T I S	L V A I S	I A M	559	70.54
3	NMRG234	26	49.25	74.43	2	2	T I S	L V V V A	I A M	555.5	44.82
3	NMRG235	22	33.31	65.67	1	2	T I S	L V A I A	I A M	555.5	59.55
4	NMRG246	8	29.65	67.66	1	2	T I S	M V A I S	I A M	559	86.7
4	NMRG247	10	29	69.74	1	2	T I S	M V A I A	I A M	555.5	88.7
5	NMRG274	29	33.25	68.55	1	2	T I S	L V A I S	I A M	559	58.38
5	NMRG275	28	50.95	51.74	1	1	T I S	L V A I A	I A M	555.5	66.97
6	NMRG289	22	53.45	52.81	1	1	T I S	M V V I S	I A V	559	67.07
6	NMRG290	20	72.84	68.83	3	1	T I S	M V A I S	I A M	559	87.62
7	NMRG413	21	33.6	67	1	2	T I S	L V A I S	I A M	559	48.10
7	NMRG427	16	43.97	45.06	1	1	T I S	L V A I S	I A M	559	64.17
8	NMRG030	24	58.79	49.01	1	1	T I S	L V A I S	I A M	559	79.53
8	NMRG182	18	44.1	52.19	1	1	T I S	L V A I S	I A M	559	67.97
8	NMRG183	20	47.5	53	1	1	T I S	L V A I S	I A M	559	78.15
9	NMRG210	25	51.97	53.01	1	1	T I S	M V V V S	I A V	559	74.45
9	NMRG211	18	54.11	53.53	1	1	T I S	M V V V S	I A V	559	73.38
10	NMRG265	34	32.06	59.28	1	2	T I S	L V A I S	I A M	559	65.86
10	NMRG266	29	31.1	61.8	1	2	T I S	L V A I S	I A M	559	60.00
11	NMRG314	20	32.71	61.83	1	2	T I S	L V A I S	I A M	559	64.69
11	NMRG315	22	31.06	62.87	1	2	T I S	L V A I S	I A M	559	63.55
12	NMRG366	22	53.16	54.22	1	1	T V S	L V A I S	I A V	559	55.59
12	NMRG367	27	53.84	56.01	1	1	T V S	L V A I S	I A V	559	59.23
13	NMRG372	19	40.24	54.93	1	1	T I S	L V A I S	I A M	559	74.07
13	NMRG373	17	48.12	47.46	1	1	T I S	L V A I S	I A M	559	76.46
14	NMRG418	23	33.46	66.97	1	2	T I S	M V A I S	I A M	559	55.52
14	NMRG424	19	31.2	57.65	1	2	T I S	M V A I S	I A M	559	58.60
15	NMRG434	13	35.75	71.64	1	2	T I S	L V A I S	I A M	559	66.82
15	NMRG435	15	35.6	71.29	1	2	T I S	L V A I S	I A M	559	53.02
16	NMRG214	24	27.39	72.31	1	3	T I S	L I A I S	I A M	559	59.49
16	NMRG215	34	29.91	69.51	1	3	T I S	L I A I S	I A M	559	51.30
16	NMRG249	16	21.94	69.68	1	3	T I S	L I A I S	I A M	559	59.65
16	NMRG370	33	29.62	79.19	1	3	T I S	L I A I S	I A M	559	49.21
16	NMRG248	30	41.69	50.56	1	1	T I S	L V V V S	I A M	559	63.48
17	NMRG285	25	45.99	49.92	1	1	T I S	L V A I S	I A M	559	62.27
17	NMRG287	22	48.47	52.7	1	1	T I S	L V A I S	I A M	559	70.67
17	NMRG296	32	46.25	51.59	1	1	I V Y	L V A I A	I A M	553	63.18

[1]Brothers in families 1-7 were determined to have different L/M gene arrays, while brothers in families 8-15 had the same L/M array. The multi-sibling families 16 & 17 were "mixed", with some brothers having the same L/M gene array and others having different L/M arrays.

[2]Single letter amino acid code: A = alanine, I = isoleucine, L= leucine, M = methionine, S = serine, T = threonine, V = valine, Y = tyrosine.

There is a significant complication regarding the statistical analyses of our data. Our data set is not a collection of independent brother pairs. Our sample of 39 males consisted of 14 independent pairs, two families of three brothers, and one family of five brothers. To determine if the non-independence was affecting the difference between the two groups, we removed all of the three families with more than two brothers, reducing the analysis to only the 14 independent brother pairs. In this analysis the brothers who shared the same L/M gene array were again found to have very similar L:M ratios – the mean difference was 4.4%L (range = 1.1 – 13.8%L). The brothers with different L/M gene arrays again had larger L:M ratio differences, with a mean difference of 15.12%L (range = 2.0 – 33.6%L). This difference was significant ($p = 0.026$, two-tailed Mann-Whitney test).

Taken together, these results are consistent with the hypothesis that sequences associated with the L/M gene array on the X-chromosome are the major genetic factors responsible for variations in cone ratio among males with normal color vision, [16] an idea first put forth by DeVries 70 years ago.[17-19] However, the fact that we observe substantial variability in some brothers with the same L/M gene array indicates that there may be other regulatory factors to consider. More importantly, it provides a mechanism by which a deleterious haplotype (*i.e.*, LIAVA or LVAVA) could have dramatically different effects within brothers harbouring the same L/M array.

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