Supplemental Data

Mix and Match Color Vision: Tuning Spectral Sensitivity by Differential Opsin Gene Expression in Lake Malawi Cichlids

Juliet W.L. Parry, Karen L. Carleton, Tyrone Spady, Aba Carboo, David M. Hunt, and James K. Bowmaker

Supplemental Results and Discussion

Spectral Tuning

Between Different Genes within the Same Opsin Family

SWS2A and SWS2B opsin subtypes are spectrally separated by 20–40 nm. A trio of potential tuning sites, A94C, T97C, and F103M, can be identified, with site 94 being closest to the retinal binding pocket. This site causes a small short-wave shift in the SWS2 pigment of the newt, when Ser is replaced by Ala [S1], and all the SWS2B pigments so far sequenced contain Cys at this site. Site 94 is one helical turn beyond site 90 where a Cys substitution is known to cause a significant short-wave shift in avian SWS1 pigments [S2, S3].

The spectral difference between the RH2A and RH2B cone pigments is about 30–40 nm and this may in part be attributed to the presence of Gln at site 122 in the RH2B opsins. Glu122 is thought to cause a 15 nm long-wave shift in teleost RH2 pigments [S4–S7]. Conserved substitutions Ala295Ser and Ala98Cys may be candidates for tuning the remaining difference.

Between the Same Gene in Different Percomorph Species

The *P. acei* SWS1 sequence has eight amino acid differences compared to other species in this study, four of which (83/114/160/204) could be relevant for spectral tuning (Table S2), although their effect on UV sensitivity of the pigments is likely to be minimal since this is largely determined by Phe at site 86 UV [S6, S8, S9]. However, they may be relevant to fine tuning, since the *O. niloticus* pigment expresses with λ_{max} 360 nm (T.S., personal communication), 8 nm shorter than that of *M. zebra* and 18 nm shorter than that of *P. acei* (Table S2). These differences correlate with sites 114, 118, and 298, all previously considered for tuning in SWS1 pigments [S2, S10].

Two sites, 118 and 269, have been shown to be important in tuning within the SWS2A group of pigments in cottoid fish [S11] where an Ala or Gly substitution of Thr at 118 leads to a short-wave shift of about 20 nm. Site 118 appears to be also relevant to tuning within the SWS2B group. The three species of cichlid, *M. vermivorus*, *P. acei*, and *M. zebra*, for which both λ_{max} and opsin sequences are available, have λ_{max} values between 415 and 423 nm (Table S2) with Thr at site 118. *O. niloticus* has the same sequence at sites 118 and 269 and its pigment expresses with λ_{max} 425 nm (T.S., personal communication). In contrast, in the killifish *L .goodei*, the SWS2B pigment has a λ_{max} at 405 nm [S12] with Gly at site 118. The presence of A269T in *T. intermedius* (and *D. compressiceps*) would predict the violet pigment to be long-wave shifted to around 434 nm.

The RH2B pigment from *O. niloticus*, which expresses at 472 nm (T.S., personal communication), is 12 nm shorter than that from *M. zebra* (Table S2). This difference correlates with S292A, an established site for tuning in LWS pigments, and the cause of a 10 nm shift in rod opsins [S13, S14].

Supplemental References

S1. Takahashi, Y., and Ebrey, T.G. (2003). Molecular basis of spectral tuning in the newt short wavelength sensitive visual pigment. Biochemistry *42*, 6025–6034.

S2. Wilkie, S.E., Robinson, P.R., Cronin, T.W., Poopalasundaram, S., Bowmaker, J.K., and Hunt, D.M. (2000). Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. Biochemistry *39*, 7895–7901.

Table S1. Primers Used for Amplification for Sequencing and Cloning Opsin Genes

Opsin	Primers Used for PCR Amplification	Primers Used for Expression Cloning[a]
RH1	F: GGCTGATCGCAACCACAAG	
	R: GGAGCAGAAGTCTTCGTCTTGG	
SWS1	F: **GCGCGGAATTC**AAAGAGCTCAGGGTCACAATG	**GGCGGGAATTCCCACC**ATGGGAAAACACTTCCACCTGTACGA
	R: **GCGCGCAAGCTT**GCTCAGTCAACGCCCTCTTA	**GGCGGGTCGAC**GAAGCTGTGGACACTTCAGTCTTTG
SWS2B	F1: **GCGCGGAATTC**TAGATTTTGATCGCAAACTCCAT	**GGCGGGAATTCCCACC**ATGAGAGGAAATCGTCCC
	R1: CCAAACAGAGGTGGAAGTGC	**GGCGGGTCGAC**CAGGTCCAACTTTAGAGACTTCAGTGG
	F2: GCTTGTGGTCTCTTGCTGTGG	
	R2: **GCGCGCAAGCTT**CGGTTATTCACAACCCAGATG	
	F3: GATTATGGTGCTGGGCTTTC	
	R3: CAGTATGCGAGCTGTCCAAA	
SWS2A	F1: **GCGCGGAATTC**GCGCGATACCTAATTTGAGC	
	R1: **GCGCGCAAGCTT**AGCCTTTGAGAAACAGGACG	
	F2: **GCGCGGAATTC**GCAGAGAGGGAAGTGACCAG	
	R2: **GCGCGCAAGCTT**AAATCAGCGAGCATTGACG	
RH2B	F1: CAGTACTCCAAGGAGCTTAGCAG	**GGCGGGAATTCCCACC**ATGGCTTGGGATGGAGGACTTGAGCCT
	R1: GCCATTCCAGACATGGGTAG	**GGCGGGTCGAC**GAAACAGAGGAGACTTCTGTCTTGCTG
	F2: CCTGATACTTCATATTCAACTAACCTT	
	R2: CAGGAAGGAGTATGGCTGGA	
RH2Aβ	F: **GCGCGGAATTC**GGGATATTCCATCAGCTGAAAC	**GGCGGGAATTCCCACC**ATGGCTTGGGAAGGAGGAAT
	R: **GCGCGCAAGCTT**GCTTCTTAAATCCATTTGGCA	**GGCGGGTCGAC**GACACAGAGGACACCTCTGTCTTGC
RH2Aα	F: ACGCAGACTCAACTAAACAGC	**GGCGGGAATTCCCACC**ATGGTTTGGGATGGAGGAATTGAG
	R: GGAAGCAATCATCAATGTCCA	**GGCGGGTCGAC**GACACAGAGGACACCTCTGTCTTGC
LWS	F1: **GCGCGGAATTC**GGCTAACAGCTCAGGACCTC	
	R1: **GCGCGCAAGCTT**GCCCTCAAAGATACACATTGG	
	F2: **GCGCGGAATTC**TTTGAGGGTCCCAATTACCA	
	R2: **GCGCGCAAGCTT**TCCACACAGCAAGGTAGCAC	
	F3: **GCGCGGAATTC**ACTGGCCTCATGGACTGAAG	
	R3: **GCGCGCAAGCTT**TCCCAAAATGGAGAACATGG	

[a] Sequence nonhomologous with the target opsin is shown in bold, restriction enzyme sites are underlined.

Table S2. Opsin Amino Acid Sequence Differences

Gene	Site	*M. zebra*	*P. acei*	*M. vermivorus*	*T. intermedius*
LWS		AF247126	DQ088627	DQ088628	DQ088629
	13	A	A	A	V
	42	L	L	F	L
	164	**A**	**S**	**S**	**A**
	172	M	M	M	I
RH2Aα		DQ088651	DQ088630	DQ088631	DQ088632
		V	A	A	A
	214	V	I	I	V
	263	V	I	I	V
RH2Aβ		DQ088650	DQ088633	DQ088634	DQ088635
	56	G	G	G	S
	100	N	S	S	N
	107	S	S	S	P
	133	I	I	I	V
	205	M	M	M	I
	218	I	VI	V	I
RH2B		DQ088652	DQ088645	DQ088646	DQ088647
	27	P	Q	P	Q
	44	**I**	**M**	**I**	**M**
	46	S	C	S	C
	119	I	I	I	V
	235	S	S	S	A
	304	I	IV	V	V
SWS2A		AF247114	DQ088636	DQ088637	DQ088638
	39	T	T	A	A
SWS2B		DQ088649	DQ088639	DQ088640	DQ088641
	1	R	R	H	R
	11	V	V	I	V
	79	L	L	L	M
	182	G	SG	S	G
	269	**A**	**A**	**A**	**T**
	273	**I**	**I**	**F**	**I**
SWS1		DQ088648	DQ088642	DQ088643	DQ088644
	21	I	V	I	I
	83	**G**	**S**	**G**	**G**
	114	**S**	**A**	**S**	**S**
	130	V	L	V	V
	160	**T**	**A**	**T**	**T**
	165	I	V	I	I
	166	G	A	G	G
	204	**T**	**I**	**T**	**T**
	214	I	M	M	I

Only sites that differ are shown. Sites are numbered according to the bovine rod opsin sequence. Sites directed into the retinal binding pocket are in bold. GenBank accession numbers are included for each pigment. All residues are shown at variable sites.

S3. Yokoyama, S., Radlwimmer, F.B., and Blow, N.S. (2000). Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change. Proc. Natl. Acad. Sci. USA *97*, 7366–7371.

S4. Chinen, A., Hamaoka, T., Yamada, Y., and Kawamura, S. (2003). Gene duplication and spectral diversification of cone visual pigments of zebrafish. Genetics *163*, 663–675.

S5. Chinen, A., Matsumoto, Y., and Kawamura, S. (2005). Reconstitution of ancestral green visual pigments of zebrafish and molecular mechanism of their spectral differentiation. Mol. Biol. Evol. *22*, 1001–1010.

S6. Hunt, D.M., Cowing, J.A., Wilkie, S.E., Parry, J.W.L., Poopalasundaram, S., and Bowmaker, J.K. (2004). Divergent mechanisms for the tuning of shortwave sensitive visual pigments in vertebrates. Photochem. Photobiol. Sci. *3*, 713–720.

S7. Pointer, M.A., Cheng, C.H., Bowmaker, J.K., Parry, J.W.L., Jeffery, G., Cowing, J.A., and Hunt, D.M. (2005). Adaptations to an extreme environment: retinal organisation and the spectral properties of photoreceptors in Antarctic notothenioid fish. J. Exp. Biol. *208*, 2363–2376.

S8. Cowing, J.A., Poopalasundaram, S., Wilkie, S.E., Robinson, P.R., Bowmaker, J.K., and Hunt, D.M. (2002). The molecular mechanism for the spectral shifts between vertebrate ultraviolet- and violet-sensitive cone visual pigments. Biochem. J. *367*, 129–135.

S9. Parry, J.W.L., Poopalasundaram, S., Bowmaker, J.K., and Hunt, D.M. (2004). A novel amino acid substitution is responsible for spectral tuning in a rodent violet-sensitive visual pigment. Biochemistry *43*, 8014–8020.

S10. Shi, Y.S., and Yokoyama, S. (2003). Molecular analysis of the evolutionary significance of ultraviolet vision in vertebrates. Proc. Natl. Acad. Sci. USA *100*, 8308–8313.

S11. Cowing, J.A., Poopalasundaram, S., Wilkie, S.E., Bowmaker, J.K., and Hunt, D.M. (2002). Spectral tuning and evolution of short wave-sensitive cone pigments in cottoid fish from Lake Baikal. Biochemistry *41*, 6019–6025.

S12. Fuller, R.C., Fleishman, L.J., Leal, M., Travis, J., and Loew, E. (2003). Intraspecific variation in retinal cone distribution in the bluefin killifish, *Lucania goodei*. J. Comp. Physiol. [A] *189*, 609–616.

S13. Yokoyama, S. (2000). Color vision of the coelacanth (*Latimeria chalumnae*) and adaptive evolution of rhodopsin (RH1) and rhodopsin-like (RH2) pigments. J. Hered. *91*, 215–220.

S14. Hunt, D.M., Dulai, K.S., Partridge, J.C., Cottrill, P., and Bowmaker, J.K. (2001). The molecular basis for spectral tuning of rod visual pigments in deep-sea fish. J. Exp. Biol. *204*, 3333–3344.