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The relationship between lens transmission and opsin gene expression in cichlids from Lake Malawi

Christopher M. Hofmann [a,*], Kelly E. O'Quin [a], N. Justin Marshall [b], Karen L. Carleton [a]

[a] Department of Biology, University of Maryland, College Park, MD 20742, United States
[b] Queensland Brain Institute, The University of Queensland, Brisbane 4072, Australia

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ABSTRACT

The lens plays an important role in regulating the wavelengths of light that reach the retina. However, the evolutionary relationship between lens transmission and retinal sensitivity remains cloudy at best. We examined the relationship between lens transmission and opsin gene expression in a group of rapidly radiating cichlids from East Africa. Lens transmission was bimodal, either cutting off around 360 or 400 nm, and appeared to be quite labile evolutionarily. We found a strong correlation between lens transmission and SWS1 (UV) opsin gene expression, suggesting that UV transmitting lenses are adaptive in cichlids. Species which expressed high levels of SWS2B (violet) opsin varied in their lens transmission while most species that expressed high levels of SWS2A (blue) opsin had UV blocking lenses. In no instance did lens transmission appear to limit retinal sensitivity. Finally, the strong correlation that we observe between SWS1 expression and lens transmission suggests that these two traits might be coupled genetically.

1. Introduction

The process of visual transduction begins when a photon passes through ocular media and is absorbed by a photoreceptor. Thus, ocular media present the first stage at which spectral sensitivity can be tuned or modified. This modification involves blocking or filtering short wavelengths of light, typically in the ultraviolet (UV) to blue region of the spectrum (300–450 nm) (Douglas & Marshall, 1999). Ocular media can be divided up into three primary components, the lens, cornea, and vitrea (Douglas & Marshall, 1999; Siebeck & Marshall, 2001). Although all three have the potential to filter light, the lens is most commonly the limiting filter (Douglas & Marshall, 1999; Losey et al., 2003; Siebeck & Marshall, 2001).

Previous studies have documented considerable variation in lens transmission among fishes (Thorpe, Douglas, & Truscott, 1993). For example, coral reef fish have lens cutoff wavelengths ranging from 320 to 440 nm (Losey et al., 2003; Siebeck & Marshall, 2001; Siebeck & Marshall, 2007). Rather than being continuously distributed, these cutoff wavelengths tend to be bimodal, with lenses either blocking or transmitting UV light (Losey et al., 2003; Siebeck & Marshall, 2001). There also appears to be a relationship between retinal sensitivity and lens cutoff. Species with visual pigments that absorb maximally in the UV tend to have lenses that transmit into the UV (Losey et al., 2003). Interestingly the reverse was not always true. Although many of the species that have visual pigments that absorb maximally in the blue or violet region of the spectrum have UV blocking ocular media, the ocular media of some species still transmit UV light (Losey et al., 2003).

Several adaptive benefits for blocking UV light have been proposed. High-energy UV light has the potential to damage the retina, especially in tropical species that inhabit clear, shallow waters (Losey et al., 2003; Siebeck & Marshall, 2001; Zigman, 1971). In addition, chromatic aberration at shorter wavelengths may cause loss of image resolution, particularly in species with larger eyes, which have a longer focal length (Douglas & Marshall, 1999; Lythgoe, 1979; Muntz, 1976). However, in some cases the ability to detect UV light is advantageous. UV vision is believed to improve foraging on plankton in open water by silhouetting the UV absorbing plankton against a UV scattering background (Browman, Novales-Flamarique, & Hawryshyn, 1994; Loew, McFarland, Mills, & Hunter, 1993; Losey et al., 1999). UV vision may also provide private wavelengths of communication that predators cannot detect or that are scattered rapidly (Marshall, 2000). It may even aid in distinguishing Mullerian mimics from their models (Cheney & Marshall, 2009).

Cichlids in Lake Malawi are a classic example of an adaptive radiation (Kocher, 2004; Seehausen, 2006; Streelman & Danley, 2003). Between 500 and 1000 species have arisen from riverine ancestors within the past 2 million years (Genner et al., 2007;

* Corresponding author. Address: University of Maryland, Department of Biology, 1210 Biology Psychology Blding #144, College Park, MD 20742, United States.
E-mail address: chofmal@umd.edu (C.M. Hofmann).

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Meyer, Kocher, Basasibwaki, & Wilson, 1990). Vision is believed to play an important role in this radiation, for example to aid in foraging or selecting a mate, and the visual systems of Malawi cichlids are incredibly diverse (Carleton, 2009; Hofmann et al., 2009; Spady et al., 2006). The cichlid genome contains seven different cone opsin genes, of which six are functionally and genetically distinct (Carleton, 2009). Most cichlids express only a subset of three or four of these genes, although which genes are expressed varies considerably, even among closely related species (Carleton, 2009; Carleton & Kocher, 2001; Hofmann et al., 2009; Spady et al., 2006). Photoreceptor sensitivities determined by microspectrophotometry and heterologously expressed opsin proteins suggest there is a direct relationship between photoreceptor abundance and opsin gene expression (Carleton, Harosi, & Kocher, 2000; Carleton, Parry, Bowmaker, Hunt, & Seehausen, 2005; Carleton et al., 2008; Jordan et al., 2006; Parry et al., 2005; Spady et al., 2006). In addition, we have demonstrated that opsin gene expression is related to foraging and environmental light (Hofmann et al., 2009). The importance of vision in these fishes, as well as the lability of their opsin gene expression, makes them an ideal system for investigating the relationship between lens transmission and retinal sensitivity determined by gene expression.

2. Materials and methods

2.1. Sampling

We collected cichlids from southern Lake Malawi near Cape Maclear, Malawi in 2005 and 2008. Following an overdose of MS222, eyes were enucleated and hemisected. The lenses were removed for immediate analysis of transmission and the retinas were dissected from the eye cup and stored in RNAlater. All procedures were conducted according to approved IACUC protocols (UMd R09–73).

2.2. Measuring lens transmission

We measured the lens transmission of 272 fish from 65 species following previously published protocols (Siebeck & Marshall, 2001; Siebeck & Marshall, 2007). Initial measurements of whole eyes and corneas showed that the lens was the limiting ocular media in all species; therefore, we focused our measurements on lens transmission alone. Light from a quartz halogen bulb or pulsed xenon light source (Ocean Optics, PX2) was directed through a lens mounted above a pinhole and into a quartz fiber optic cable coupled to an Ocean Optics USB2000 or 4000 spectrometer (Siebeck & Marshall, 2001; Siebeck & Marshall, 2007). Two to five measurements were made and averaged from each fish.

2.3. Analyzing lens transmission

We analyzed lens transmission using two methods. In the first method, spectra were normalized using their transmission at 600 nm and we calculated the 50% cutoff wavelength (T50) by finding the wavelength halfway between T_{min} and T_{max} in the 300–600 nm interval (Douglas & McGuigan, 1989; Siebeck & Marshall, 2001). This method is commonly used, although it is sensitive to deviations from a perfect sigmoidal curve, especially when transmission continues to increase at longer wavelengths due to sampling artifacts (e.g., lens clouding). In the second method, spectra were normalized using their maximum transmission and we calculated the wavelength of maximum slope in the 300–700 nm interval. The maximum slope is essentially the inflection point of the sigmoidal lens transmission curve. These two measures of lens transmission were highly correlated ($R^2 = 0.81$, $p < 10^{-101}$, Fig. S1); however, because the latter reduced the influence of sampling artifacts generated by field conditions, we used the wavelength of maximum slope in all further analyses.

2.4. Quantifying opsin gene expression

We quantified the cone opsin expression of 100 fish from 33 species collected in 2008 following previously published methods (Carleton & Kocher, 2001; Carleton et al., 2005; Spady et al., 2006). In brief, RNA from each retina was extracted and reverse transcribed using commercially available kits (RNeasy, Qiagen). Real-time, quantitative PCR reactions for the six cone opsins were run in parallel using opsin specific primers and probes. Reaction efficiencies were normalized using a construct that contained tandem segments of each gene in a linear array (Spady et al., 2006). Critical cycle numbers and reaction efficiencies were then used to calculate the relative expression of each opsin (see equations in Carleton & Kocher, 2001; Spady et al., 2006). Each reaction was run at least twice on separate plates (using separate reaction master mixes) and then averaged. We combined these data from 2008 with the 110 samples from 53 species collected in 2005 that had been analyzed previously (Hofmann et al., 2009). In total, our opsin expression data set consisted of 210 wild-caught fish from 65 species.

2.5. Retinal sensitivity

We examined retinal sensitivity in two ways: first by calculating relative SWS1 (UV) opsin expression, and second by estimating single cone sensitivities. Previous studies of cichlids suggest that their retinas are arranged into organized mosaics of single and double cones. The shorter-wavelength SWS1 (UV), SWS2B (violet), and SWS2A (blue) opsins are expressed in the single cones and the longer-wavelength RH2B (blue-green), RH2A (green), and LWS (red) opsins are expressed in the double cones. Therefore, we normalized SWS1 opsin expression by the total expression of SWS1, SWS2A, and SWS2B using the equation:

$$f_{SWS1} = \frac{SWS1}{SWS1 + SWS2B + SWS2A}$$

where f_{SWS1} is the fraction of SWS1 expression in the single cones. We then calculated the average sensitivity of single cones (Carleton, 2009; Carleton et al., 2008; Hofmann et al., 2009). Peak spectral sensitivities for each single cone opsin were weighted by the fraction of their expression. Because the λmax of single cone visual pigments are unknown for most of the species included in this study, we used the λmax values of heterologously expressed *O. niloticus* opsins (SWS1 = 360, SWS2B = 425, SWS2A = 456) (Spady et al., 2006; see also Hofmann et al., 2009). *O. niloticus* is a riverine ancestor and serves as an outgroup to the Malawi radiation (Kocher, Conroy, McKaye, Stauffer, & Lockwood, 1995). There are two caveats to this calculation. First, it is not meant to imply that there are actually photoreceptors with maximum spectral sensitivities at a specific wavelength, but rather provides a useful descriptive statistic that captures the overall sensitivity of single cones. The second is that variation across (or within) species due to amino acid tuning is eliminated. However, previous studies suggest this variation is quite small (~10 nm) compared to changes in opsin gene expression (e.g., expressing SWS2A instead of SWS1 shifts expression by about 100 nm) (Carleton, 2009; Hofmann & Carleton, 2009; Hofmann et al., 2009).

2.6. Phylogenetic comparisons

We used two phylogenetic comparative methods to examine the relationship between lens transmission and our two measures

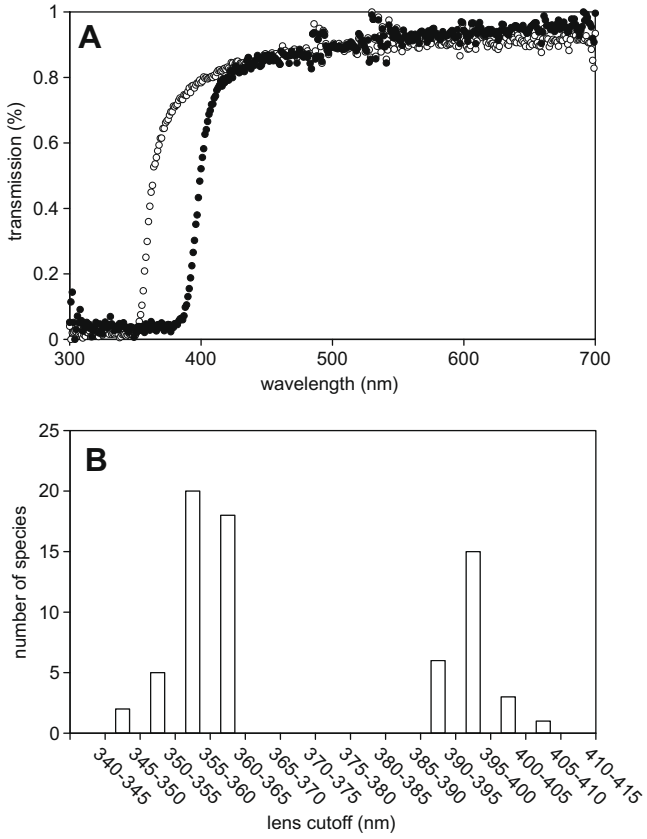

Fig. 1. Lenses fell into two groups, UV transmissive and UV blocking. (A) Transmission spectrum of a typical UV transmissive (*M. zebra*) and UV blocking lens (*M. mola*). (B) Distribution of lens cutoff values for all species surveyed.

of retinal sensitivity. The first method compared mean SWS1 expression or estimated single cone sensitivity values among species with alternate lens transmission properties using phylogenetic generalized least squares (PGLS; see Garland, Bennett, & Rezende, 2005 for a description of this method). The second method assessed the correlation between retinal sensitivity and lens transmittance using a modified version of the independent contrasts method (Purvis & Rambaut, 1995). Because of the rapid radiation of Lake Malawi cichlids, no species-level phylogenies have been resolved for these fishes. Therefore, we used three different phylogenetic hypotheses to take into account shared evolutionary history when estimating the relationship between retinal sensitivity and lens transmittance. The first phylogeny was a generic tree representing taxonomic relationships between species; the second was a star tree where the sand- and rock-dwelling lineages were each collapsed into distinct clades; and the third was a previously published mitochondrial tree for a subset of our samples. For all analyses, we coded species with lens transmittance values < 390 as 'UV-transmitting' and those with transmittance values >390 as 'UV-blocking'.

For PGLS, the R package 'ape' (Paradis, Claude, & Strimmer, 2004) was used to generate a correlation matrix describing the relationships among the species sampled based on our phylogenetic hypotheses. Construction of this matrix assumed a Brownian motion model of character evolution. We then fit a linear model to our data, using generalized least squares, while taking into account this correlation matrix. For independent contrasts, the 'brunch' algorithm, implemented in the R package 'CAIC' (Orme, Freckleton, Thomas, Petzoldt, & Fritz, 2009), was used to identify tips and nodes with alternate lens transmittance values, and only these nodes were used to calculate independent contrasts. This method is highly conservative, since not every node can be used in the analysis and each node can be used only once (Purvis & Rambaut, 1995). For both PGLS and independent contrasts, we repeated the analysis for each of the three phylogenetic hypotheses discussed above.

3. Results

Most lenses had a typical sigmoidal transmission spectrum (Fig. 1A). Lens cutoffs ranged from 347–409 nm (Table 1) and their distribution was bimodal, with one peak centered around 350–370 nm and a second around 390–400 nm (Fig. 1B). We did not observe a relationship between lens size and lens transmission across our entire data set (i.e., both large and small lenses were UV blocking and UV transmitting; Fig. S2). This finding suggests that lens size alone does not determine which discrete class of transmission a lens will fall into.

Overall, lens transmission appeared to be labile evolutionarily, with variation within and across genera (Fig. 2). We also found five potential examples of intraspecific variation in lens transmission (Table 1). However, deviant individuals for these species could have been misidentified in the field. Most mbuna, or rock dwelling genera, had UV transmissive lenses. Sand dwellers appeared to have more variation both within and across genera (Fig. 2).

When we compared lens transmission to SWS1 opsin expression, we found that all retinas with high levels of SWS1 opsin expression had UV transmissive lenses, although the reverse was not true – some species with UV transmissive lenses did not express the UV opsin (Fig. 3A,B). This relationship was highly significant under all three of the phylogenetic hypotheses that were used (Table 2). Interestingly, most species with high levels of SWS2A (blue) opsin expression had UV blocking lenses (Fig. 3B).

Comparing lens transmission to estimated single cone sensitivities suggested a similar relationship. Species calculated to have the shortest-wavelength single cones all had UV transmissive lenses, while species with the longest-wavelength single cones tended to have UV blocking lenses (Fig. 3C; note that no species fall below the diagonal). Species in between these two extremes, either because they express the violet opsin or co-express different SWS opsin genes, alternated between UV transmissive and UV blocking lenses. These relationships were also highly significant under all three phylogenetic hypotheses (Table 2).

4. Discussion

We found that cichlid lens transmission was quite labile evolutionarily. Despite the fact that cichlids are some of the most rapidly radiating species on the planet, lens transmission varied in a discrete manner. We also found a strong correlation between opsin gene expression and lens transmission, even when shared evolutionary history was accounted for. For the most part, evolutionary changes in lens transmission mirrored changes in SWS1 (UV) cone opsin expression, although about half of the species that expressed high levels of SWS2B (violet) cone opsin also had UV transmissive lenses. There did not appear to be any cases where lens transmission limited single cone λmax (i.e., no species fall under the diagonal line in Fig. 3C), although further studies using microspectrophotometry are needed to confirm this definitively.

Our findings agree with those of previous studies in coral reef fish which suggested that eyes fall into three groups: UV specialized, UV sensitive, and UV blocking (Losey et al., 2003). UV specialized eyes have ocular media that transmit UV light and have visual pigments that absorb maximally in the UV. UV sensitive eyes transmit UV light and likely detect some UV light, either through

Table 1
Average opsin gene expression and average lens cutoff values for each species.

Genus	# Retinas	LWS 560 nm	RH2A 523 nm	RH2B 472 nm	SWS2A 456 nm	SWS2B 425 nm	SWS1 360 nm	Relative SWS1[a]	Single cone λmax	# Lenses	Lens cutoff	SE
Aristochromis christyi	2	3.5	47.7	25.4	0.8	17.6	4.9	21.1	412	1	403	–
Aulonocara sp "blue fin"	4	21.3	29.8	22.9	1.3	18.3	6.4	24.5	411	2	363	2.50
Aulonocara hansbaenschi	2	15.3	55.2	19.0	0.5	8.5	1.4	13.4	418	9	356	1.83
Buccochromis lepturus	1	50.3	25.6	2.9	7.9	10.8	2.6	12.1	429	1	398	–
Buccochromis rhodesii	1	43.8	46.3	0.2	5.0	1.9	2.9	29.3	422	1	403	–
Copadichromis cf virginalis	1	12.4	54.7	9.4	0.1	1.8	21.6	92.0	365	1	350	–
Copadichromis eucinostomus	9	22.5	35.9	22.0	0.1	0.8	18.7	95.2	363	10	355	0.67
Copadichromis jacksoni	1	27.7	50.3	6.7	0.0	0.2	15.0	98.7	361	1	358	–
Cyathochromis obliquidens	4	41.4	13.9	28.7	0.2	0.6	15.3	95.5	363	2	347	0.67
Cynotilapia afra	3	0.1	51.4	31.8	0.0	0.1	16.5	99.1	361	1	358	–
Cyrtocara moorii	5	46.5	42.4	0.2	7.9	1.8	1.2	11.1	440	7	401	0.71
Dimidiochromis compressiceps	1	43.3	45.7	0.0	8.5	1.3	1.2	10.9	442	1	400	–
Dimidiochromis kwinge	1	10.1	58.5	11.1	2.3	1.4	16.6	81.8	375	1	363	–
Genyochromis mento	2	0.5	48.6	33.4	0.2	1.6	15.9	90.3	367	2	360	0.00
Hemitilapia oxyrhynchus	2	25.5	36.9	22.4	0.1	0.2	14.9	98.6	361	2	358	0.00
Labeotropheus fuelleborni	2	11.5	41.5	29.5	0.6	0.6	16.3	93.2	365	2	361	3.75
Labeotropheus trewavasae	9	5.3	47.2	22.6	0.3	0.9	23.7	95.1	364	14	361	0.48
Labidochromis "blue sp"	1	23.7	42.3	20.0	2.3	7.7	3.9	28.0	412	1	363	–
Labidochromis gigas	6	18.7	42.9	17.9	1.8	8.3	10.3	50.6	395	9	360	0.76
Labidochromis maculicauda	1	29.5	55.6	0.4	6.2	8.0	0.3	2.0	437	1	400	–
Labidochromis maculicauda[b]	1	60.4	13.0	5.0	5.1	8.1	8.4	38.9	407	1	357	–
Labidochromis vellicans	4	45.3	26.7	5.1	1.3	13.2	8.4	36.8	403	5	360	0.76
Labidochromis vellicans[b]	1	52.7	21.3	1.0	1.3	22.9	0.7	2.9	425	1	396	–
Lethrinops aurita	8	35.0	29.0	13.6	3.9	5.3	13.2	59.1	392	8	361	4.87
Maravachromis mola	5	19.5	45.7	10.9	3.1	15.0	5.8	24.4	413	6	398	0.77
Maravichromis plagiotaenia	1	71.9	16.3	0.2	5.7	4.2	1.7	14.5	431	1	399	–
Maravichromis sp	1	31.6	47.5	2.4	1.6	9.6	7.3	39.6	402	1	394	–
Melanochromis auratus	8	4.3	43.8	20.3	0.1	30.5	1.1	3.3	423	13	394	0.63
Melanochromis sp "B&W johanni"	5	3.0	49.0	27.0	0.2	0.4	20.3	97.0	362	15	358	0.84
Melanochromis chisumulu	5	5.4	61.0	15.3	0.1	0.3	18.0	98.1	361	5	358	1.25
Melanochromis labrosus	1	14.6	33.2	2.1	5.3	24.6	20.2	40.3	402	1	391	–
Melanochromis parallelus	6	2.2	49.9	20.9	0.3	24.1	2.5	9.2	419	8	395	1.13
Melanochromis parallelus[b]	1	1.1	57.4	10.4	0.1	29.2	1.9	6.1	421	1	353	–
Melanochromis vermivorus	4	2.3	46.0	29.8	0.1	20.0	1.8	8.3	420	3	395	2.46
Melanochromis vermivorus[b]	3	3.6	56.3	14.8	0.1	24.2	0.9	3.7	423	7	355	1.71
Metriaclima aurora	5	4.0	46.1	32.4	0.2	1.2	16.1	92.0	366	2	362	3.00
Metriaclima barlowi	5	13.5	43.6	14.8	3.4	4.0	20.8	73.7	381	10	351	0.96
Metriaclima benetos	1	17.1	39.9	4.5	5.2	13.6	19.6	51.0	396	1	361	–
Metriaclima callainos	2	5.1	40.8	36.1	0.2	0.5	17.3	96.1	363	2	363	2.50
Metriaclima livingstonii	1	4.1	46.4	33.9	0.0	0.0	15.6	99.5	360	1	355	–
Metriaclima sp	1	7.2	44.5	33.8	1.4	0.3	12.9	88.6	370	1	365	–
Metriaclima zebra	7	2.3	55.4	24.0	0.8	0.8	16.7	91.3	367	9	360	0.47
Nimbochromis linni	2	5.1	35.2	33.4	0.8	16.1	9.3	35.5	403	2	364	0.84
Nimbochromis polystigma	5	10.9	43.6	19.3	0.3	11.3	14.7	55.9	389	9	355	1.35
Oreochromis sp	1	66.0	21.9	0.4	8.3	2.3	1.2	9.8	440	1	400	–
Otopharynx heterodon	1	14.3	20.2	16.7	9.8	10.8	28.1	57.6	394	1	391	–
Otopharynx pictus	2	7.5	47.1	22.6	0.6	15.2	6.9	30.3	406	1	360	–
Petrotilapia nigra	5	12.4	45.1	19.5	0.6	0.4	21.9	95.3	364	11	360	0.49
Placidochromis johnstoni	1	46.6	40.4	0.1	10.7	1.1	1.2	8.9	445	1	409	–
Placidochromis milomi	1	8.5	74.3	8.7	0.2	6.6	1.7	19.6	413	1	360	–
Protomelas annectens	1	1.0	29.5	30.5	0.2	27.7	11.0	28.3	407	1	400	–
Protomelas fenestratus	1	50.2	25.5	8.1	6.0	8.1	2.1	12.7	428	1	361	–
Protomelas similis	2	59.5	26.6	2.1	5.3	4.7	1.7	14.5	430	2	361	0.63
Protomelas spinolotus	1	15.4	32.6	12.0	7.3	16.7	16.0	40.1	405	1	363	–
Protomelas taeniolatus	7	9.5	42.2	22.4	1.2	13.7	11.1	42.8	399	15	361	0.52
Pseudotropheus elongatus slab	3	3.5	60.2	16.2	0.2	0.6	19.4	96.1	363	3	358	1.51
Pseudotropheus heteropictus	1	0.7	48.7	36.7	0.2	0.7	13.1	93.6	365	1	360	–
Pseudotropheus microstoma	2	4.7	34.3	32.6	0.1	0.2	28.0	98.8	361	1	360	–
Rhamphochromis esox	1	56.7	20.7	3.6	12.1	5.3	1.6	8.2	439	1	400	–
Rhamphochromis sp	1	0.0	78.7	15.5	1.5	2.9	1.4	23.9	417	1	400	–
Stigmatochromis woodi	2	7.9	48.4	15.1	4.0	12.4	12.3	42.8	402	2	359	0.95
Taeniolatus praeorbitalis	5	18.4	55.4	5.2	1.8	13.1	6.0	28.8	409	7	396	1.00
Tramitichromis brevis	1	59.9	31.9	0.1	7.0	0.1	1.1	13.0	443	1	391	–
Trematocranus placodon	4	30.6	42.9	0.1	8.5	2.5	15.4	58.1	397	5	394	0.60
Trematocranus placodon[b]	1	40.9	46.5	0.2	8.5	2.5	1.5	12.2	438	1	358	–
Tropheops gracilior	13	4.2	44.9	21.4	1.0	1.0	27.5	93.2	365	12	356	0.49
Tropheops sp "orange chest"	5	18.1	44.4	6.3	3.5	8.9	18.8	60.4	389	6	359	1.15
Tropheops sp "red cheek"	4	13.2	43.2	19.6	1.0	1.1	21.9	91.1	367	11	363	0.72
Tyrranochromis macrostoma	2	36.0	35.5	9.7	2.0	9.3	7.5	40.0	402	1	400	–
Tyrranochromis maculiceps	1	46.0	36.9	0.9	10.5	4.5	1.2	7.7	440	1	400	–

[a] Relative SWS1 expression refers to the percentage of single cone SWS1 expression.
[b] Taxa with potential intraspecific variation.

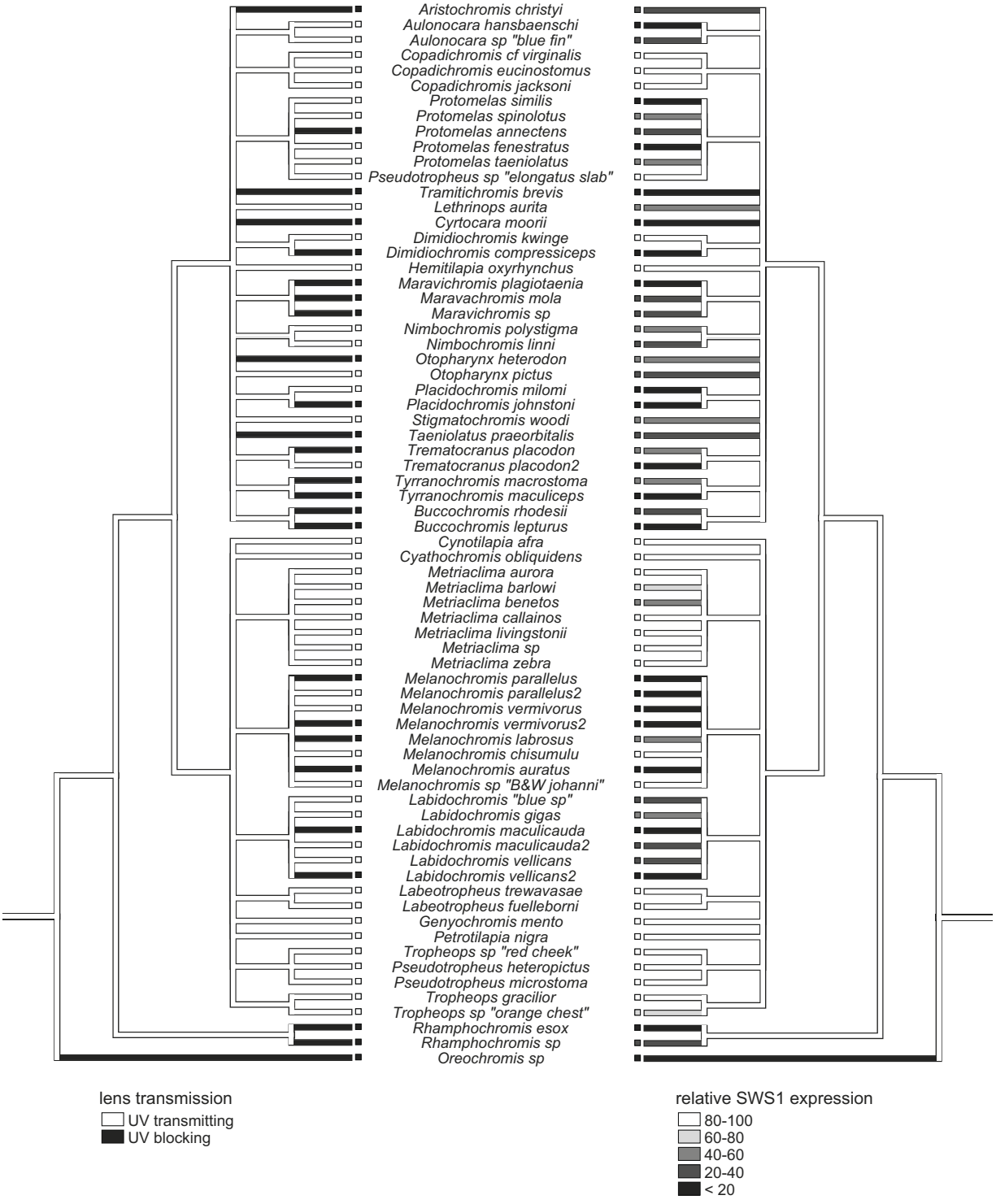

Fig. 2. Both lens transmission and SWS1 opsin expression are labile evolutionarily with considerable variation within and across genera. Character states have been discretized and mapped onto the generic phylogeny. Because of the lack of resolution ancestral states at internal nodes were not inferred.

the short wavelength tail of a SWS2B (violet) or SWS2A (blue) visual pigment, and/or through the beta bands of longer-wavelength visual pigments. They do not, however, have visual pigments that are maximally sensitive in the UV. Finally, UV blocking eyes do not transmit UV light and do not have photoreceptors with peak sensitivity in the UV. We observed all three of these eye types in cichlids from Lake Malawi.

It is worth noting that, while our findings may seem self evident, such relationships must still be tested using a phylogenetic framework. The rapidness of the cichlid radiation, combined with the large changes in opsin expression we have observed among closely related taxa suggest that discordance between lens transmission and opsin expression could easily arise. This scenario might occur due to shared evolutionary history, for example, a species that recently gained SWS2A opsin expression might maintain a UV transmissive lens, or a species that gained a UV blocking lens might not lose SWS1 opsin expression immediately. The fact that these two scenarios were never observed suggests there is either

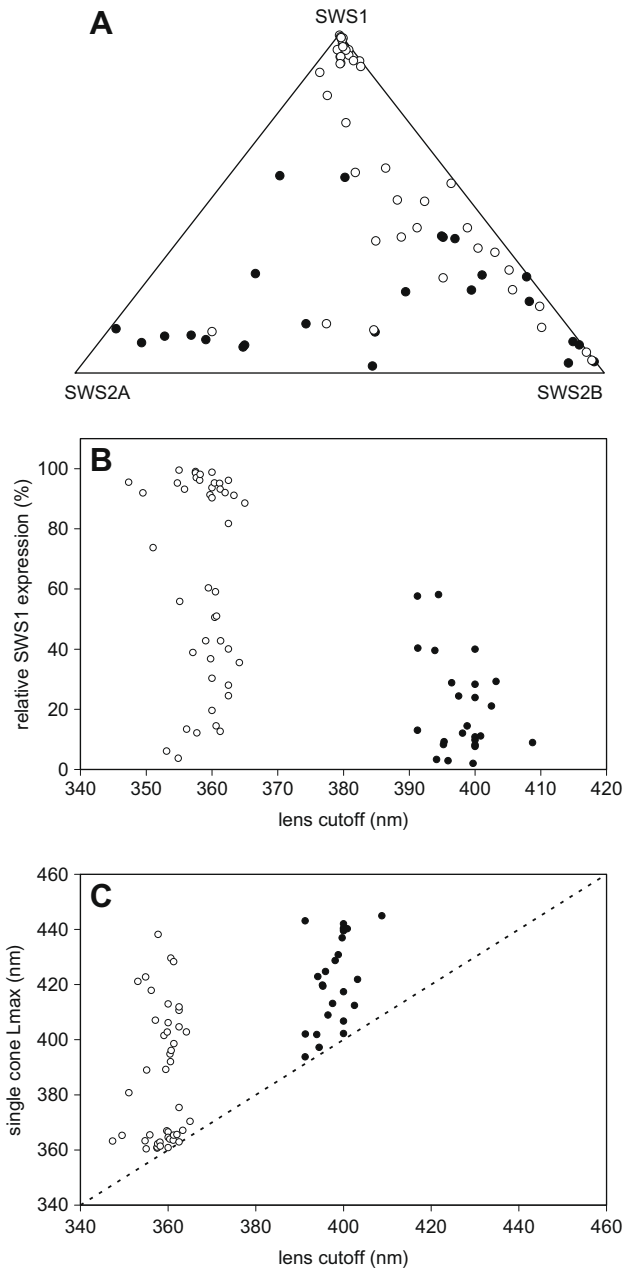

Fig. 3. There is a strong relationship between opsin gene expression, single cone sensitivity and lens transmission. (A) Triangle plot of single cone opsin expression. Species with UV transmissive lenses are illustrated using open circles and UV blocking lenses with black circles. (B) Graph of relative SWS1 expression in the single cones versus lens cutoff. All species with a high percentage of SWS1 expression (>60%) have UV transmitting lenses. (C) Graph of single cone λmax versus lens cutoff. Note that no values fall below the diagonal, suggesting that lens transmission does not limit retinal sensitivity.

a genetic correlation or strong selection for the co-evolution of these two traits.

4.1. Adaptive benefits

We have previously demonstrated that SWS1 opsin expression is correlated with foraging mode in Lake Malawi. Although the riverine ancestors that colonized Lake Malawi lacked SWS1 opsin expression as adults (Carleton et al., 2008; Spady et al., 2006), species feeding on zooplankton, phytoplankton, and algae have gained high levels of SWS1 expression, and shorter wavelength single cone λmax (Hofmann et al., 2009). Our findings here suggest that these species also have lenses that transmit UV light. In addition to foraging, vision plays an important role in mate choice and many cichlids have colors that reflect in the UV (Pauers, McKinnon, & Ehlinger, 2004; Dalton, Cronin, Marshall, and Carleton, unpublished data). While our results do not definitively address whether there are any adaptive benefits for blocking UV transmission and losing UV sensitivity, the correlation between UV transmitting lenses, SWS1 opsin expression, and single cone λmax suggests strongly that gains of UV transmission in Lake Malawi cichlids are adaptive.

Table 2
Comparative analyses.

Phylogenic hypothesis	Method	DF[a]	*t*	*P*
SWS1 Expression vs. lens cutoff				
Generic	BRUNCH	7	2.62	0.034
	PGLS	21	4.34	0.000
Rock-sand	BRUNCH[b]	–	–	–
	PGLS	5	5.30	0.002
Mitochondrial	BRUNCH	10	2.28	0.046
	PGLS	23	3.26	0.002
Single Cone λmax vs. lens cutoff				
Generic	BRUNCH	7	2.58	0.037
	PGLS	21	4.63	<0.0001
Rock-sand	BRUNCH[b]	–	–	–
	PGLS	5	5.72	0.001
Mitochondrial	BRUNCH	10	2.27	0.047
	PGLS	23	3.39	0.001

[a] We subtracted 47, 63, and 26 degrees of freedom from the PGLS analyses using the generic, rock-sand, and mitochondrial trees, respectively, due to the presence of numerous polytomies in each tree.
[b] Independent contrasts of the rock/sand tree yielded only two contrasts, and so could not be used to confidently estimate the relationship between retinal sensitivity and lens transmittance.

4.2. Physiological and genetic implications

The light absorbing compounds in fish lenses are typically either tryptophan derivatives or mycosporine-like amino acids (MAAs) (Thorpe et al., 1993). While the former may be synthesized, the latter must be obtained from the diet (Dunlap & Shick, 1998; Thorpe et al., 1993). A previous study found the tryptophan derivative 3-hydroxykynurenine in the lenses of the Central American cichlid *A. pulcher* and the MAAs palythene and palythine in the lenses of the riverine cichlid *O. niloticus* (Thorpe et al., 1993).

The strong correlation that we observed between lens transmission and UV sensitivity suggests that the two are somehow coupled. We have also shown that opsin expression is genetically determined by only a few loci (Carleton, Hofmann, Klisz, Patel, Chircus, Simenauer, Soodoo, Albertson, and Ser, unpublished data). Since lens transmission is correlated with opsin gene expression, this raises the possibility that lens transmission also has a genetic basis and that these two traits are somehow genetically linked. This raises many interesting questions. For example, do UV transmitting species block specific MAAs from being deposited in the lens or from being absorbed altogether? Is there concordance between MAAs in the lens and the mucus covering the skin (which also contains UV blocking compounds) across species? Can MAAs be modified enzymatically? Finally, previous work also suggests that UV light stimulates the production of MAAs in some tissues (Dunlap & Shick, 1998). We are currently investigating whether cichlids that have been raised indoors (under fluorescent lights) still produce UV blocking compounds in their lenses.

4.3. Evolutionary changes in lens transmission

To our knowledge, our study is the first to examine the correlation between lens transmission and retinal sensitivity in a phyloge-

netic context. It is also the first to examine variation in lens transmission among so many closely related species. Although we had limited phylogenetic resolution – due to the rapid radiations that have occurred in Lake Malawi – several observations stand out. One is that lens transmission is quite labile evolutionarily, and can vary among closely related species, including sister taxa (Fig. 2). A side by side comparison of SWS1 opsin gene expression and lens transmission suggests that opsin gene expression may be more labile than lens transmission, although it should be noted that this comparison is between traits with potentially different modes of character evolution (continuous variation in gene expression versus discrete changes in lens cutoff). Finally, although our limited phylogenetic resolution precludes the reconstruction of the ancestral state at many nodes, both the tilapian outgroup *Oreochromis sp*. and the basal branch containing the two *Rhamphochromis spp*. both have UV blocking lenses, suggesting that UV transmitting lenses represent an evolutionary gain in Lake Malawi. Future phylogenetic studies of lens transmission in older radiations, such as Lake Tanganyikan cichlids and damselfish (which also appear to have diverse visual systems) will provide interesting new insights into the co-evolution of these two traits.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.visres.2009.12.004.

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