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

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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;

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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