

Reprint

# COMMUNICATION IN FISHES

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# Visual Communication in East African Cichlid Fishes: Diversity in a Phylogenetic Context

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## ABSTRACT

The flocks of cichlid fishes in the African Great Lakes are a fascinating system for studies of visual ecology. Separate radiations have produced a diverse flock of several hundred closely related cichlid species in each of the three large lakes (Malawi, Tanganyika and Victoria). Closely related species exhibit a diverse range of color patterns. The three lakes have dramatically different photic environments, from the murky waters of Lake Victoria to the clearer waters of lakes Tanganyika and Malawi. The cichlid species flocks in these lakes, therefore, offer a unique opportunity to examine the forces driving the evolution of visual sensitivity and communication. In this chapter, we shall summarize what is currently known about visual communication in this intriguing system. An especially diverse range of visual sensitivities is found among the species occurring in Lake Malawi. The range of visual sensitivities in Lake Victoria is much narrower, and tends to be at longer wavelengths. These differences suggest an important role for the photic environment in shaping visual sensitivities. There is also evidence for links between color usage and visual sensitivities among the flocks in the different lakes. We shall discuss here the possible relationships between visual sensitivity, ecology, photic environment and color usage, and suggest future work in order to examine the forces acting on visual communication. There is a special need for studies at finer phylogenetic scales, to quantify the variability of visual sensitivities and color between sister taxa. Such studies will provide insights into the possible role of visual communication in cichlid speciation.

**Key Words:** Vision; Opsin gene; Visual communication; Color; Cichlid fish; Cichlidae.

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## 1. INTRODUCTION

The large endemic species flocks of cichlids in the African rift lakes have fascinated scientists and lay people alike. Their rapid diversification has made them a textbook example of vertebrate adaptive radiation and has inspired generations of researchers to study the mechanisms responsible for their rapid speciation. Cichlids are adorned with a striking array of colors comparable with coral reef fishes, which makes them enormously popular in the aquarium trade. These same bright colors have attracted the attention of cichlid biologists and visual ecologists to questions about cichlid visual communication.

Visual communication occurs when a receiver views a signal from a sender. For cichlid fishes, this might involve solar illumination being transmitted through the water, reflecting off a displaying individual, being transmitted through the water, and finally received by another individual. A number of environmental factors are important for both sender signal design and receiver sensitivity (Lythgoe, 1979; Endler, 1992; Loew and Zhang, this volume). The most important is the aquatic photic environment. This includes water quality, substrate, depth, and time of the day. These key elements control the spectral wavelengths, which are available for communication (Loew and McFarland, 1990; McFarland, 1991; Loew and Zhang, this volume).

Visual systems are optimized for tasks including food identification, mate selection, and predator avoidance (Endler, 1992,1993). Like most fishes, cichlids have multiple cone types and are likely to utilize color vision to assist in these tasks (Douglas and Hawryshyn, 1990). Cones detect different wavelengths of light depending on the absorption properties of the visual pigment contained in each cone (Bowmaker, 1990, 1995; Bowmaker and Hunt, 1999; Yokoyama, 2000; Hunt and Bowmaker, this volume). Color vision relies on neural comparisons of the different cone types in the retina.

Many studies have examined how visual pigment sensitivities of fishes correlate with the photic environment (Munz and McFarland, 1973, 1977; Loew and Lythgoe, 1978; Levine and MacNichol, 1979; Bowmaker et al., 1994; Lythgoe et al., 1994; Douglas et al., 1998). These include models, which describe the optimal pairing of visual pigment sensitivities to maximally discriminate certain targets in various waters. Typically, one visual pigment is matched to the wavelengths best transmitted through the water, while other pigments are offset from this maximum to provide contrast (Munz and McFarland, 1973; Lythgoe and Partridge, 1991; Loew, 1995; Partridge and Cummings, 1999; Marshall and Vorobyev, 2003).

Another important factor, which drives visual sensitivity is the mode of foraging. For example, fish which feed on zooplankton often possess ultraviolet sensitivity (Loew et al., 1993; Browman et al., 1994; Losey et al., 2003). Since zooplankton absorb light at ultraviolet wavelengths, a fish which can sense ultraviolet light will detect the darker zooplankton against the bright,

background spacelight. Fish which are not planktivorous have often lost sensitivity in this range (Siebeck and Marshall, 2001; Losey et al., 2003; Siebeck et al., this volume). In addition, UV sensitivity can vary through ontogeny, either being lost after the juvenile stage or gained in adults (Beaudet and Hawryshyn, 1999).

Not as much is known about signaling, but it is assumed that signalers also match their signals to those that are best transmitted through the water (Endler, 1992). Signals are also optimized to contrast with the background against which they are viewed. However, it has been shown in coral reef fishes that signals may also match the background at greater viewing distances. This may allow communication with conspecifics at close range while providing crypsis from predators at larger distances (Marshall, 2000a; Marshall et al., this volume).

In this chapter, we will discuss the ecology of visual communication in cichlid fishes, beginning with the evolutionary history of cichlids, with emphasis on the African Great Lake species, and discuss their importance as an evolutionary model. We will then describe what is currently known about cichlid visual communication, including visual sensitivities and coloration. We will conclude by discussing future directions for studies aimed at unraveling the possible role of visual communication in cichlid speciation.

## **2. CICHLID FISHES**

### **2.1. Evolutionary History**

There are literally thousands of species currently described in the family Cichlidae, with two-thirds (~1500 species) occurring in the Great Lakes of Africa (Turner et al., 2001). Cichlids also inhabit lakes and rivers around the globe from Central and South America to Madagascar and India. This Gondwanan distribution suggests that the family originated over 100 million years (MY) ago, prior to the break up of the major continents (Stiassny, 1991; Kumazawa et al., 1999).

Cichlids in the Great African lakes are a classic example of rapid vertebrate radiation. Each of the major African lakes (Victoria, Tanganyika, and Malawi) harbors a separately evolved flock containing hundreds of species. The Victorian flock is less than 500,000 years old and contains an estimated 700 species of cichlids, giving it the most rapid rate of speciation known in vertebrates (Seehausen, 1996, 2002; Verheyen et al., 2003). The Malawian flock is approximately one million years old and contains from 500 to 1000 species (Konings, 1995; Turner, 1996; Kornfield and Smith, 2000; Turner et al., 2001). The Victorian and Malawian flocks are reciprocally monophyletic and thought to have arisen from a common riverine ancestor within the last two million years (Meyer et al., 1990; Kocher et al., 1995; Seehausen et al., 2003; Verheyen

et al., 2003). The Tanganyikan flock is older, having arisen sometime in the past 5–8 million years, and also contains several hundred species (Snoeks et al., 1994; Konings, 1998). This flock may be the result of multiple invasions of distinct riverine taxa (Salzburger et al., 2002). The search for the identity and characteristics of the nearest riverine ancestors of these species flocks is ongoing (Salzburger et al., 2002; Seehausen et al., 2003; Verheyen et al., 2003). The Nile tilapia, *Oreochromis niloticus*, is a riverine species which diverged from these species ~10 mya, before the emergence of the lacustrine flocks (Kocher et al., 1995). As an outgroup to species from all three lakes, it is representative of the riverine ancestor.

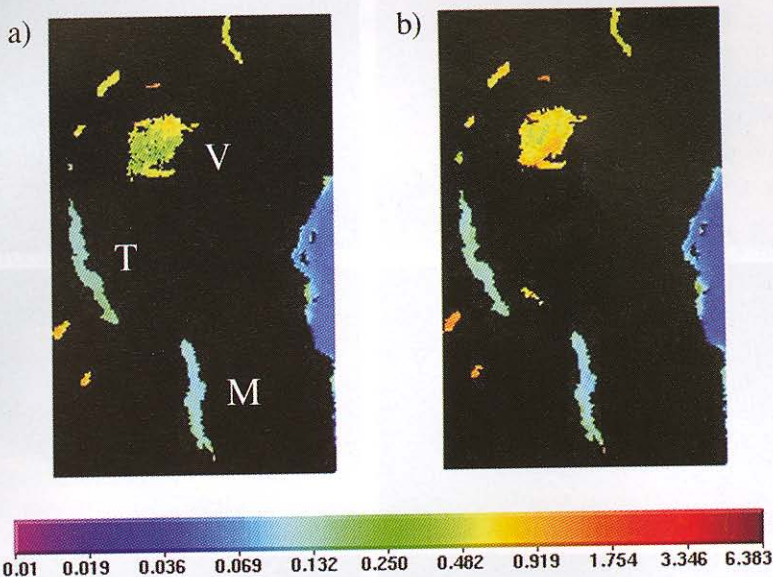
Understanding the mechanisms by which so many species have arisen and are maintained in each of the Great African lakes is an evolutionary puzzle with important implications for understanding the origins and maintenance of biodiversity. Visual communication probably plays an important role in the evolution and preservation of this diversity. Seehausen et al. (1997) demonstrated that degradation of the visual environment can lead to a decrease in cichlid species diversity, through hybridization of previously distinct forms.

## 2.2 Ecological Diversity

### 2.2.1 Photic Environment

The water clarity in which cichlids live varies immensely with light transmissions differing over an order of magnitude. Secchi disc readings in Lake Malawi can be as high as 15 m (Muntz, 1976). However, readings in the more shallow Lake Victoria are much lower, with 1.5–3 m being the best recorded in recent years (Seehausen et al., 1997). There are large variations within each lake over a period of time and across microhabitats as cichlids live everywhere from murky inshore bays to clearer offshore waters. No transmission data are available for surrounding rivers, but they are noticeably less clear than the deep lakes (Muntz, 1976).

These *in situ* measurements of lake-based differences agree with global satellite imagery, which samples attenuation coefficients on a daily basis (Fig. 17.1). The attenuation coefficients at 490 nm averaged over the past several years in both Malawi (0.09–0.18 m<sup>-1</sup>) and Tanganyika (0.12–0.2 m<sup>-1</sup>) are five to ten times lower than those in Lake Victoria (0.4–1 m<sup>-1</sup>). This suggests that light transmission in these two lakes is much greater than in Lake Victoria. Chlorophyll concentrations are similarly diverse with Lake Victoria having an order of magnitude higher concentration than Lakes Malawi or Tanganyika in recent times. This contributes to the decrease in light transmission in Lake Victoria, particularly at shorter wavelengths. While water quality has decreased in the previous century (Seehausen et al., 1997; Verburg et al., 2003), it seems likely that water transmission has always been lower in Lake Victoria because



**Fig. 17.1:** SeaWiFS satellite images of East Africa showing the diffuse attenuation coefficient at 490 nm (K490) for the major African lakes (V, Victoria; T, Tanganyika, M, Malawi). The two panels show averages from 1998–2002 for: (a) June–August and (b) December–February. The color calibration scale is given below with K490 values in  $\text{m}^{-1}$ . Images obtained from <http://seawifs.gsfc.nasa.gov/SEAWIFS.html>.

of its different geology. These spectral differences must influence the optimal visual sensitivities and visual signals of cichlids living in these different lakes.

### 2.2.2 Trophic Diversity

The foraging styles of cichlids include feeding on almost every possible food source (Fryer and Iles, 1972; Liem, 1991; Yamaoka, 1991; Barlow, 2000). Their specialized trophic morphologies provide an indication of their primary modes of feeding (Fig. 17.2). Some species have terminal mouths which are well suited for suction feeding on zooplankton. Others have subterminal mouths, which are better adapted for grazing the algae off rocks in shallow water. Some cichlids comb diatoms and insect larvae out of the algae. Others feed on insect larvae plucked out of the sand. Some can even eat snails, crushing the shells with powerful pharyngeal jaws or removing them from their shells. There are also specialized piscivores, paedophages and scale eaters. Each species has a jaw morphology, which is adapted for a particular approach to capturing prey.

These diverse foraging styles likely have different visual requirements. Some Malawi species detect insect larvae in the sand by vibration (*Aulonocara*), while others sift insect larvae out of the sand (*Lethrinops*). These species do



**Fig. 17.2:** Photographs of male Lake Malawi cichlids with different foraging strategies and mating colorations. While many of these species will feed on more than one food source, their preferred food source is listed. (a) *Astatotilapia calliptera*, omnivore; (b) *Labeotropheus trewavasae*, attached algae; (c) *Cynotilapia afra*, plankton; (d) *Aulonocara jacobfreibergi*, invertebrates dug from sand; (e) *Labidochromis caeruleus*, invertebrates picked from biocover; (f) *Chilotilapia rhoadesii*, snails; (g) *Corematodus taeniatus*, scales; (h) *Dimidiochromis compressiceps*, fish. Photos provided by A. Konings, Cichlid Press.

not rely solely on vision for foraging. Piscivores (*Dimidiochromis*) or scale eaters (*Corematodus*) hunt relatively large prey and do not require high acuity vision. However, planktivorous cichlids, including the sand-dwelling utaka (*Copadichromis*) and several rock dwellers (*Cynotilapia* and *Metriaclima*) need extremely good visual capabilities to pluck zooplankton from the water column.

### 2.2.3 Breeding Behavior

The breeding and parental behavior of cichlids is also diverse. Mating systems include monogamous pair bonding and polygamy. Polygamous systems can be harem or involve females choosing mates from among a group of territorial males (Barlow, 1991, 2000). Parental care can include bi-parental, maternal or, in a few cases, paternal care (Keenleyside, 1991). Egg care includes substrate spawning and mouth brooding. Monogamous pairs are typically substrate spawning with bi-parental care, while mouthbrooders are typically polygamous with females choosing mates and caring for the young.

In Victoria and Malawi lakes, polygamous breeding with maternal mouthbrooding is the most common system. Species breed in large leks, as well as in mixed feeding and breeding territories. Males compete for and then hold territories, while displaying bright breeding coloration. Females choose from among many potential mates and breed with one or several males. Females mouthbrood the young for the first few weeks of life. This differential parental investment leads to strong intersexual selection through female mate choice. There is a corresponding sexual dimorphism in color pattern: males display bright coloration during the breeding season, while the females' colors usually remain more muted, at least to the human eye (Konings, 1995, 1998, 2001; Seehausen, 1996). Sexual selection is thought to be an important driver in the rapid diversification of these species as male color patterns rapidly evolve, driven by female preference (Dominey, 1984). As a result, the most closely related species differ in male color pattern but not in other morphological or behavioral traits (Albertson et al., 1999; Danley and Kocher, 2001; Streelman and Danley, 2003).

## 2.3 Visual Ecology

With such a variety of cichlids living in diverse habitats and utilizing different foraging behaviors and mating signals, cichlids would seem an ideal system to examine the various factors which impact visual sensitivity and visual communication. Since these fishes are phylogenetically so close, most differences in visual sensitivity can be directly related to adaptation to different environments or visual tasks. This makes cichlids an excellent addition to other studies, which have examined the effects of depth (Bowmaker et al., 1994; Hunt et al., 1996; Cowing et al., 2002) and water quality (Lythgoe et al., 1994;



Loew and Zhang, this volume) on fish visual systems. Cichlids also complement studies on the visual ecology of the phylogenetically diverse coral reef fishes (Losey et al., 2003; Marshall et al., 2003a, b).

The replicate speciation events, which have occurred within the three large African lakes, enable some interesting comparisons. It is known that there has been convergent evolution in trophic morphologies between the lakes (Kocher et al., 1993). If visual sensitivity is strongly correlated with foraging behavior, this should also lead to convergent evolution of visual sensitivities. If photic environment is a strong driver of visual sensitivity, then adaptation of riverine ancestors to the two clear lakes may cause similar shifts in visual sensitivity relative to the ancestral state. Comparisons of cichlids from these two clear lakes with lake Victoria can examine the effects of photic environment on cichlid sensitivities. Since a similar set of foraging styles has evolved in the Victorian flock, as well as in lakes Malawi and Tanganyika lakes, these comparisons can help to partition the selective effects of feeding and the photic environment.

In addition to understanding the ecological forces acting on cichlid visual sensitivities, it is also important to examine the effects that visual sensitivities may have on cichlid visual communication. If cichlid visual sensitivities are selected by ecology to favor shorter or longer wavelengths, the strong effects of female mate choice may have important implications for the evolution of male color patterns. Male-male competition may also play a role in causing character displacement of male color in order to reduce male-male aggression (Seehausen and Schluter, 2004). The effects of sensory bias or sensory drive have been demonstrated in other systems and may play a role in cichlid diversification (Endler, 1992; Ryan, 1998). If visual sensitivities evolve rapidly and sister taxa differ in their visual sensitivities, this would have implications for female mate choice, male-male aggression and the evolution of male color patterns. Visual sensitivities could exert divergent selection on male color patterns and precipitate speciation.

The importance of visual cues in cichlid mate choice has been demonstrated. Laboratory experiments have shown that when females are given only visual cues from males, they correctly choose conspecifics for mating (Seehausen et al., 1997; Couldridge and Alexander, 2002; Jordan et al., 2003). However, if spectral cues are removed, females make random mating choices (Seehausen and van Alphen, 1998; Jordan et al., 2003). Despite the importance of vision in cichlid mate choice and perhaps cichlid speciation, little is known about the diversity of cichlid visual systems. In addition, little has been done to objectively quantify cichlid color patterns and spectral reflectances. These visual cues are an important part of sender-receiver signaling and are likely responsive to the same environmental variables as the visual sensitivities. Here we shall describe the work that has been done to date to examine cichlid visual communication.

### 3. CICHLID VISUAL SENSITIVITY

#### 3.1 Molecular Basis of Visual Pigment Sensitivity

Visual pigments comprise an opsin protein bound to retinal, a chromophore derived from vitamin A. Opsins contain seven transmembrane alpha helices, which surround and form a binding pocket for the retinal chromophore. Phylogenetic studies have shown five major classes of opsin proteins, which arose early in vertebrate evolution (Yokoyama, 2000; Ebrey and Koutalos, 2001; Collin et al., 2003; Collin and Trezise, this volume). These include the rod opsin (Rh1) and four cone opsins: very short wavelength sensitive (SWS1), short wavelength sensitive (SWS2), rhodopsin-related medium wavelength sensitive (Rh2) and long wavelength sensitive (LWS). Reconstitution of photopigments from opsin proteins expressed *in vitro* has demonstrated that each of the opsin classes has a unique range of sensitivities. For the species studied to date, the spectral ranges of the peak wavelengths ( $\lambda_{\max}$ ), when combined with 11-cis retinal, are Rh1: 470–510 nm; SWS1: 358 to 425 nm; SWS2: 420 to 474 nm; Rh2 opsins: 466 to 511 nm; and M/LWS: 521 to 575 nm (Ebrey and Koutalos, 2001; Hisatomi and Tokunaga, 2002).

#### 3.2 Tuning Visual Sensitivities

The several known mechanisms for tuning visual sensitivity include: opsin sequence tuning, gene expression tuning, chromophore exchange, and filtering (Loew, 1995; Hunt and Bowmaker, this volume). These mechanisms can act in concert or independently in order to determine the final visual pigment absorption and the wavelengths of light reaching the retina.

##### 3.2.1 Opsin Sequence Tuning

Opsin sequence tuning involves a change in the amino acid sequence of the opsin protein (Bowmaker and Hunt, 1999; Yokoyama, 2000; Ebrey and Koutalos, 2001). The amino acid substitutions that are most effective in causing spectral shifts are those which change the polarity of sites, which are directed into the retinal binding pocket (Chang et al., 1995; Kochendoerfer et al., 1999). Single amino acid changes typically result in  $\lambda_{\max}$  differences of 2 to 15 nm, although a few substitutions are known to cause larger shifts, particularly in the SWS1 class (summarized in Takahashi and Ebrey, 2003). Because the sequence differences occur in individual opsin genes, these changes result in shifts of a single pigment relative to the others. Ionic tuning is a special case of opsin sequence tuning and is the result of chloride ion binding in those M/LWS opsins, which have a histidine in the second extracellular loop (H197 in human red and green opsins, Kleinschmidt and Harosi, 1992; Wang et al., 1993; Sun et al., 1997).

### 3.2.2 Gene Expression Tuning

There are a number of fish species whose visual sensitivities change significantly through ontogeny (reviewed in Bowmaker, 1995). This is thought to be the result of differential opsin gene expression at different life stages. Typically, this occurs in association with a major change in habitat, either from freshwater to marine (Beaudet and Hawryshyn, 1999) or from pelagic to benthic (Evans et al., 1993; Shand, 1994; Shand et al., 1999). Changes in opsin expression may also correlate with changes in foraging style between larval and adult stages (McFarland and Loew, 1994; Britt et al., 2001; Loew et al., 2002; Shand et al., 2002).

### 3.2.3 Chromophore Exchange

Chromophore 'tuning' is the result of the ability of fishes to generate two different retinal chromophores: 11-cis retinal (vitamin A1 derivative) and 3,4-didehydroretinal (vitamin A2 derivative). Shifts from one to the other require several weeks and are correlated with changes in day length, temperature, age/developmental stage, and migration (Munz and McFarland, 1977; Beatty, 1984). A1-based visual pigments (derived from a given opsin) absorb at shorter wavelengths and tend to occur at warmer temperatures, longer day lengths and in clearer waters. A2-based pigment (derived from the same opsin) absorb at longer wavelengths and are correlated with colder temperatures, shorter day lengths and murkier waters. Yearly shifts in A1/A2 usage have only been observed to date in salmonids and a few other migratory species (Loew and Dartnall, 1976; Beatty, 1984; Bowmaker, 1995). Cyprinids and sticklebacks remain primarily A2 (Bowmaker, 1995). Therefore, the likelihood of whether cichlid fishes possess a labile visual system which can be tuned through chromophore shifts in different environments remains uncertain.

The absolute magnitude of the A1 to A2 shift (for a given opsin) is a strong function of the spectral region of peak absorption and can be parameterized in terms of the  $\lambda_{\max}$  of the A1 pigment (Harosi, 1994; Parry and Bowmaker, 2000). Figure 17.3 shows the relative shift of the A1/A2 pigment pair over the complete range of vertebrate visual pigments (Harosi, 1994). The medium to long wavelength pigments have much larger shifts (30–60 nm) than the shorter wavelength opsin classes (5–10 nm). If the entire retina uses the same chromophore, any changes from A1 to A2 will increase the relative peak sensitivity differences between the long and short wavelength cone classes. Chromophore mixtures in single photoreceptors will also broaden the absorption peaks.

### 3.2.4 Filtering

Filtering can change spectral sensitivities by absorbing certain wavelengths before they reach the outer segment of the photoreceptor. This can occur

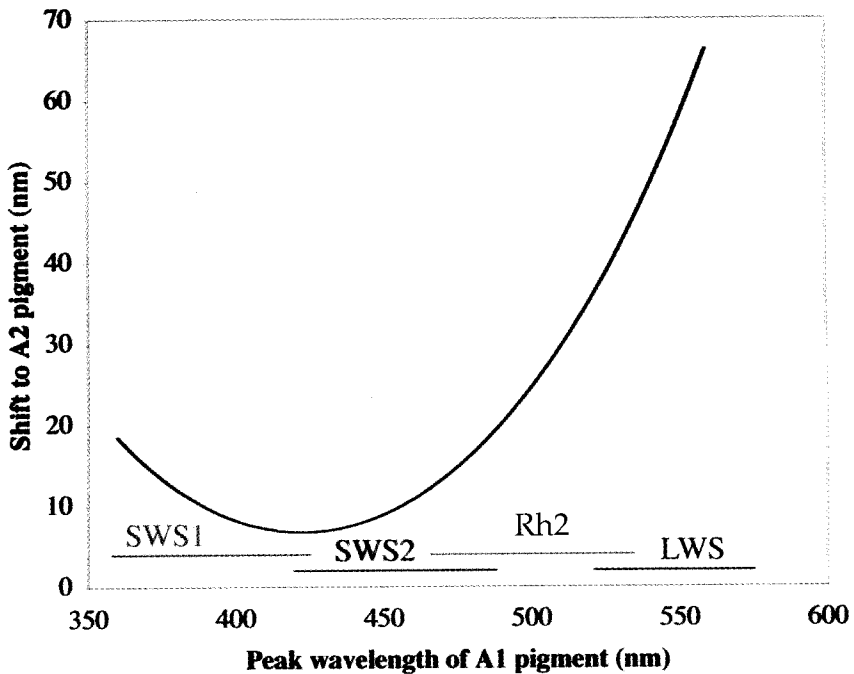


Fig. 17.3: Magnitude of the shift in peak absorption for a visual pigment made from a given opsin protein with A2 versus A1 chromophore plotted as a function of the A1 peak absorption (Harosi, 1994). The spectral range of the visual pigments corresponding to the known cone opsin gene classes is shown by the colored bars. These values have been extended from those of Ebrey and Koutalos (2001) to include cichlid visual pigments. The new ranges are SWS1: 358 to 425 nm; SWS2: 420–488 nm; Rh2 466–535 nm; and LWS: 521–575 nm.

because of pigments deposited either in the lens (Thorpe and Douglas, 1993; Thorpe et al., 1993; Siebeck and Marshall, 2001) or in the inner segment of photoreceptors. These filters typically pass long wavelengths (absorbing at shorter wavelengths) to refine the wavelengths reaching the photoreceptor outer segments.

### 3.3 Photoreceptors in African Lake Cichlids

Spectral absorptions for cichlid photoreceptors have been determined for only a handful of the thousands of cichlid species. The best studied cichlid is *Haplochromis burtoni* (also known as *Astatotilapia burtoni*) from Lake Tanganyika. This species has been used by Fernald and co-workers to study retinal growth and development for over 25 years (Fernald, 1984, 1990). Adults of this species have three visual pigments with peak sensitivities of 455, 523, and 562 nm (Table 17.1, see Fernald and Liebman, 1980). The photoreceptors form a square retinal mosaic with single cones surrounded by pairs of double cones (Fernald,

**Table 17.1:** Peak absorption of visual pigments from African Great Lake cichlid species determined by MSP.

Lake	Species	Rods	Single cone	Medium	Long double	Reference
Tanganyika	<i>H. burtoni</i>	500	455	523	562	a
Victoria	<i>H. argens</i>	506	456	533	567	b
	<i>H. ishmaeli</i>	507	459	535	569	b
	<i>H. piceatus</i>	503	465	522	565	b
	<i>H. pyrrhocephalus</i>	519	462	539	595	b
Malawi	<i>D. compressiceps</i>	505	447 <sup>c</sup>	536	569	d
	<i>M. zebra</i>		368 <sup>e</sup>	488	533	d

<sup>a</sup> Fernald and Liebman, 1980

<sup>b</sup> van der Meer and Bowmaker, 1995

<sup>c</sup> R.K. Jordan and E. Loew (pers. comm.)

<sup>d</sup> Levine and MacNichol, 1979

<sup>e</sup> Carleton, Harosi and Kocher, 2000

1981). This square mosaic forms at approximately 4 days post fertilization and is present throughout ontogeny (Hagedorn and Fernald, 1992).

Four haplochromine species from Lake Victoria have also been studied (van der Meer and Bowmaker, 1995). These species possess three cone pigments with short, medium and long wavelength sensitivity similar to *H. burtoni* (Table 17.1). There is some variation in long wavelength sensitivity among these species, which may partially be the result of chromophore shifts. Histological studies show that both *H. argens* and *H. ishmaeli* utilize a square retinal mosaic, while *H. piceatus* and *H. pyrrhocephalus* have reduced numbers of single cones and a row-patterned retina. Ontogenetic studies in a related Victorian cichlid, *H. sauvagei*, as well as in *H. argens*, show that the square retinal mosaic is retained throughout ontogeny (van der Meer, 1994, 1995).

Two species from Lake Malawi have also been studied. Levine and MacNichol (1979) measured double cones from *Haplochromis compressiceps* (now *Dimidiochromis compressiceps*, Eccles and Trewavas, 1989) and *Pseudotropheus zebra* (now *Metriaclima zebra*, Stauffer et al., 1997). *D. compressiceps* has double cone sensitivities quite similar to other African cichlids with peak absorptions of 536 and 569 nm (Table 17.1). Recent microspectrophotometry (MSP) has shown that the single cones in *D. compressiceps* are also similar to other cichlids with a peak sensitivity of 447 nm (R.K. Jordan and E. Loew, pers. comm.). However, peak absorptions from *M. zebra* visual pigments are radically different from values for *D. compressiceps*. *M. zebra* has ultraviolet-sensitive single cones with a  $\lambda_{\max}$  value of 368 nm (Carleton et al., 2000), while the double cones have  $\lambda_{\max}$  values of 488 and 533 nm (Levine and MacNichol, 1979). Relative differences are 79, 48 and 36 nm for the short, medium, and

long wavelength sensitive cones, respectively. This is the largest known difference in visual sensitivities between such closely related cichlid species. These changes do not result from changes in chromophore usage. A1-A2 shifts would be quite small for the short wavelength pigments and much larger for long wavelength pigments, exactly opposite to the trend observed here. In addition, the MSP spectral widths are consistent with the use of an A1 chromophore for all these species (R.K. Jordan and E. Loew, pers. comm.; Carleton et al., 2000). These shifts in  $\lambda_{\max}$  have occurred during the brief 1-million-year history of the Lake Malawi flock, and if they are the result of opsin sequence tuning, this would suggest a rapid rate of opsin gene evolution (see below).

### 3.4 Cichlid Opsin Genes

MSP studies on cichlid species to date suggest they have a trichromatic retina. Three cone visual pigments are typically arrayed in a square retinal mosaic with the shortest wavelength pigment in the central single cones and the longer pigments in the two members of the double cones. In spite of the presence of three adult visual pigments, recent studies have shown that there are actually five classes of cone opsin genes (Carleton and Kocher, 2001). This includes one gene for each of the SWS1, Rh2 and LWS classes, as well as two genes in the SWS2 class (denoted SWS2a and b). The latter two genes are the result of a gene duplication event that occurred approximately 140 mya. The duplicate SWS2 genes have also recently been found in a freshwater killifish, which is in keeping with the estimated time of duplication (Fuller et al., 2003). All five cone opsin genes appear to be fully functional, with no frame shifts or stop codons in three species from Lake Malawi and the riverine tilapia, *O. niloticus*.

### 3.5 Cichlid Tuning Mechanisms

#### 3.5.1 Opsin Sequence Tuning

Not much is known about the interspecific variation in cichlid visual sensitivities based on the few species studied by MSP. A more rapid way to survey cichlid visual pigment diversity is to examine opsin gene sequences for a large number of species. Opsin sequence diversity for the LWS and SWS2b genes has been recently examined in the Lake Victoria flock (Terai et al., 2002). Not only is the LWS opsin gene diverse among populations it is, in fact, the most variable of any gene identified to date in this species flock (even more variable than MHC genes). Many of the variable amino acid sites are in the retinal binding pocket and involve changes in amino acid polarity. Several of them are known to cause spectral shifts in other species. This suggests that at least some of the LWS opsin gene variation could be functional, producing visual pigments with different peak sensitivities (reviewed in Carleton and Kocher, 2003).

The diversity of the LWS gene stands in sharp contrast to the near invariance of the SWS2b in the same set of Victorian cichlids. While the LWS gene determines visual sensitivity at the long end of the cichlid spectral range, the SWS2b visual pigment is sensitive to the mid part of the spectrum. This suggests that different selective forces are acting on visual sensitivity in these two spectral regions. The different variability is even more impressive when one considers the fact that these two genes are only 6 kb apart in the genome. Selection must act at quite a fine genomic scale to produce the LWS variability next to an essentially fixed SWS2b gene.

The Rh1 opsin gene of the rod photoreceptors has also been studied in some detail (Sugawara et al., 2002). Genetic variation was examined in cichlids from all three lakes as well as in five riverine species. This gene also shows a high level of variability, particularly among the lacustrine species. The ratio of nonsynonymous changes (DNA sequence differences resulting in a different amino acid in the opsin protein) to synonymous changes (DNA sequence differences, which do not change the amino acid) was very low (0.15) for the riverine species. This is indicative of purifying selection acting to maintain function (spectral sensitivity) in these species. However, the ratio of nonsynonymous to synonymous changes was just above 1 for the lacustrine species, suggesting neutral or positive selection. Sugawara et al. (2002) suggest that this change in selection on the Rh1 gene is the result of adaptation to the different lake environments. A few of the identified amino acid changes are in the retinal binding pocket and one site (A292S) is known to cause large shifts in visual pigment sensitivity in human red/green pigments (A308S). The A292S substitution has occurred in several deepwater species and is consistent with shifts in light spectrum to shorter wavelengths with depth (see also Hunt and Bowmaker, this volume). This suggests that rhodopsin pigments do have unique spectral characteristics dependent on the photic environment.

Comparisons of the five cone opsin gene sequences among the three Malawi species (*M. zebra*, *Labeotropheus fuelleborni*, and *D. compressiceps*) examined to date show that the corresponding genes in these three species are nearly identical. For all but the SWS2b gene, there are no differences in the amino acids directed into the retinal binding pocket. This further indicates that opsin sequence changes have not played a significant role in tuning visual pigment absorptions in these species.

### 3.5.2 Gene Expression Tuning

The rock dwelling *M. zebra* and the sand dwelling *D. compressiceps* have some of the largest known differences in visual pigment absorptions amongst cichlids. In spite of this, their opsin gene sequences are nearly identical. The only resolution to this paradox is that these two species do not express the same opsin genes. A pattern of differential opsin gene expression was confirmed using quantitative RT PCR on retinal cDNA from these species.

Both species express the Rh2 opsin. However, *M. zebra* also expresses SWS1 and SWS2b, while *D. compressiceps* utilizes SWS2a and LWS (Carleton and Kocher, 2001).

This unique mechanism for tuning adult visual systems explains the different visual pigment absorption data for these two Malawi species. Figure 17.4 shows a comparison of the relative cone opsin gene expression and the spectral absorbances of the visual pigments for both *M. zebra* and *D. compressiceps*. Due to the narrow tuning range of each of the opsin classes, these data can be used to predict the spectral sensitivities of the corresponding visual pigments. Both species express an Rh2 gene and both have a visual pigment sensitive around 535 nm. *D. compressiceps* has shorter (447 nm) and longer (569 nm) wavelength-sensitive pigments, which are likely to correspond to the SWS2a and LWS opsin genes, respectively. Similarly, *M. zebra* has a very short (368 nm) and a short (488 nm) wavelength-sensitive pigment which may correspond to the SWS1 and SWS2b genes. *M. zebra* also expressed a small amount of a fourth opsin, the LWS (see discussion in Sect. 3.6.2). The predicted spectral sensitivities of the pigments corresponding to these opsin genes needs to be confirmed by in vitro protein expression (Oprian, 1993).

### 3.5.3 Chromophore Tuning

Fish from fresh water are often thought to utilize mixtures of A1 and A2 chromophores to adapt to the more turbid, longer wavelength-transmitting conditions characteristic of fresh water habitats. However, evidence from MSP suggests that cichlids from the clear waters of Lake Malawi use predominantly A1 chromophores (R.K. Jordan and E. Loew, pers. comm.; Carleton et al., 2000). In visual pigment extraction studies, Muntz (1976) showed that Lake Malawi species utilized A1-based pigments. This was in contrast to four species (mostly tilapia) from the Shire river at the southern end of the lake. These riverine cichlids had longer wavelength sensitive porphyropsins (rod pigments based on vitamin A2 chromophores), probably produced by a shift from A1 to A2 chromophores.

Cichlids from Lake Victoria have been examined with MSP (van der Meer and Bowmaker, 1995). The results suggest that the Victoria flock also utilize mixed chromophores. In particular, the species *H. pyrrhocephalus* had a visual pigment  $\lambda_{\max}$  of 595 nm. Such long-wavelength pigments normally require the presence of an A2 chromophore in combination with a LWS opsin.

These data demonstrate that chromophore exchange is used by cichlid species. This mechanism will have the greatest impact on the LWS pigments where shifts are largest. Species living in turbid water are most likely to use A2 chromophores. This finding is consistent with the turbid photic environment found in Lake Victoria. Further studies are needed to confirm the generality of A2 usage and test for its lability in the Victorian flock.



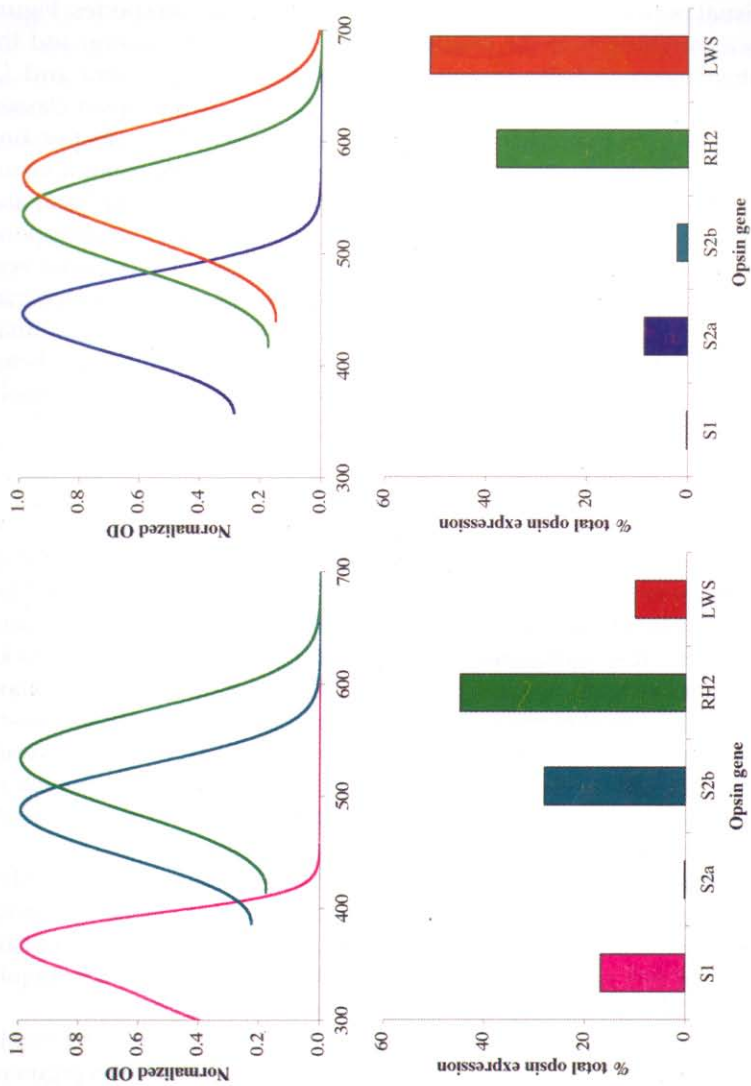


Fig. 17.4: Comparison of MSP visual pigment absorbances and cone opsin gene expression for the Lake Malawi cichlids *M. zebra* (left) and *D. compressiceps* (right). MSP curves are calculated based on templates of Govardovskii et al. (2000) and scaled to unit absorption. Cone opsin gene expression for each gene is given as a percent of the total opsin mRNA expressed.

## 3.5.4 Filtering

The potential for filtering of short wavelength light by cichlid lenses has been examined by transmission studies (Thorpe and Douglas, 1993; Thorpe et al., 1993). Ten species from Lake Victoria and two from Lake Tanganyika were studied (Table 17.2). These data show that some cichlid lenses do have blocking pigments that reduce lens transmission at shorter wavelengths. Interestingly, the Lake Victoria planktivore, *H. argens* does not have any pigment, while the rest of the Victorian species do. Such differences in lens properties have also been observed among closely related species of coral reef fishes, where the differences are associated with planktivory (Losey et al., 2003).

The Lake Victoria species for which lens pigments were extracted had two different pigments, with peak absorptions at 320 and 360 nm. These likely correspond to palythine (320 nm) and palythene (360 nm), which were also detected in *O. niloticus* (Thorpe et al., 1993). However, two Lake Tanganyika species had only the 320 nm pigment. This suggests that the Lake Tanganyika species have the potential for shorter wavelength sensitivity than the majority of the Lake Victoria species. This would be in keeping with the water transmission properties of these two lakes.

Several Lake Victoria cichlids as well as *O. niloticus* were examined to study changes in lens transmission as a function of age and lens size. These studies suggest that the cut off wavelength increases with age and then levels

**Table 17.2:** Lens transmission properties of East African lake cichlids (Thorpe et al., 1993). The 50% transmission point is given for the range of lens sizes considered. In addition, the peak absorption wavelength of any pigments extracted are also given. For some species, pigments were detected but not extracted and quantified.

Habitat	Species	No. of fish	Lens diameter (mm)	Lens 50% transmission (nm)	Pigment $\lambda_{max}$ (nm)
Nile River	<i>O. niloticus</i>	66	0.86–6.81	339–402	324, 360
Victoria	<i>H. argens</i>	11	1.43–3.34	315–328	–
	<i>H. ishmaeli</i>	16	0.98–3.08	323–390	320, 358
	<i>H. nyererei</i>	1	3.01	358	+
	<i>H. piceatus</i>	2	1.94–2.03	344–360	+
	<i>H. pyrrhocephalus</i>	2	2.01–2.09	325–339	+
	<i>H. sauvagei</i>	5	1.50–3.57	327–394	+
	<i>H. xenognathus</i>	4	1.41–3.41	335–390	323, 360
	<i>H. "velvet black"</i>	1	3.10	392	+
Tanganyika	<i>Lamprologus dahlia</i>	1	1.82	351	320
	<i>L. tertiocephalus</i>	1	2.82	348	321

– no detectable pigment

+ pigment detected but not extracted

off at the point where the 50% transmission point is just below 400 nm (Thorpe and Douglas, 1993). Since fish lenses grow throughout life, the amount of pigment deposited in the lens must first increase to effect the transmission change, but then must also decrease to enable the leveling off of the 50% transmission point at 400 nm.

These data suggest probable variations in lens transmission between African cichlids. The lenses of some species do transmit into the ultraviolet wavelength range, particularly in species from the clear waters of Lake Tanganyika. UV transmission has also been demonstrated in recent studies of Lake Malawi cichlids, where lenses of both *M. zebra* and *D. compressiceps* were shown to be UV transmissive (50% transmission at 350 nm; R.K. Jordan and E. Loew, pers. comm.). This suggests that ultraviolet-sensitive cones in the cichlid retina should be able to receive and detect ultraviolet wavelengths.

### 3.6 Discussion of Cichlid Visual Sensitivities

Cichlids have evolved a unique mechanism for tuning visual sensitivity using differential gene expression. Through this mechanism, closely related species within a given lake can radically differ in visual pigment absorptions. This results in major changes in visual sensitivity, which would not be possible through the slower evolutionary process of opsin sequence tuning. To accomplish large spectral shifts of 30–80 nm would have required substitutions of several amino acids in each of the three cone opsin genes. Such large changes might not even be possible because of the limitations in spectral tunability of each of the opsin classes. By turning opsin genes on or off, cichlids bypass this evolutionary constraint, and so broaden the spectral options available for a given photoreceptor class.

#### 3.6.1 Possible Ontogenetic Change?

All cichlid adults studied to date utilize only three cone visual pigments. However, they all seem to carry five cone opsin genes in their genome. This raises the interesting question of why cichlids have so many opsin genes. One possibility is that some of these genes are expressed at another life stage. Certainly, fish species are known to utilize different visual pigments in the juvenile stage. For example, juvenile salmon have corner cones that express an SWS1 opsin gene, which corresponds to an ultraviolet-sensitive pigment (Beaudet and Hawryshyn, 1999; Allison et al., 2003). The corner cone and SWS1 opsin gene expression are lost at smoltification prior to migration to the ocean. However, studies of the cichlid retinal mosaics suggest that both *H. burtoni* and several of the Lake Victoria species have stable retinal mosaics throughout ontogeny (Hagedorn and Fernald, 1992; van der Meer, 1994, 1995). Therefore, they do not appear to lose or gain cone types.

In addition to stable retinal mosaics, most cichlids do not have significant changes in their visual environments during their lifetimes. The African lakes

provide a fairly constant environment. All three lakes are near the equator (Victoria: 0.5° N to 3° S Tanganyika: 3–8° S, Malawi: 9–14° S latitude) with roughly constant solar illumination and temperature throughout the year. There is typically a rainy season, and changes in water clarity can vary as a function of local water runoff. However, in general, there are no major changes in photic environment over the several-year lifetimes of these fish.

Cichlids also do not experience significant changes in habitat and do not migrate significant distances. In Lake Malawi, rockdwellers are particularly stenotopic and tend to remain throughout their life within a short distance (~100 m) of where they are released by the mother (Danley et al., 2000). Sand dwellers do tend to come to the shallows for a month of breeding and return to deeper waters for the rest of the year. However, in general, there are no major habitat changes during their lifetimes.

There are changes in foraging styles from the larval to juvenile to adult stage of these species. Fry typically start out eating zooplankton and other microorganisms before switching to the adult specialty (Barlow, 2000; Genner et al., 2003). This change could be linked to a change in visual sensitivity.

Future studies are needed to determine whether there are changes in opsin expression through ontogeny in any of these cichlid species. Such changes could explain why these species have five fully functional cone opsin genes but only utilize three of them in the adult stage. If the genes are utilized at some other life stage, they will be maintained by selection.

### 3.6.2 Possible Tetrachromaticity?

In addition to the three cone opsin genes corresponding to the known visual pigments, there can be expression of additional opsins, detected in retinal mRNA. In *M. zebra*, significant LWS opsin was detected even though the corresponding long wavelength-sensitive visual pigment has not yet been observed by MSP for this species. However, MSP cannot be used to prove the absence of a pigment because of its low sampling rate and insensitivity to unequal mixtures of pigments. There are other possible explanations for LWS opsin expression in *M. zebra*. The LWS gene is in a tandem array with the SWS2 genes. They are arrayed SWS2a–SWS2b–LWS with 4.5 kb separating the first two genes and 6 kb separating the latter two (Carleton and Kocher, 2001). Since the SWS2b gene is expressed in this species, the chromatin must be opened up for transcription. This may enable some low level expression of the LWS gene. The result may be LWS expression in some small region of the retina or co-expression with another opsin gene in the same photoreceptor. Both of these outcomes would be difficult to detect with MSP.

Electroretinograms (ERG) allow a global test for a functioning LWS visual pigment. Preliminary ERG data is available for a closely related species *M. thapsinogen* (Garner et al., 2003; Olson et al., 2004). ERG data was gathered over the spectral range from 320 to 680 nm. The spectral sensitivity data was

then compared to a model combining the summed response to all three visual pigments based on MSP (368, 488 and 535 nm). The model did not accurately reproduce the sensitivity data in the long wavelength part of the spectrum. A small amount of additional long wavelength sensitivity (570 nm) was required to match the simulated response with the actual data. This suggests that the LWS visual pigment is present in retinas of shorter wavelength-sensitive cichlids. However, ERG data cannot determine whether the LWS pigment occurs in unique LWS cones or whether it is co-expressed with another pigment. It is also unclear whether it is localized in some special part of the retina or is present throughout. Future studies using *in situ* hybridization or immunohistochemistry will be required to determine visual pigment distributions. In addition, behavioral studies will be required to test the implications of four possible pigments and determine whether cichlids possess the ability to discriminate color more finely than would be possible with just a trichromatic visual system (Douglas and Hawryshyn, 1990).

## 4. CICHLID COLOR

### 4.1 Cellular Basis of Color

Fish color patterns are generated through specialized pigment cells called chromatophores (Bagnara and Hadley, 1973; Fox, 1979; Fujii, 1993). The pigment cells absorb and reflect light. The wavelength content of this reflected light is then interpreted by the receiver as color. There are three primary cell types, each of which contain characteristic pigments. Melanophores (black, brown) contain the pigment melanin. Erythrophores (red) and xanthophores (orange and yellow) contain pteridines and carotenoid pigments. Iridophores contain guanine, which is colorless, but has a high index of refraction. Guanine is held in reflecting platelets, which cause constructive interference to preferentially reflect certain wavelengths of light. Iridophores produce the shorter wavelength-reflected colors such as blues and greens.

The organelles which contain the pigments are highly motile in many fish chromatophores. This motility enables fish to turn on and off certain colors. Some of the chromatophores are under hormonal control so that long-term changes occur over several weeks. However, in cichlids, many of the chromatophores are under neural control, so these color changes can be quite rapid (<1 sec). The patterns are often a function of a fish's social status and emotional state (Muske and Fernald, 1987a, b). The ephemeral nature of cichlid color patterns complicates their study.

### 4.2 Cichlid Color Patterns

Several studies have examined the diversity of cichlid color. Much of this work has been through human eyes and involved analyses of photographs. In spite of the obvious limitations of this approach, some interesting results were obtained regarding the selective forces acting on cichlid color and the possible

role of phylogenetic and developmental constraints. Two studies examined the diversity of cichlid color usage within the rock dwellers of Lake Malawi (the mbuna) and tested for correlations between color usage and ecological and behavioral factors (McElroy et al., 1991; Deutsch, 1997). The most important conclusion from these studies was that most of the variation in male breeding color was distributed within, rather than among, genera. There were no significant correlations between habitat and color usage. This supports the idea that male color patterns have evolved rapidly among closely related species. This is assumed to be the result of female mate preference and intersexual selection as well as male-male competition and intra-sexual selection (Danley and Kocher, 2001; Allender et al., 2003; Seehausen and Schluter, 2004).

Although color usage in the mbuna is diverse, it is somewhat limited to the yellow and blue hues of the human visual system. This was attributed to the water transmission properties of Lake Malawi (McElroy et al., 1991; Deutsch, 1997). Since Lake Malawi is extremely clear, it will have spectral properties similar to that of pure water with peak transmission near 500 nm. Blue to yellow hues should be maximally transmitted in these waters. This differs from Lake Victoria, where water transmission is shifted to longer wavelengths. Not surprisingly, a sample of the Lake Victoria cichlids suggests that hues are shifted to longer wavelengths, with more blue-greens and reds (Seehausen, 1996, 1999).

These differences make sense in light of our new knowledge on cichlid visual sensitivities. If many of the mbuna have a short wavelength-shifted visual system similar to that of *M. zebra*, then males may shift their color usage to the shorter blue and yellow hues. This may help maximize male brightness by matching the shorter wavelength sensitivities of females. The Lake Victoria species examined have longer wavelength-shifted visual sensitivities. This correlates well with the greater use of longer wavelength hues such as blue-greens and reds in this species flock.

A larger scale study of color pattern evolution in the East African cichlid fishes corroborates the Lake Malawi studies. Seehausen et al. (1999) examined male nuptial coloration and stripe patterns in multiple individuals from 100 species of cichlids. These included species from all three African lakes as well as neotropical, Indian and Malagasy cichlids. By mapping color pattern characters onto a molecular phylogeny, the researchers determined that the evolution of stripe patterns was often associated with habitat. However, the evolutionary origins of nuptial coloration were most closely associated with changes in the mating system. In addition, sister taxa were more likely to differ from each other in hue if they were promiscuous. This suggests that changes in hue are most common in cichlids subject to strong intersexual selection.

Little information is available on the spectral content of cichlid reflectivities. Kornfield (1991) published reflectance curves demonstrating that the blue color from *M. zebra* is highly reflective in the ultraviolet. This correlates well with

the UV sensitivity of this species and supports the idea that UV wavelengths can contribute to cichlid visual communication.

## **5. IMPLICATIONS FOR CICHLID COMMUNICATION**

Work on cichlid visual communication is just beginning. Preliminary evidence suggests a large variability in visual sensitivity between species. Below, we shall describe some of the questions which must be addressed before we can understand cichlid visual communication and its possible role in the rapid speciation of cichlid fishes.

### **5.1 How Variable are Cichlid Visual Sensitivities?**

We need to know the phylogenetic scale at which cichlid visual sensitivities vary. To date, MSP has been used to examine only two species from Lake Malawi, one species from Lake Tanganyika and four species from Lake Victoria. The two Malawi species are representatives of the sand- and rock-dwelling clades, which diverged about 1 mya, early within the history of the lake. Therefore, comparisons between more closely related species are needed. Ideally, we would like to compare visual sensitivities between sister taxa to see if they ever differ.

The rapid evolution of cichlid species and the confounding problems with ancestral polymorphisms can make it difficult to identify such taxa (Parker and Kornfield, 1997; Kornfield and Smith, 2000; Kocher, 2003). New molecular methods have recently begun to identify sister taxa, which will allow us to examine visual variability at the fine scale needed (Albertson et al., 1999, 2003). MSP studies in progress on cichlid species within one genus from Lake Malawi (R.K. Jordan and E. Loew, pers. comm.) and another genus from Lake Victoria (J. K. Bowmaker, J. Parry, K.L. Carleton and O. Seehausen, unpubl. data) should provide useful information in this regard.

### **5.2 What is the Molecular Basis for Differences in Visual Sensitivity?**

We know that some species within Lake Malawi can express different sets of opsin genes resulting in large differences in visual pigment absorption. However, we need to know at what phylogenetic scale these differences arise. Does this change in opsin expression happen multiple times within the Lake Malawi species flock or did it occur only once? Has it occurred in either the Lake Tanganyika or Lake Victoria flocks? Is it a common factor in tuning visual sensitivities, or a single event that happened early within the divergence of just one flock?

There is also evidence that at least some opsin genes—such as the LWS opsin gene in the Victorian cichlids—can be highly variable at sites likely to contribute to different  $\lambda_{\max}$  values (Terai et al., 2002). Further studies are needed to examine the variability of the other opsin genes in each of the species flocks. Evidence of differences between the most closely related species would be most significant. The functional significance of any sequence changes would need to be confirmed by *in vitro* expression of the opsin proteins (Oprian, 1993) or through careful MSP measurements.

There is also a potential role for shifts in chromophore usage. Cichlid species have already been shown to differ in whether they utilize A1, A2 or a mixture of retinals. Shifts are greatest for the longer wavelength-sensitive pigments, which would modify the relative spacing of the visual pigment absorption peaks in the various cone classes and impact the perception of color. As chromophore usage can shift over a few weeks in response to changes in lighting, temperature and even diet (Bowmaker, 1995), studies are needed on newly caught cichlids from different lake habitats to answer several questions. How prevalent is chromophore exchange within the different lakes? Is chromophore usage linked to photic environment? Is there any evidence for temporal changes in chromophore shifts throughout the year? Do closely related species in the same habitat ever differ in chromophore usage?

### 5.3 What Selective Forces Shape Cichlid Visual Sensitivity?

The selective force most likely to play a role in shaping the cichlid visual system is the photic environment. Comparisons between Lake Malawi and Lake Victoria suggest that many of the Victorian cichlids utilize a long wavelength-sensitive visual system, as expected for the murky waters of that lake. However, both long and short wavelength-shifted visual systems are found in Lake Malawi. This suggests that a broader range of visual systems is possible in the broadly transmissive waters of Lake Malawi, but only the long wavelength systems are adaptive for the limited spectral range found in Lake Victoria.

There may be other important ecological or behavioral factors controlling visual sensitivity, such as foraging. Indeed, foraging on zooplankton has been found to be a critical factor in determining whether coral reef fishes have ultraviolet sensitivities (Losey et al., 2003; Siebeck et al., this volume). Analysis of correlations with foraging can be complex, as some cichlids are opportunistic and feed on multiple sources dependent on the time of the year and availability. However, there are some groups (sand-dwelling utaka; certain species in *Cynotilapia* and *Metriaclima*), which tend to be more planktivorous than others. The spectral sensitivities of these species need to be compared to non-planktivorous species to determine the effect of foraging on visual sensitivity.

There may also be different forces, which act on juvenile life stages separately from adults. Therefore, it is important to compare visual sensitivities of both adults and juveniles. Examination of which opsin genes are expressed



in the juvenile stage may also help explain why some opsin genes are retained even though they are not expressed in the adult stage.

#### **5.4 What is the Spectral Content of Cichlid Colors?**

While a number of studies have examined cichlid color usage through human eyes, studies have yet to be carried out to objectively quantify cichlid color reflectances. It seems likely, based on reflectances from other fishes (Marshall et al., 2003; Marshall et al., this volume) and the little cichlid data available (Kornfield, 1991) that a broad range of wavelengths will be present in cichlid colors as well. The spectral content of cichlid colors needs to be quantified for species in different lakes and different habitats. In light of the ultraviolet sensitivity of certain cichlid species (and the lack of human UV sensitivity), particular attention needs to be taken to measure reflectivities in this specular range.

#### **5.5 What are the Forces Acting on Cichlid Color Patterns?**

When interpreted through the human visual system, the colors and patterns of breeding male cichlids appear conspicuous, while the females appear cryptic. However, human visual interpretations can be misleading and lead to erroneous conclusions about the selective factors at work (Marshall, 2000a, b; Marshall and Vorobyev, 2003). Cichlid coloration is likely subject to a number of selective pressures. First, predation can have an important effect where stronger predation can lead to reduced male conspicuousness (Endler, 1991). Observations of the sand- and rock-dwellers in Lake Malawi suggest that sand dwellers are more muted in coloration than rock dwellers, perhaps as a result of higher predation pressure over the open sand. However, a comparative study of brightness among populations of two Lake Victoria species found male brightness unaffected by or positively correlated with predation pressure (Seehausen et al., 1997). Second, female mate choice requires male colors to be conspicuous under the viewing conditions of the females during selection. The distance of females from males and the background against which males are viewed are important. There will also be an impact of photic environment in such a way that spectral colors will match those wavelengths that can be maximally transmitted, simultaneously providing a contrast against the background against which it is viewed (spacelight, sand or rocks). Third, cichlid visual sensitivities will be an important force. If females have shorter wavelength-visual sensitivities, which have evolved for optimizing prey detection, this may increase selection for males with spectral reflectances that match. There is some indication that this has occurred. Male cichlids in Lake Malawi utilize UV, blue and yellow, which correspond with the shorter wavelength sensitivities found in some of these fish. Conversely, male cichlids in Lake Victoria utilize more blue-greens and reds, which correspond to the

longer wavelength sensitivities found in these fish. This characterization needs to be quantitatively tested on the basis of spectral measurements made for all the key factors: cichlid spectral sensitivities, cichlid spectral reflectances, water transmission, and spectral reflectance of backgrounds.

## **5.6 What is the Role of Cichlid Visual Sensitivities in Cichlid Speciation?**

Previous studies have demonstrated a clear link between ecology, visual sensitivities and color usage (Boughman, 2001; Fuller, 2002; Fuller and Travis, 2004; Fuller et al., 2004). The exact roles of these different factors in cichlid visual communication and speciation remain to be seen. If sensory biases have played a significant role in driving speciation, we expect closely related taxa to differ in visual sensitivity, and for these sensitivity differences to be correlated with differences in male coloration. However, this may be too simplistic a picture. Color discrimination (and pattern detection) involves more than just a difference in photoreceptor absorption. Higher order neural processing in the visual cortex is also needed to integrate differences in hue and various melanin patterns. Neural processing beyond the visual cortex is likely to contribute to female mating preferences or decisions by males to be aggressive.

It is possible, therefore, that cichlid visual communication may be gently molded by broad effects of photic environment and other ecological factors. These factors might define the palette of colors that cichlids choose from in a given lacustrine or riverine habitat. However, the individual colors for a particular species may be the product of more random processes of female choice, male competition, and genetic variability of color pattern generation. We hope that with more studies of cichlid diversity, the roles of vision and color in creating this diversity will become clearer so that we might learn how the rainbow of cichlid species have come to be.

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