Minireview

Will he still look good with the lights on? Spectral tuning of visual pigments in fish

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Abstract

Visual perception is a key element in evolution, as it is required for many life processes. Two recent studies in *BMC Biology* shed light on the genetic determinants of color detection in strikingly colored fish.

Early work on animal behavior by Jakob Uexküll defined each animal's perceived world as its *Umwelt*. In this regard every species lives in its own world. Bats 'hear' their world mostly by echolocation, elephants communicate with very low-frequency sounds and, likewise, the ultraviolet (UV) world of insects is hard for us to imagine. We live in a world that we perceive to a large extent through vision, as do many other organisms. But not all visual worlds are the same; each species perceives only a subset of light wavelengths, which are determined by various evolutionary pressures. For example, color-driven sexual selection is rife among fish, including sticklebacks, cichlids, and poeciliids (guppies and swordtails) [1-3]. (The family that cichlids belong to is aptly named *Buntbarsche* in German, which translates as 'colorful perches'. Cichlids and guppies display stunning color diversity, whereby males differ markedly in coloration from females [4,5], but they pay a price for this by increasing their risk of predation. Furthermore, the vision of each species is tuned to its spectral environment and must enable a balance between successful foraging, predator avoidance and the choice of attractive mates. Also, during development, the requirements of the fishes' visual worlds might change because larva and adults feed on

different foods, live in different places or are preyed on by different predators. Therefore, it is important to understand better how sensory cells might change not only during their evolution, but also during their development. Specifically, what are the developmental and genetic mechanisms that shape the unique visual palettes of different species?

The vertebrate retina is a complex structure that can detect wavelengths that span from UV at about 350 nm to far red at about 630 nm [6]. This range is determined primarily by the ratio of rods to cones and the visual pigments that they contain [7]. The opsin pigment genes are a central factor in determining the visual landscape that species can detect. Vertebrate visual pigments are classified into six evolutionarily distinct classes on the basis of the parts of the visual spectrum they are most sensitive to. These classes are RH1 (rhodopsin; about 500 nm absorbance), RH2 (rhodopsin-like; 470-510 nm), SWS1 (short wavelength; 360-430 nm), SWS2 (SWS1-like; 440-460 nm), LWS/MWS (long- or medium wavelength; 510-560 nm) and the P group (pineal-gland specific; 470-480 nm). Gene duplication within these classes can, in concert with mutation of key amino-acid residues in the light-absorbing portions of the proteins,
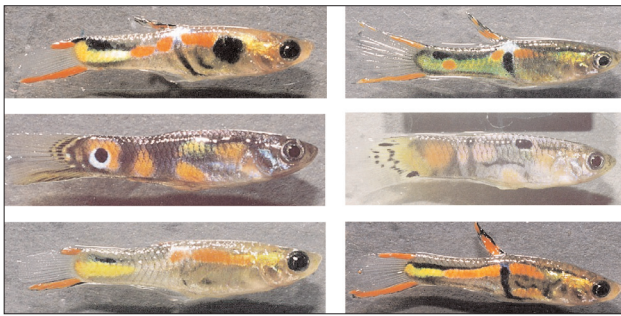
Figure 1
Examples of adult male guppies (*Poecilia reticulata*). Sexual selection in guppies is based on their striking color patterns. Images kindly provided by Felix Breden.

expand their absorbance spectra further still [7]. Two papers recently published in *BMC Biology* explore the genetic basis of spectral absorbance in colorful fish [8,9].

Ward and co-workers [8] examined spectral tuning in the vision of guppies (*Poecilia reticulata*), a popular model for studying the role of male color pattern in sexual selection. They describe four LWS opsin genes, *LWS S180*, *LWS S180r*, *LWS P180*, and *LWS A180* [8]. Through the analysis of five key amino acids in the light-absorbing portions of the proteins encoded by these LWS genes, Ward *et al.* predicted that the proteins are most sensitive to three separate wavelengths in the orange/red spectra. In addition, in an in-depth phylogenetic analysis, the LWS sequences were separated into three well supported clades that included a range of fish lineages. Maximum parsimony analysis indicated that the four guppy LWS opsins are the consequence of three gene-duplication events, which have provided *Poecilia* species with a larger repertoire of LWS pigments than any other fish taxon studied to date.

One might predict that the spectral absorbance of guppies is strongly shifted towards orange, as this color is important in sexual selection in this species. Males with orange spots are particularly attractive to females [5,10] (Figure 1). In this study [8], quantitative PCR showed that all four LWS genes are coexpressed in the adult eye. This equips guppies with the ability to distinguish narrow spectral shifts in the red/orange color range, thus enabling them to appear brighter and more conspicuous to conspecifics, but not to predators with different wavelength sensitivities. These observations are in line with the sensory exploitation hypothesis for preference evolution, which suggests that sensory systems are involved not only in mate choice, but also in a multitude of other biological tasks, and thus will evolve in response to several different pressures [10].

Ward and co-workers [8] have built on the research of Weadick and Chang [11] and Hoffmann *et al.* [12], who also cloned LWS genes from the guppy. Some differences in LWS copy number are found between these papers, and future work will be necessary to determine whether these are laboratory artifacts or real differences between guppy populations. In particular, the Southern blot results of Weadick and Chang [11] showed three copies of LWS in guppies sampled from a tributary of the Paria River, Trinidad, whereas Ward et al. [8] found four copies of LWS in guppies sampled from Cumaná, Venezuela. These differences, if they turn out to be real, could possibly enable local guppy populations to tune their visual perception to the turbidity and light penetration in the local area.

In a similar vein, in African cichlid fish the LWS gene sequences are surprisingly differentiated between populations living at different depths in the turbid waters of Lake Victoria [13]. In fact these sequences show clear signs of strong positive selection [14,15]. Murky waters, such as those of Lake Victoria and many East African rivers, scatter and absorb light of short wavelengths, causing a spectral shift towards longer wavelengths [16]. This results in very different light environments at different depths, which may have contributed to the rapid divergence in sexual display coloration in the males of some cichlid species, in addition to a shift in the perception of these colors.

But what happens to opsin gene evolution in cichlid fish in crystal clear lakes? Lake Malawi in East Africa is one of the best examples of this degree of clarity [13,15]. Carleton and co-workers [9] have explored the evolution of opsin gene function by comparing Lake Malawi cichlids with a distantly related riverine ancestral cichlid lineage. Unlike the Lake Victoria cichlids, the opsin gene sequences of Lake Malawi cichlids show only limited variation [14,15]. This is surprising because the cichlid species flock of Lake Malawi is several times older than that of Lake Victoria. Nonetheless, the spectral absorbance of the Lake Malawi cichlid opsins varies between species, through differences in expression of the various classes of opsin genes [14,17,18].

The novelty of this research is that it examines fine-scale ontogenetic changes in opsin gene expression for Lake Malawi cichlids and compares them with the riverine, more basal, tilapia cichlid lineage (*Oreochromis niloticus*). Tilapia has seven cone opsins, including SWS1, SWS2b, SWS2a, RH2b, RH2aβ, RH2aα, and LWS (Figure 2). Lake Malawi's cichlid species flock contains an estimated record number of up to 1,000 species. Although detailed knowledge of phylogeny is still lacking, two major groups of cichlids make up the vast majority of this adaptive radiation - those that live over rock (which are also called mbuna) and the
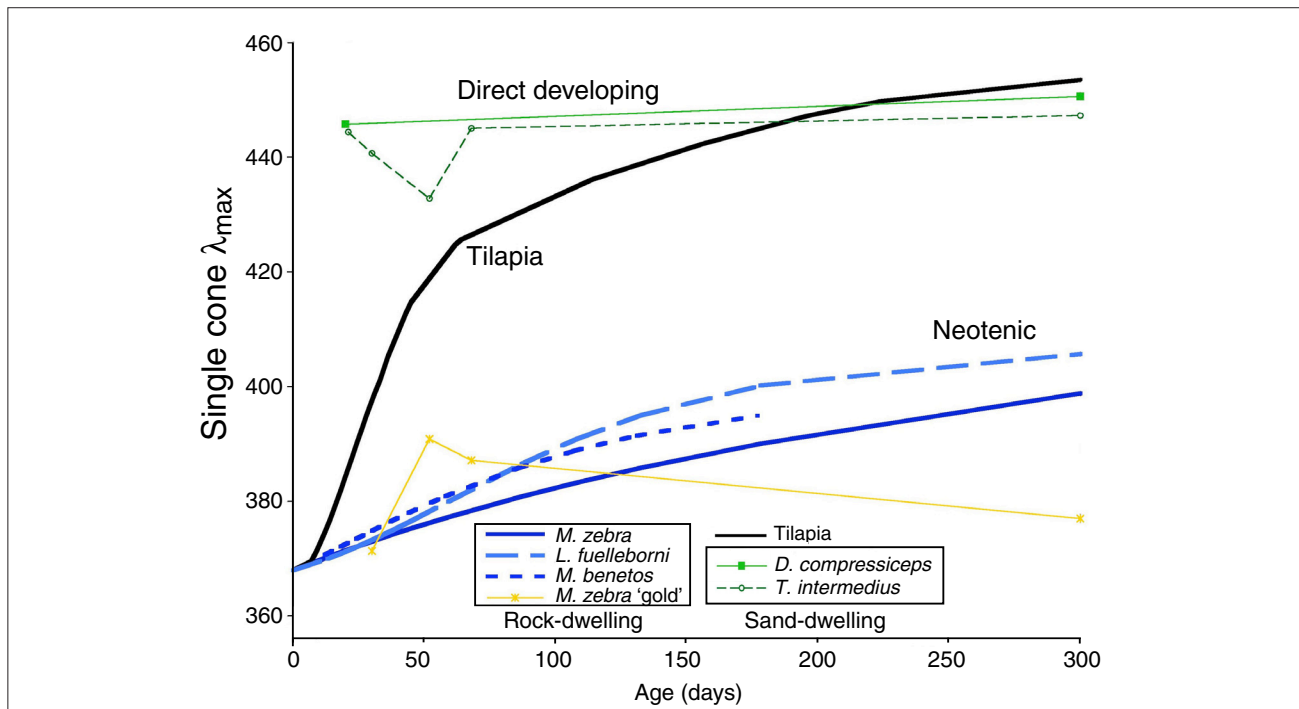
Figure 2
Spectral peak absorbance for the Lake Malawi cichlids follows a heterochronic shift compared with that of tilapia, as inferred from opsin gene expression. Tilapia opsins shift in their peak absorbance from lower to higher wavelengths during development. Rock-dwelling clades, such as *Metriaclima zebra*, *M. zebra* 'gold' (another member of the *Metriaclima* species complex), *Labeotropheus fuelleborni* and *Metriaclima benetos*, show a neotenic pattern, in which peak wavelengths increase during development but at a slower rate than in tilapia. Sand-dwelling clades, such as *Dimidiochromis compressiceps* and *Tramitichromis intermedus*, undergo direct development, with the peak wavelengths high right through development.

others that live over sand [19]. Interestingly, the rock- and sand-dwelling Malawi species express only a subset of the total visual palette of tilapia, a fact that is reflected in their dramatically different spectral absorbance capabilities [9,14].

More interestingly, opsin gene expression changes during ontogeny in mbuna but not in the sand-dwelling cichlids of Lake Malawi. A high proportion of the total larval opsin gene expression in tilapia consists of SWS opsins, which are downregulated in juveniles and adults compared with the LWS opsins. Carleton *et al.* [9] interpret the ontogenetic changes in gene expression within an evolutionary framework and infer heterochronic shifts relative to each other. Traditionally, heterochrony describes an alteration in the timing of ontogenetic events relative to an ancestral sequence, which can result in distinct adult morphologies [20]. One example of a heterochronic shift is neoteny, defined as the process of producing a pedomorphic descendant by retardation in growth and/or differentiation [20]. Carleton *et al.* [9] suggest that compared with the ancestral tilapia pattern, opsin gene expression in Lake Malawi cichlids shows heterochronic shifts that are in either

a neotenic mode (retention of larval or juvenile gene expression in adults) or a direct-development mode (expression of adult opsin gene sets in juveniles). For example, mbuna have a neotenic pattern of SWS1 (UV-sensitive) expression. This could potentially enable them to feed more efficiently on zooplankton throughout their lives [9]. By comparison, sand-dwelling cichlids, not known for zooplanktivory, do not change the expression pattern of LWS and RH2a opsins throughout their life and are therefore considered to be direct developers.

These heterochronic changes in opsin gene expression, in relation to the presumed ancestral condition of tilapia, are likely to reflect functional changes in peak absorbance of the cones. Heterochronic shifts in developmental programs have long been seen as a potential source of morphological variation in a range of organisms, including cichlids [20,21]. It should be noted that reconstructions of ontogenetic patterns are crucially dependent on the phylogenetic framework on which they are based. If, in this example, an even more basal lineage than tilapia were included and were found to have, for example, a 'direct developing'

pattern, then the most parsimonious assumption would be that this, and not the 'tilapia pattern', is ancestral. This would necessitate a reinterpretation of the evolution of the ontogenetic patterns of opsin expression in cichlids.

Vertebrate vision is shaped by the spectral absorbance of opsins, which can be determined through both amino-acid sequence and differential expression. Finding food, avoiding predators and choosing mates all depend on vision, and an understanding of vision evolution at the gene level might shed light on the relative importance of these different forces on the evolution of the visual system. A wider range of species data will help determine how common heterochronic shifts in opsin expression are. In addition, spatial localization of opsin genes to specific cones will solidify the relationship between spectral absorbance and gene sequence. Finally, further field observations will create a more in-depth connection between genetic changes and ecological context and ultimately aid the discovery of genes associated with species divergence.

References

1. Bakker TCM: Positive genetic correlation between female preference and preferred male ornament in sticklebacks. *Nature* 1993, 363:255-257.
2. Breden F, Stoner G: Male predation risk determines female preference in the Trinidad guppy. *Nature* 1987, 329:831-833.
3. Salzburger W, Niederstätter H, Brandstätter A, Berger B, Parson W, Snoeks J, Sturmbauer C: Colour assortative mating among populations of *Tropheus moorii*, a cichlid fish from Lake Tanganyika, East Africa. *Proc R Soc B* 2006, 273:257-266.
4. Dominey W: Effects of sexual selection and life history on speciation: species flocks in African cichlids and Hawaiian *Drosophila*. In: *Evolution of Fish Species Flocks*. Edited by Echelle AA, Kornfield I. Orono: University of Maine Press; 1984: 231-249.
5. Endler JA: Natural and sexual selection on color patterns in poeciliid fishes. *Env Biol Fish* 1983, 9:173-190.
6. Lamb TD, Collin SP, Pugh EN Jr: Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nat Rev Neurosci* 2007, 8:960-975.
7. Yokoyama S: Molecular evolution of vertebrate visual pigments. *Prog Retin Eye Res* 2000, 19:385-419.
8. Ward MN, Churcher AM, Dick KJ, Laver CRJ, Owens GL, Polack MD, Ward PR, Breden F, Taylor JS: The molecular basis of color vision in colorful fish: four long wave-sensitive (LWS) in guppies (*Poecilia reticulata*) are defined by amino acid substitutions at key functional sites. *BMC Biol* 2008, 8:210.
9. Carleton KL, Spady TC, Streelman JT, Kidd MR, McFarland WN, Loew ER: Visual sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC Biol* 2008, 6:22.
10. Ryan MJ, Rand AS: Sexual selection and signal evolution: the ghost of biases past. *Philos Trans R Soc Lond B Biol Sci* 1993, 340:187-195.
11. Weadick CJ, Chang BSW: Long-wavelength sensitive visual pigments of the guppy (*Poecilia reticulata*): six opsins expressed in a single individual. *BMC Evol Biol* 2007, 7:S1-S11.
12. Hoffmann M, Tripathi N, Henz SR, Lindholm AK, Weigel D, Breden F, Dreyer C: Opsin gene duplication and and diversification in the guppy, a model for sexual selection. *Proc Biol Sci* 2007, 274:33-42.
13. Terai Y, Seehausen O, Sasaki T, Takahashi K, Mizoiri S, Sugawara T, Sato T, Watanabe M, Konijnendijk N, Mrosso HD, Tachida H, Imai H, Shichida Y, Okada N: Divergent selection on opsins drives incipient speciation in Lake Victoria cichlids. *PLoS Biol* 2006, 4:2244-2251.
14. Spady TC, Parry JWL, Robinson PR, Hunt DM, Bowmaker JK, Carleton KL: Evolution of the cichlid visual palette through ontogenetic subfunctionalization of the opsin gene arrays *Mol Biol Evol* 2006, 23:1538-1547.
15. Terai Y, Mayer WE, Klein J, Tichy H, Okada N: The effect of selection on a long wavelength sensitive (*LWS*) opsin gene of Lake Victoria cichlid fishes. *Proc Natl Acad Sci USA* 2002, 99:15501-15506.
16. Seehausen O, Jacques JM, van Alphen FW: Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science* 1997, 277:1808-1811.
17. Carleton KL, Kocher TD: Cone opsin genes of African cichlid fishes: tuning spectral sensitivity by differential gene expression. *Mol Biol Evol* 2001, 18:1540-1550.
18. Parry JWL, Carleton KL, Spady T, Carboo A, Hunt DM, Bowmaker JK: Mix and match colour vision: tuning spectral sensitivity by differential opsin gene expression in Lake Malawi cichlids. *Curr Biol* 2005, 15:1734-1739.
19. Meyer A, Kocher TD, Basasibwaki P, Wilson AC: Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* 1990, 347:550-553.
20. Alberch P, Gould SJ, Oster GF, Wake DB: Size and shape in ontogeny and phylogeny. *Paleobiology* 1979, 5:296-317.
21. Meyer A: Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* 1987, 41:1357-1369.