

Table S1

[Click here to Download Table S1](#)

Table S2. Data for limits of fish cone spectral sensitivities

Gene	Species	$\lambda_{\max}$ value	Reference
SWS1	<i>Chitala ornata</i>	347 nm	(Liu et al., 2019)
	<i>Metriaclima emmiltos</i>	383 nm	(Jordan et al., 2006)
SWS2	<i>Lucania goodei</i>	397 nm	(Yokoyama et al., 2007)
	<i>Verasper moseri</i>	482 nm	(Kasagi et al., 2018)
RH2	<i>Oryzias latipes</i>	452 nm*	(Matsumoto et al., 2006)
	<i>Lucania goodei</i>	537 nm	(Fuller et al., 2005)
LWS	<i>Chitala ornata</i>	501 nm	(Liu et al., 2019)
	<i>Lucania goodei</i>	573 nm <sup>#</sup>	(Fuller et al 2005)

\*This may be as short as 435 nm based on estimations of the RH2 sensitivity for *Maurolicus mucronatus*, but this has not been experimentally confirmed (de Busserolles et al., 2017).

<sup>#</sup>This limit is for vitamin A<sub>1</sub> based visual pigments. Species using vitamin A<sub>2</sub> derived chromophores can have LWS based pigments as long as 625 nm as observed in neotropical cichlids (Torres-Dowdall et al., 2017).

## CORRECTED

Supp Table 2: Data for limits of fish cone spectral sensitivities

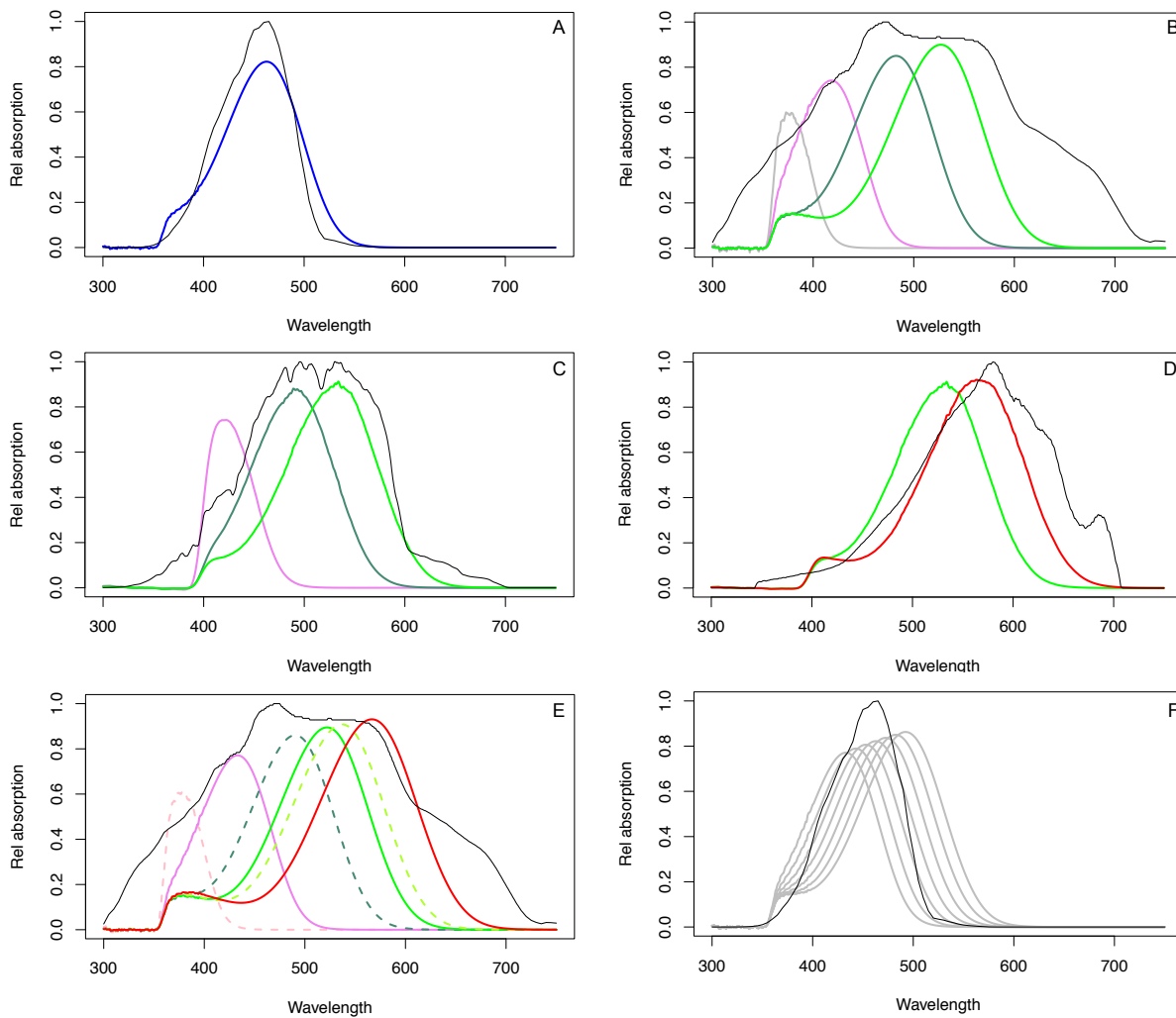
Gene	Species	$\lambda_{\max}$ value	Reference
RH1	<i>Diretmus argentus</i>	447 nm	(Musilova et al., 2019)
	<i>Aristostomias scintillans</i>	525 nm <sup>%</sup>	(Yokoyama et al., 2008)
SWS1	<i>Chitala ornata</i>	347 nm	(Liu et al., 2019)
	<i>Metriaclima emmiltos</i>	383 nm	(Jordan et al., 2006)
SWS2	<i>Lucania goodei</i>	397 nm	(Yokoyama et al., 2007)
	<i>Verasper moseri</i>	482 nm	(Kasagi et al., 2018)
RH2	<i>Oryzias latipes</i>	452 nm <sup>*</sup>	(Matsumoto et al., 2006)
	<i>Lucania goodei</i>	537 nm	(Fuller et al., 2005)
LWS	<i>Chitala ornata</i>	501 nm	(Liu et al., 2019)
	<i>Lucania goodei</i>	573 nm <sup>#</sup>	(Fuller et al 2005)

\*This may be as short as 435 nm based on estimations of the RH2 sensitivity for *Maurolicus mucronatus*, but this has not been experimentally confirmed (de Busserolles et al., 2017).

#This limit is for vitamin A<sub>1</sub> based visual pigments. Species using vitamin A<sub>2</sub> derived chromophores can have LWS based pigments as long as 625 nm as observed in neotropical cichlids (Torres-Dowdall et al., 2017).

%The 525 nm is an estimate based on reconstitution of a slightly modified pigment sequence for this species.

Fig S1. Visual Ecology, Mostly to do with Fish, and Why There Are Too Many Opsins.



The visual system of animals have evolved, co-adapting to the environmental constraints and behavioral needs of each species. Natural sunlight provides an available light spectrum for vision of 300-700nm, but in underwater light environments this may be attenuated by water color and particulate scatter. Aquatic animals' spectral sensitivity necessarily follows or is limited to the spectral envelope available to them (solid black lines above), e.g., deep sea fishes see blue (A). Within this spectral 'envelope' some photoreceptors are situated to absorb as much light as possible, acting as luminance detectors (typically rods and double cones in some animals). Such light may come from the background spacelight or even bioluminescent sources. Cone photoreceptors operating in daytime are also placed within the light envelope. However,

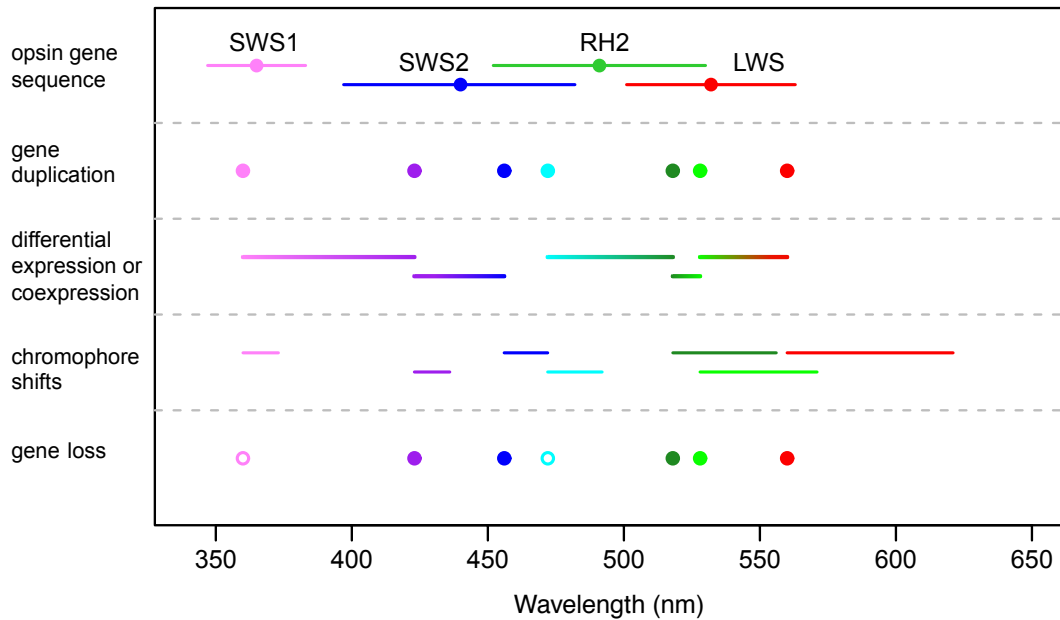
they vary in number and placement according to behavioral task and to some extent micro-habitat, where light availability might have subtle but critical differences. Some species inhabit clear marine habitats (B) while others are in more productive green-shifted (C) or even red-shifted (D) waters.

Co-evolution of photoreceptor spectral sensitivity and specific task may exist, but color vision is often for more than one task and may be set up by several evolutionary constraints. Color vision in any animal is best thought of as 'general purpose'. Animal colors function for sexual communication, aggression or warning, and camouflage or mimicry. They also evolve in response to light availability and visual system. However there are few examples of tight adaptation or fixation (in either direction) due to a single color-driven function (e.g., deep-sea bioluminescence). Exceptions to this may include sensory drive where sensitivity and/or colors evolved for one task (foraging) become co-opted for another task (mating) as they are easy to detect. This relationship is rarely quantified. Spectral sensitivities that become focused on a color code, or colors that respond to spectral sensitivities present due to available light may help drive speciation. Some animals evolve simple color vision such as dichromacy as this is good enough for their needs (D). Others take advantage of full spectrum and may become tri- or tetrachromats (B, C). Beyond tetrachromacy, additional pigments are generally not used for polychromacy (e.g., penta-chromaticity), the extra opsins more likely vary due to ontogenetic stage (E with dashed pigments for larvae and solid pigments for adults), retinal area, or extra-ocular use. Recent molecular evidence suggests levels of plasticity in photoreceptor number and spectral positioning on timescales that can be surprisingly short.

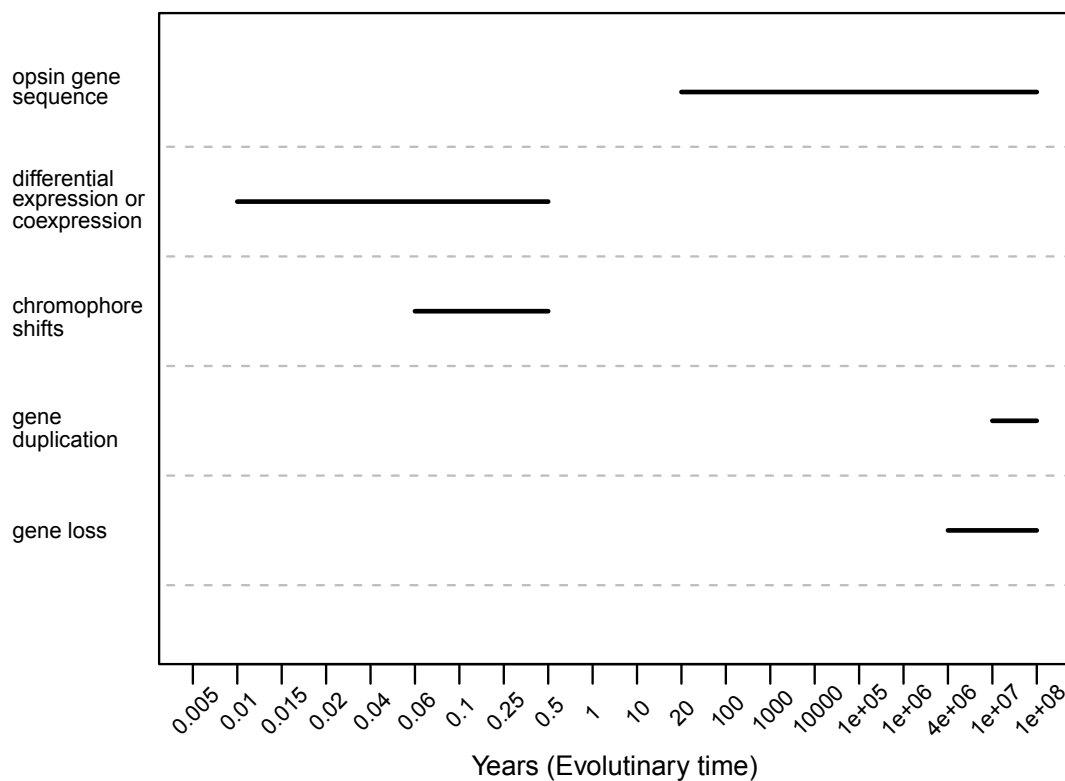
Several mechanisms may increase spectral sensitivity number or tune spectral positioning in an eye including: use of photostable filters, subdivision of the retina with task-division between visual areas, duplications of opsin genes, differential degree of expression of opsins over several timescales (including evolutionary to hourly). Several species are known to express far more visual pigments (situated in rods or cones) than would seem necessary (F). Rather than jumping to conclusion, such as this driving polychromatic color vision or the use of all opsins for visual purposes, other functional reasons should be sought.

Fig S2. Effect size and speed of different tuning mechanisms

**A Shifts in spectral sensitivities**



**B Changes in spectral sensitivities through time**



The mechanisms that contribute to photoreceptor tuning cause different spectral shift sizes (A). Variation in opsin gene sequence can shift each of the different opsin

classes. The variation shown denotes the known fish spectral absorbance range for each opsin class including opsin duplicates (e.g. SWS2 and RH2). Gene duplication values are based on data from a typical perciform, the tilapia *Oreochromis niloticus* which has two SWS2 genes (purple and blue) and three RH2 genes (cyan to green) (Spady et al., 2006). Differential expression shifts sensitivity by mixing closely related opsins. Chromophore shifts are calculated based on Parry and Bowmaker (2000). Gene losses are shown as typical of South American cichlids (Escobar-Camacho et al., 2017).

The amount of time needed to alter visual sensitivity through different spectral tuning mechanisms also varies (B; note x axis is log scale). Changes in gene expression (Fuller and Claricoates 2011, Nandamuri et al., 2017) and chromophore shifts (Enright et al., 2015) can be days to weeks or perhaps months (Escobar-Camacho et al., 2019). Changes in spectral sensitivity by opsin sequence typically require mutations occurring over millions of years. However, more rapid changes by fixation of standing genetic variation have been shown to shift opsin sequence within a given population in 20 yrs (Marques et al., 2017). Gene duplications occur on average every 100 MY but with 10 opsins could occur every 10 MY, while gene losses occur on average every 4 MY (Lynch, 2002).

## References

- de Busserolles, F., Cortesi, F., Helvik, J. V., Davies, W. I. L., Templin, R. M., Sullivan, R. K. P., Michell, C. T., Mountford, J. K., Collin, S. P., Irigoien, X. et al.** (2017). Pushing the limits of photoreception in twilight conditions: The rod-like cone retina of the deep-sea pearlshades. *Sci Adv* **3**, eaao4709.
- Fuller, R. C., Carleton, K. L., Fadool, J. M., Spady, T. C. and Travis, J.** (2005). Genetic and environmental variation in the visual properties of bluefin killifish, *Lucania goodei*. *J Evol Biol* **18**, 516-23.
- Jordan, R., Kellogg, K., Howe, D., Juanes, F., Stauffer, J. R. and Loew, E. R.** (2006). Photopigment spectral absorbance of Lake Malawi cichlids. *J Fish Biology* **68**, 1291-9.
- Kasagi, S., Mizusawa, K. and Takahashi, A.** (2018). Green-shifting of SWS2A opsin sensitivity and loss of function of RH2-A opsin in flounders, genus *Verasper*. *Ecol Evol* **8**, 1399-1410.
- Liu, D. W., Wang, F. Y., Lin, J. J., Thompson, A., Lu, Y., Vo, D., Yan, H. Y. and Zakon, H.** (2019). The Cone Opsin Repertoire of Osteoglossomorph Fishes: Gene Loss in Mormyrid Electric Fish and a Long Wavelength-Sensitive Cone Opsin That Survived 3R. *Mol Biol Evol* **36**, 447-457.
- Lynch, M.** (2002). Genomics. Gene duplication and evolution. *Science* **297**, 945-7.
- Matsumoto, Y., Fukamachi, S., Mitani, H. and Kawamura, S.** (2006). Functional characterization of visual opsin repertoire in Medaka (*Oryzias latipes*). *Gene* **371**, 268-78.

**Parry, J. W. and Bowmaker, J. K.** (2000). Visual pigment reconstitution in intact goldfish retina using synthetic retinaldehyde isomers. *Vision Res* **40**, 2241-7.

**Spady, T. C., Parry, J. W., Robinson, P. R., Hunt, D. M., Bowmaker, J. K. and Carleton, K. L.** (2006). Evolution of the cichlid visual palette through ontogenetic subfunctionalization of the opsin gene arrays. *Mol Biol Evol* **23**, 1538-47.

**Torres-Dowdall, J., Pierotti, M. E. R., Harer, A., Karagic, N., Woltering, J. M., Henning, F., Elmer, K. R. and Meyer, A.** (2017). Rapid and Parallel Adaptive Evolution of the Visual System of Neotropical Midas Cichlid Fishes. *Mol Biol Evol* **34**, 2469-2485.

**Yokoyama, S., Takenaka, N. and Blow, N.** (2007). A novel spectral tuning in the short wavelength-sensitive (SWS1 and SWS2) pigments of bluefin killifish (*Lucania goodei*). *Gene* **396**, 196-202.