

## The Visual Opsin Gene Repertoires of Teleost Fishes: Evolution, Ecology and Function

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### Abstract

Visual opsin genes expressed in the rod and cone photoreceptor cells of the retina are core components of the visual sensory system of vertebrates. Here, we provide an overview of the dynamic evolution of visual opsin genes in the most species-rich group of vertebrates, teleost fishes. The examination of the rich genomic resources now available for this group reveal that fish genomes contain more copies of visual opsin genes than are present in the genomes of amphibians, reptiles, birds, and mammals. The expansion of opsin genes in fishes is due primarily to a combination of ancestral and lineage-specific gene duplications. Following their duplication, the visual opsin genes of fishes repeatedly diversified at the same key spectral tuning sites, generating arrays of visual pigments sensitive from the ultraviolet to the red spectrum of the light. Species-specific opsin gene repertoires correlate strongly with underwater light habitats, ecology, and color-based sexual selection.

### Key words:

fish vision, eye, retina, photoreceptor cell, gene duplication, key-spectral tuning sites

# **1. INTRODUCTION**

Many animals rely upon vision – that is, the ability to perceive a narrow waveband of electromagnetic radiation flanking the peak of the solar emission spectrum in the range of 350-700 nm – for a number of essential tasks. Among other things, their ability to perceive light and see permits animals to adjust their circadian rhythm; to obtain a real-time overview of their immediate surroundings; to navigate through their environments; to track down edible items; to recognize predators and flee and/or hide from them; and to seek for potential mating partners (Cronin et al. 2014; Land & Nilsson 2012). The various tasks and demands that vision has to fulfill in different animals, together with the varying light environments that the different species are exposed to, are manifested in a diverse array of adaptations and modifications concerning the visual sensory system (Cronin et al. 2014). This becomes apparent in the great structural and morphological diversity of animal eyes and the associated parts of the central nervous system including the retina and the visual cortex (Land & Nilsson 2012).

Visual opsin genes expressed in the photoreceptor cells of the retina constitute a core component of vision at the molecular level (Lamb 2020; Yokoyama 2008). Numerous adaptations in visual opsin genes and their regulation have recently been documented, which is not least because of advances in next-generation sequencing technologies and a broader taxonomic sampling. In this review, we focus on the visual opsin genes of teleost fishes. With currently more than 34'000 species catalogued, the infraclass Teleostei within the class Actinopterygii (the ray-finned fishes) represents the by far most species-rich clade of vertebrates, with over half of all vertebrate species included in it. We provide an overview of the general trends in visual opsin evolution in teleosts and delve deeper into some specific cases of opsin gene proliferation in places such as the deep sea. We then take a closer look at attempts to explain, at least in part, the enormous diversity of visual opsin genes found in fishes.

## **2. THE VISUAL SENSORY SYSTEM OF TELEOST FISHES**

In this section, we give a short introduction to the visual sensory system of vertebrates and some of its main components such as the eye, the retina, and the visual opsin genes, highlighting features that are specific to teleost fishes.

### **2.1. The Vertebrate Eye**

Eyes are organs of the visual sensory system and present in almost all animal phyla (Land & Nilsson 2012). However, the eyes in most phyla are rather simple and permit only directional photoreception or low-resolution vision, whereas high-resolution image-forming eyes are

restricted to arthropods (compound eyes in insects and crustaceans), mollusks (camera-style eye in cephalopods), and chordates (camera-style eye in vertebrates) – and perhaps annelids (alciopid polychaetes) (Land & Nilsson 2012; Nilsson 2013; Randel & Jékely 2016).

The vertebrate eye (**Figure 1a**) is almost entirely surrounded by a light-impermeable and protective sclera on the outside and a choroid coat on the inside, discontinued only in the areas where light enters and where the optic nerve exits the eye. The point of entry, consisting of the pupil surrounded by an iris, is shielded from the ambient medium by the cornea, which the incoming light has to penetrate before entering the eye. While pupil and iris control the amount of light that enters the eye, cornea and lens are responsible for focus adjustment, which may be achieved by moving the lens forward and backwards (as in teleost fishes and amphibians), or by dynamically changing the shape of the lens or the cornea using specific muscles and ligaments (as in mammals, reptiles and birds) (Ott 2006). The inner surface of the vertebrate eye, especially in the sphere vis-à-vis the lens, is lined with the retina, a membrane consisting of multiple layers of neurons including the photoreceptor cells through which the inverted mirror image projected by the lens is perceived (Cronin et al. 2014; Land & Nilsson 2012). This basic blueprint of a camera-style eye is common to the jawless lampreys and all jawed vertebrates (therefore also to teleost fishes), suggesting that this feature was already present in the last common vertebrate ancestor (Fain 2020; Lamb et al. 2007).

The eyes of fishes are similar in structure to those of other vertebrates, except that the diameter of the pupil is fixed in lampreys and almost all teleosts, whereas rays and sharks do possess a muscular iris to regulate aperture (Helfman et al. 2009). There are nevertheless a number of adaptations and constraints in the fishes' eyes in response to their waterborne lifestyles. For example, because the refraction index of water is similar to that of the cornea, the light is refracted at the lens, favoring spherical lenses with a relatively short radius (Collin 2009). Such lenses are, in turn, susceptible to spherical aberration, where light passing through the lens is focused at different points, which is compensated by a graded refraction index from the center to the outside of the lens (Collin 2009). To minimize chromatic aberration, where different wavelengths are focused at different focal planes or at different points of the same focal plane, fishes have multifocal lenses (Kröger et al. 1999). Moreover, many fishes have pigmented corneas and lenses that contain mycosporine-like amino acids or yellow pigments to filter out shorter, ultraviolet (UV), wavelengths (<400 nm) and to shift the spectral sensitivity towards longer wavelengths (Muntz 1973; Siebeck & Marshall 2001; Thorpe et al. 1993). Some fishes, especially nocturnal and deep-sea species, have a reflective tapeta at the back of their retina, which reflect unabsorbed photons back to the photoreceptors to increase sensitivity [reviewed in (de Busserolles et al. 2020)].

## 2.2. The Vertebrate Retina

The retina of vertebrates is a multi-layered neural tissue that, depending on the species, may contain more than one hundred types of neurons, broadly classified into amacrine, bipolar, ganglion, horizontal, and photoreceptor cells (Baden et al. 2020; Masland 2012; Sanes & Masland 2015). Amacrine, bipolar, and horizontal cells are interneurons that process the output of the light-detecting photoreceptors, while the axons of retinal ganglion cells transmit visual information to the brain via the optic nerve (Sanes & Masland 2015). The basic makeup of the retina is such that its boundary layer towards the vitreous humour inside the eye is composed of retinal ganglion cells followed by a stratum containing a mosaic of amacrine, bipolar, and horizontal cells, whereas the light-detecting photoreceptors are located at its outside, that is, towards the choroid-coated sclera (Land & Nilsson 2012) (**Figure 1b**). This means that the vertebrate retina is “inverted” or – in other words – that photons have to pass through several layers of retinal neurons before reaching the photoreceptors (Cronin et al. 2014; Lythgoe 1979).

There are two basic types of photoreceptor cells in the vertebrate retina, cones and rods (Schultze 1866) (**Figure 1c**). Cones typically have shorter, but relatively wide cone-shaped outer segments and operate in bright-light (photopic) conditions where they convey colour vision, while the longer and thinner outer segments of rods maximize photon capture in dim-light (scotopic) conditions (Land & Nilsson 2012; Yokoyama 2008). Cones can further be subdivided into single and double cones (i.e., two single cones that are joined together and may be optically coupled or that may still work as independent units; Pignatelli et al. 2010). In teleost fishes, single cones usually express short-wavelength-sensitive opsins, while double cones express medium- and long-wavelength-sensitive opsins (Carleton et al. 2020). In teleosts, single and double cones often form regular mosaics, either in a row (e.g. zebrafish, cods and herrings), a triangular (e.g. pike), or a square arrangement (e.g. medaka, tilapia and many percomorph fishes) [see (Ali & Anctil 1976)] (**Figure 1b**). In rare cases, fishes can have triple and quadruple cones, but their functions remain unknown (Bowmaker 1995; de Busserolles et al. 2021).

## 2.3. The Vertebrate Phototransduction Cascade

The biochemical process by which a stimulus in the form of photons of light is converted into a neuronal – that is, electro-chemical – signal is referred to as phototransduction (Arshavsky et al. 2002; Hunt et al. 2014; Lamb 2020). The phototransduction cascade is initiated by the absorption of photons through visual pigments, which are located in the membranes of the outer segments of photoreceptors cells (**Figure 1c**). Visual pigments consist of a Vitamin A1 (11-cis-retinal) or Vitamin A2-based chromophore (11-cis-3,4-dehydroretinal) that is covalently bound to the visual opsin protein via a Schiff base linkage to a conserved lysine residue at amino-acid position

296 (Wald 1968) [note that by convention, the alignment positions in visual opsins are referenced to the bovine rhodopsin (Palczewski 2000)]. Visual pigments have a bell-shaped absorption profile with varying peak spectral sensitivities ( $\lambda_{\max}$ ), depending on the chromophore type (A2 is longer wavelength-shifted compared to A1) and the opsin protein it is bound to (Hunt et al. 2014; Wald 1968) (**Figure 1d**).

Visual opsins are G protein-coupled receptors that – through a conformational change in response to the photon-induced isomerization of the chromophore – activate a heterotrimeric G protein signalling cascade involving transducin and a number of other phototransduction proteins (Arshavsky et al. 2002; Lamb 2020) (**Figure 1c**). Differences in the structure of the rod and cone opsins and the transduction cascade proteins are responsible for the variation in activation, shut-off, and recovery speed of the opsin pigment. Rods are highly sensitive, but take longer to recover compared to the cones, which are tolerant to higher light intensities and show faster recovery rates (Cronin et al. 2014; Hunt et al. 2014).

## 2.4. The Visual Opsin Genes of Vertebrates

Visual opsins are part of a much larger family of opsin proteins that, when bound to a chromophore, are involved in light sensation (Bowmaker 2008). Vertebrates possess five basic types of visual opsins, the rod opsin (RH1) expressed in rod photoreceptors and four cone opsins expressed in the various cone photoreceptors. These visual pigments can be classified according to photoreceptor specificity, phylogeny, and their range of  $\lambda_{\max}$ : RH1 typically operates in the blue-green part of the light spectrum (teleost  $\lambda_{\max}$  = 447 - 525 nm), while for the cone opsins, the short-wavelength sensitive opsins absorb in the UV (SWS1: teleost  $\lambda_{\max}$  = 347 - 383 nm) and violet-blue (SWS2: teleost  $\lambda_{\max}$  = 397 - 482 nm) wavebands, rhodopsin-like 2 (RH2) is most sensitive in the green fraction of the spectrum (teleost  $\lambda_{\max}$  = 452 - 537 nm), and the long-wavelength-sensitive opsin (LWS) covers the red part (teleost  $\lambda_{\max}$  = 501 - 573 nm) (Carleton et al. 2020) (**Figure 1d,e**). In some species, only a subset of these photopigment types is present, while in others certain types may occur in more than one copy. Note that a single photoreceptor containing only one visual pigment cannot distinguish differences in intensity or luminance (achromatic vision) from a shift in wavelength (chromatic vision). Therefore, to distinguish color, the relative excitation ratios from at least two differently tuned photoreceptors are required (Kraukopf et al. 1982). Teleost fishes use between two to four differently tuned cone photoreceptors (dichromatic to tetrachromatic vision) to distinguish colors during the day (Marshall et al. 2018; Carleton et al. 2020). Whether higher chromacy exists in fishes and if some species can also see color using their rod photoreceptors (Musilova et al 2019a) remains to be investigated.

### 3. EVOLUTION OF VISUAL OPSIN GENES IN TELEOST FISHES

While in most vertebrate lineages the ancestral number of visual opsin genes has been maintained (e.g., in birds and diurnal lizards) or became smaller (e.g., in mammals and snakes), the visual opsin genes of teleosts have continued to proliferate (Hunt et al. 2014) (**Figure 2**). This is likely a response to the various light environments that fishes inhabit – ranging from clear mountain streams to the deep sea – as well as to the varied ecologies and lifestyles they exhibit. In this section, we will dive into the evolutionary history of visual opsin genes in teleosts in an attempt to synthesize the large body of literature that has emerged on this topic since the dawn of the genomic era. The picture that emerges is that of teleosts varying greatly in their numbers and types of visual opsin genes. Also, the molecular processes causing this variability differ between lineages and species. Predicting the number and types of visual opsin genes in a given fish species, and what this species can see by virtue of these genes, is thus a precarious endeavor.

#### 3.1. Molecular Mechanisms involved in Opsin Gene Evolution in Fishes

It has long been recognized that gene (and genome) duplications and the subsequent diversification of the newly emerged gene copies can provide the substrate for functional novelty (Ohno 1970). This is also the case for visual opsins, in which arguably the most crucial functional modifications relate to shifts in  $\lambda_{\text{max}}$ . Teleosts feature an extended set of functionally distinct visual opsins compared to other vertebrates (Carleton et al. 2020; Cortesi et al. 2020; Musilova et al. 2019a). That opsin gene evolution is more dynamic in teleosts than in other vertebrates is further illustrated by the fact that they possess the largest numbers of visual opsin gene copies for all vertebrate opsin types: 38 copies of *RH1* in the silver spinyfin, *Diretmus argenteus* (Diretmidae) (Musilova et al. 2019a); three *SWS1* copies in anemonefish (Amphiprioninae; Pomacentridae) [two functional copies and one pseudogene (Mitchell et al. 2020)]; four copies of *SWS2* in the Humphead wrasse, *Cheilinus undulatus* (Labridae) (Dong et al. 2020); eight copies of *RH2* in soldierfish (Myripristinae) (Musilova et al. 2019a); and five copies of *LWS* in wrasses (Labridae), fighting fish (Osphronemidae) and Brown trout (Salmonidae) (Cortesi et al. unpublished; Dong et al. 2020) (**Figure 2c**). In the following, we outline the main molecular mechanisms that are responsible for this diversity.

##### 3.1.1. Whole-Genome and Tandem Gene Duplications

Already the five basic types of visual opsin genes of vertebrates – that is, the four cone opsins and the rod opsin – are the product of two rounds of whole genome duplications (2R), likely starting from an initial set of two opsin genes (*LWS* and *SWS*) in their common ancestor (Lamb 2020; Larhammar et al. 2009). The evolutionary lineage leading to modern teleosts underwent an

additional (third) round of genome duplication (Meyer & Van de Peer 2005). This teleost-specific genome duplication is also traceable in the visual opsin genes of some fishes. For example, Elopomorpha (eels) and Osteoglossomorpha have retained their two ancestral rod opsins (*RH1s*) (Chen et al. 2018), and characins, bony tongues, and gobies have two ancestral types of the red-sensitive LWS opsin (Adrian-Kalchhauser et al. 2020; Cortesi et al. unpublished; Escobar-Camacho et al. 2020; Liu et al. 2019) (**Figure 2**).

Apart from the expansion through three rounds of whole-genome duplications, several additional ancestral and numerous lineage-specific opsin gene duplications have occurred in fishes (Cortesi et al. 2015; Lin et al. 2017; Liu et al. 2019; Musilova & Cortesi unpublished; Musilova et al. 2019a) (**Figure 2a,b**). The most common way of opsin gene expansion in fishes is via tandem duplication, whereby the resultant sister copies (paralogs) end up being located next to each other on the same chromosome, as exemplified by the *RH2* gene arrays found in many species (Lin et al. 2017; Musilova & Cortesi unpublished). Interestingly, while tandem duplications prevail in the cone opsins [all *SWS2* duplicates, most *SWS1* and *LWS* duplicates, and many of the *RH2* duplicates derive from tandem duplications (Lin et al. 2017)], this is usually not the case for *RH1* (Musilova et al. 2019a), probably because of the somewhat unique evolutionary history of the teleost *RH1* (see next section).

### **3.1.2. Duplication by Retrotransposition**

Gene duplication may also occur via retrotransposition, whereby mature messenger RNA post splicing is retrotranscribed and re-inserted into the genome. Two such cases have been documented in fish: The first involves *RH1*, which is a single-exon gene in all ray-finned fishes but bichirs (Fujiyabu et al. 2019) and has originated from the retrotransposition of its common ancestor with the extraocular rhodopsin (exo-rhodopsin) (Bellingham et al. 2003) (**Figure 2a**). While the new intron-less copy retained the ancestral function in vision, exo-rhodopsin expression mainly became restricted to the pineal gland in extant fishes, where it is involved in circadian regulation (Mano et al. 1999; Pierce et al. 2008). The second case occurred in Cyprinodontiformes (guppies, killifish and others), where three *LWS* copies emerged through tandem duplication and a fourth, intron-less copy through retrotransposition (Sandkam et al. 2017; Ward et al. 2008).

### **3.1.3. Pseudogenization, Gene Loss and Gene Conversion**

The evolution of opsin genes in fishes is also characterized by the frequent occurrence of gene losses and pseudogenization, often in connection to a peculiar light environment (see Chapter 4). Gene conversion, that is, the unidirectional exchange of information between sequences, is yet

another mechanism to reduce opsin diversity due to its homogenizing effect on paralogs (Cortesi et al. 2015; Sandkam et al. 2017). This can even lead to the “resurrection” of a no longer functional gene copy, as found in the *SWS2* genes of the Asian swamp eel (*Monopterus albus*) and the Roughhead grenadier (*Macrourus berglax*). In both species a segment of a functional gene was replaced by a homologous sequence derived from a pseudogene (Cortesi et al. 2015). Ultimately, it is the interplay between gene duplications, gene loss, pseudogenization, and gene conversion that determines the number of visual opsin genes in a given teleost genome.

### **3.1.4. Point Mutations and Adaptations of Teleost Visual Opsins**

Bovine RH1 was the first G-coupled protein to have its crystal structure fully resolved (Palczewski 2000). Even before this feat, there has been a plethora of studies looking into how changes in gene sequence affect amino acid composition and, thus, the function of visual opsin genes. Some point mutations affecting so called “key-spectral tuning sites” have directly been implicated with shifts in  $\lambda_{\max}$  (Yokoyama 2008) (**Figure 1d**). These sites are usually inside or close by the retinal binding pocket and have traditionally been identified on the basis of phylogenetic comparisons, that is, by correlating amino acid sequences with the spectral sensitivity a visual pigment conveys (Yokoyama 2008; Chang & Donogue 2000). *In vitro* opsin protein regenerations (Yokoyama 2008) and – as of late – atomistic molecular simulations (e.g., Patel et al. 2018) have also been used to infer the contribution to shifts in  $\lambda_{\max}$  of specific amino acid substitutions if *in situ* spectral absorbance measurements using microspectrophotometry or similar techniques are not feasible [e.g., for deep-sea fishes (de Busserolles et al. 2017)].

Although a number of key-tuning sites have been identified so far [e.g., RH2 (Yokoyama & Jia 2020); RH1 (Musilova et al., 2019a)], ongoing research on reconstituted opsin proteins and increasing phylogenetic coverage are likely to keep adding to this list. Notably, in some cases, sites found to be involved in the spectral tuning of one type of visual opsin are also relevant in others (Yokoyama & Jia 2020). For example, mutations in amino acid site 292 lead to shifts in  $\lambda_{\max}$  in RH1, RH2, LWS and SWS2 (Yokoyama 2008; Musilova et al., 2019a; Yokoyama & Jia 2020). The question remains as to which extent at least some key-tuning sites may be able to universally tune any type of visual opsin gene.

The contribution of amino acids other than the classical key-tuning sites to functional shifts in  $\lambda_{\max}$  is not very well understood. One reason is that multiple amino acid sites – be these key-tuning or not – may interact in determining  $\lambda_{\max}$  (Yokoyama 2008). For example, atomistic molecular simulations have recently uncovered a disulfide bridge between two amino-acid sites of RH1 (111, 188) that cause a substantial blue-shift in rod opsins of the deep-sea spinyfins (Musilova et al. 2019a). Also, the general background of the coding sequence may impact the



function of visual opsins, as suggested by signatures of positive selection in nucleotide substitutions that do not affect key-tuning sites (Nozawa et al. 2009).

Mutations at sites that do not alter  $\lambda_{\max}$  may also concern functions unrelated to spectral sensitivity. For example, in Andean/Amazonian catfishes a variant of RH1 (L59Q, M288L) has been identified that is specific to populations living at high altitudes and shows accelerated protein kinetics (Castiglione et al. 2017). And in several deep-sea fishes, four amino-acid sites (159, 196, 213, 275) have been implicated with lower opsin dimer compressibility and, hence, a greater stability under high hydrostatic pressure (Porter et al. 2016).

### 3.2. The Specifics of Rod and Cone Opsin Evolution in Fishes

As detailed above, visual opsin genes in teleost fishes have diversified along multiple axis and involving a variety of mechanisms. The median number of visual opsins in teleost fish genomes has been estimated at seven [six cones and one rod opsin (Musilova et al. 2019a)]. Despite this higher number compared to other vertebrates, there is no substantial overlap in the  $\lambda_{\max}$ -range of the cone opsin types in fishes (Carleton et al. 2020). It therefore appears that processes such as gene conversion and the convergent evolution of key-tuning sites are keeping different cone opsins constrained to specific spectral ranges. However, these constraints might be released once an opsin type is lost. For example, analog to what has happened in primates including human, osteoglossomorph fishes have lost the green-sensitive *RH2* gene and instead use a second *LWS* copy that has shifted its spectral sensitivity from red to green (Liu et al. 2019). It is also of note that the cone opsins that are sensitive to the edges of the light spectrum (UV sensitive, *SWS1*; and red-sensitive, *LWS*) are more variable compared to the ones sensitive to the middle, blue-green part of the spectrum [*SWS2* and *RH2*; (Carleton et al. 2020)]. This is likely a consequence of the optical properties of water, in which the short and the long wavelengths are first absorbed and scattered as a function of water depth (or of distance from the light source).

#### 3.2.1. Rod Opsin Evolution

Rods are active during dim light and, in the majority of vertebrates, contain only a single RH1-based visual pigment used to tell apart differences in brightness (Hunt et al., 2014). However, some teleost lineages possess two or more copies of *RH1* that have functionally diversified and are expressed, for example, during different developmental stages (Zhang et al. 2000), or in different areas of the retina (Morrow et al. 2017). Most Otomorpha contain two *RH1* genes that are likely derived from a duplication event in the clupeocephalan ancestor (Chen et al. 20218; Musilova et al. 2019a) (**Figure 2**). Cyprinids have up to four *RH1* copies, associated with an additional round of genome duplication in this group. A special case of convergent *RH1* gene

proliferation has occurred in three deep-sea fish lineages that possess between five and 38 *RH1* copies due to lineage- or species-specific gene duplications (Musilova et al. 2019a). Since these *RH1* copies do not all occur in tandem, it is possible that they are the product of repeated (retro)transposition events.

### 3.2.2. Cone Opsin Evolution

Teleosts, on average, have two to three *RH2* copies within their genomes (Musilova et al. 2019a). The spectral sensitivity of *RH2* in the blue-green light overlaps largely with that of *RH1*. Note that *RH1* (and the teleost exo-rhodopsin) and *RH2* share a common ancestry (**Figure 1e**) but are active during different light intensities and have evolved functional independence. Expansions of *RH2* primarily occurred in fish living in blue-green dominated marine habitats, with species with five and more *RH2* copies either inhabiting the deep-sea or the pelagic open ocean, or showing nocturnal activities on coral reefs (de Busserolles et al. 2020; Musilova et al. 2019a).

The largest numbers of the red-sensitive *LWS* copies have been found in species inhabiting shallow aquatic environments rich in long-wavelength light, such as rivers and lakes or shallow coral reefs [tropical fighting fish and temperate Brown trout (Cortesi et al. unpublished), wrasses (Dong et al. 2020)]. Some freshwater lineages (salmonids, pike, percids, livebearers) also expanded their *LWS* gene repertoire (Cortesi et al. unpublished). On the contrary, *LWS* tends to be lost in deeper-living species (Musilova et al. 2019a).

Fishes generally have fewer copies of the shorter-wavelength-sensitive opsins (*SWS1* and *SWS2*) compared to the longer-wavelength-sensitive opsin genes. Only a handful of species, such as damselfishes, smelts, and salmonids, have been found to have two UV-sensitive *SWS1* copies (Mitchell et al. 2020; Musilova et al. 2019a). These copies are derived from tandem duplications or from lineage-specific whole-genome duplications, and there is no evidence for ancestral duplications of *SWS1* within teleosts. Moreover, many species in the deep-sea and in the shallows have lost this gene altogether (see Chapter 4). Most teleosts possess between one and three copies of the violet-blue sensitive *SWS2*, which is largely due to two ancestral duplications, one specific to neoteleosts, and the other one to percomorphs, the most species-rich crown group of teleosts (Cortesi et al. 2015). Up to three copies (*SWS2A $\alpha$* , *SWS2A $\beta$*  and *SWS2B*) can be found in the genomes of several coral-reef or pelagic species (Cortesi et al. 2015), and the Humphead wrasse has four copies of *SWS2B* (Dong et al. 2020) (**Figure 2**).

### 3.3. Visual Opsin Gene Expression and its Regulation

Besides mutating the amino acid sequence shifting  $\lambda_{\max}$ , visual adaptations may also be achieved by changing the type or amount of visual opsin expressed or co-expressed within a given photoreceptor. Alterations in gene expression are very common and rather straight-forward to assess, but their genetic underpinnings remain difficult to uncover. It is also possible that changes in gene expression are plastic and under the control of epigenetic rather than genetic mechanisms. Either way, changing the type of opsin that is expressed, or co-expressing multiple opsins within a single photoreceptor type, appear to be quick ways by which fishes are able to adapt their vision to changes in the light environment (Carleton et al. 2020).

#### 3.3.1. Variation in Opsin Gene Expression

A common observation in teleosts is that only a subset of their visual opsin genes is expressed at any one time. Opsin gene expression often differs between closely related species. For example, alternative gene expression profiles (referred to as “opsin palettes”) are common between closely related cichlid species that differ in ecology and/or the light environment they inhabit (Hofmann et al. 2009; Musilova et al. 2019b; O’Quin et al. 2010). Visual opsin palettes may also differ within an individual, e.g., along a development axis. Cone opsins are typically the first visual opsins to be expressed during ontogeny, with rod opsin being switched on only later (e.g., Lupše et al. 2020). Within the cone opsins there are species that first express the shorter wavelength sensitive (*SWS1* and *SWS2*) opsins [e.g., groupers (Kim et al. 2019), salmonids (Cheng et al. 2007)], while others start their lives expressing the longer-wavelength sensitive (*RH2* or *LWS*) opsins [e.g., zebrafish, goldfish (Cheng et al. 2007)].

#### 3.3.2. Opsin Gene Regulation

We are just beginning to understand how opsin gene expression is regulated, and what we have learned so far is limited to a few species such as zebrafish and some cichlids. Generally, both *cis*- and *trans*-regulatory processes are thought to drive the expression of cone opsins, while rod opsin regulation seems to rely more on *cis*-regulation (Tsujimura 2020). A number of candidate gene regulatory elements as well as the locus control regions for some of the visual opsins in fishes have been described. For instance, the thyroid hormone receptor beta (*THRβ*), also known to play a role in the expression of mammalian cone opsins (Roberts et al. 2006), has been shown to be essential for the expression of *LWS* (Suzuki et al. 2013) and *SWS1* (Alvarez-Delfin et al. 2009) in zebrafish. The transcription factor *Tbx2a* has been shown to simultaneously regulate the expression of *LWS* and *RH2* in cichlids (Sandkam et al. 2020), and its paralog *Tbx2b* has been

shown to regulate *SWS1* in trout (Raine & Hawryshyn 2009). Also, transcription factors Six6b and Six7 have been shown to regulate the expression of *SWS2* and *RH2* in zebrafish (Ogawa et al. 2019). However, while their binding sites have been identified in the promoter regions of *RH2* and *LWS*, the complete regulatory machinery remains elusive. Clearly, more work is needed to establish the link between changes in opsin gene expression and habitat, ecology, and behavior in the tens of thousands of teleost species.

## **4. VISUAL OPSIN DIVERSITY IN FISHES: ENVIRONMENT, ECOLOGY, FUNCTION**

As shown above, recent advances in sequencing technology have made it possible to reconstruct the evolution of teleost visual opsins across a large number of species. At a first glance, it emerges that fishes possess many more opsin genes than necessary to perform a given visual task. In the following section, we will review some general trends in visual opsin evolution in fishes and highlight, in more detail, some specific cases of environmental factors driving the opsin gene diversity in this group. Caution must be taken, however, in interpreting such trends, as the adaptive advantage often remains correlative rather than causative. Hence, understanding whether the diversity of opsin genes in fishes and the resulting spectral sensitivities are tightly linked to specific functions or whether fish vision evolved to be “good enough to serve multiple purposes” remains a challenge (Marshall et al. 2015).

### **4.1. Visual Opsin Genes and the Light Environment**

It has long been established that the spectral sensitivities of photoreceptors of aquatic animals tend to correlate with – albeit not always exactly match (Munz & McFarland 1977) – the light environment of their respective habitats [e.g., crustaceans (Cronin et al. 2001; Marshall et al. 1999), cetaceans and pinnipeds (Dungan et al. 2016; Fasick & Robinson 2000; Meredith et al. 2013), squamates (Seiko et al. 2020; Simões et al. 2020), and teleosts (reviewed in (Bowmaker 1995; Munz & McFarland 1977; Schweikert et al. 2018)]. In the most extreme cases of fishes that live in constant darkness such as in caves, the deepest depths of the ocean (**Box 1**), or in deep rivers and lakes, there is a trend towards the loss or reduction of eye structures, which is often accompanied by change in the regulation and/or the loss of genes relevant for vision (Aardema et al. 2020; Gore et al. 2018; Jeffery 2009; McGaugh et al. 2014; Musilova et al. 2019a).

#### 4.1.1. Vision and Depth

Due to the absorbing properties of water and the scattering effect of particles in the water column, the light intensity decreases and the light spectrum becomes narrower (blue-light shifted) with increasing depth (Jerlov 1976) (**Figure 4a**). Consequently, fishes that inhabit shallow and clear waters tend to rely on cone-based visual systems during the day that are sensitive to a broad spectrum of light. Deeper-living species, on the other hand, feature visual systems that rely on cones and/or rods tuned towards the blue-green spectrum of the light. At water depths below 200m, the remaining downwelling light is dim and spectrally narrow, as is bioluminescence emitted by deep-sea organisms. Accordingly, most deep-sea fishes utilize purely rod-based visual systems sensitive to blue wavelengths (~480 nm) [reviewed in (Carleton et al. 2020; de Busserolles et al. 2020; Munz & McFarland 1977)]. This correlation between water depth, light environment, and visual phenotype has been reported from a great number of fish species inhabiting both freshwater [e.g., sculpins (Hunt et al. 1996; Luk et al. 2016), salmonids (Eaton et al. 2020), cichlids (Hofmann et al. 2009; Musilova et al. 2019b; Sugawara et al. 2005; Terai et al. 2006, 2017)] and marine habitats [e.g., damselfishes (Stieb et al. 2016), holocentrids (Munz & McFarland 1973; Yokoyama & Takenaka 2004), deep-sea fishes (de Busserolles et al. 2020; Douglas et al. 1998)].

Recent studies based on whole-genome sequencing data revealed that the water depth at which a species lives is not only reflected in repeated changes in the same key-tuning sites, but is also a robust predictor of the opsin gene repertoire (Lin et al. 2017; Musilova et al. 2019a) (**Figure 4a**). Shallow-living species have opsin complements rich in *SWS2*, *RH2* and particularly *LWS*, conferring sensitivity across the visible light spectrum. Notably, although the UV-sensitive *SWS1* is more prevalent in fishes experiencing UV-illuminated environments, not all shallow-living species possess this gene (Musilova et al. 2019a). UV light may damage the eye (Ivanov et al. 2018) and is also scattered quickly in clear water (Rayleigh scattering), causing unwanted visual noise, which limits contrast detection over distance (Muntz 1973). Hence, both of these properties are likely to have driven the evolution of UV-absorbing lenses and similar structures, which in turn might have facilitated the loss of *SWS1* (Escobar-Camacho et al. 2017; Hofmann et al. 2009; Losey et al. 2003; Siebeck & Marshall 2001).

The genomes of deeper-living fishes, on the other hand, tend to be rich in *SWS2* and *RH2*, conferring sensitivities to the more central blue-green part of the light spectrum, while having reduced numbers of *SWS1* and *LWS* genes (Lin et al. 2017; Musilova et al. 2019a) (**Figure 4a**). In the deep sea, where dim light and bioluminescence prevails, another phenomenon has been observed: Together with colleagues, we have recently shown that at least three deep-sea fish lineages have independently expanded and functionally diversified their rod opsin repertoires

(Musilova et al. 2019a). Why some deep-sea fishes have more copies of *RH1* is not entirely clear yet. One possible explanation is that they use them for a broader spectral absorbance to maximise photon capture; alternatively, the spectrally different rod opsins might be used to distinguish differently colored bioluminescent signals. In the silver spinyfin, there is also a difference in the expression of the various *RH1* copies in different developmental stages (Musilova et al. 2019a), which might likewise be the case for other species with multiple *RH1*s. Interestingly, in common with other deep-sea fishes, spinyfins start their larval lives in the shallow, nutrient-rich layers of the pelagic zone, at which point their vision mostly relies on the green-sensitive *RH2* (Lupše et al. 2020; Musilova et al. 2019a). Being exposed to a well-lit environment early on in life might explain why species that rely on pure rod retinas as adults still retain cone opsin genes in their genomes.

#### ***4.1.2. Vision during Twilight and at Night***

In shallow and clear waters, the light spectrum changes considerably with the time of the day: Daylight is characterized by a broad spectrum of high-intensity light; during crepuscular hours, the intensity decreases and the light environment is mostly blue-wavelength dominated; and at night, the moon and the stars are the main sources of light, whereby the light intensity is 8-9x lower than during the day and longer wavelengths predominate despite a fairly broad light spectrum (McFarland 1986). Consequently, nocturnal fishes show visual adaptations that are similar to those of deep-sea fishes, including large eyes and rod-dominated retinas to maximise sensitivity [reviewed in (Cortesi et al. 2020; Munz & McFarland 1977)]. However, because green light prevails at night, the rod spectral sensitivities of nocturnal shallow-water fishes are shifted towards longer wavelengths ( $\sim 490 - 520 \text{ nm } \lambda_{\text{max}}$ ) compared to deep-sea fishes [reviewed (Munz & McFarland 1977; Schweikert et al. 2019)].

The twilight period (also referred to as quiet period) is of special interest, because the intensity of light during the crepuscular hours leads to the simultaneous activity of both cones and rods, albeit neither of them work at their optimum (Munz & McFarland 1973; Stockman & Sharpe 2006). While many animals avoid being active during this time of the day, one group of fishes stands out by taking advantage of this “antipredation window” (Clark & Levy 1988). The pearlsides (*Maurolicus* spp.) are deep-sea fishes found in water depths of  $\sim 200 \text{ m}$  during the day. However, in contrast to other mesopelagic fishes that venture to the surface at night to find food, pearlsides migrate to the surface during crepuscular hours (Giske et al. 1990). Accordingly, their visual system shows unique adaptations to twilight conditions (de Busserolles et al. 2017) (**Figure 4b**). For example, they rely mainly on rod-looking cone cells that express *RH2* and genes belonging to the cone-photoreceptor cascade. Also, the spectral sensitivities of their

transmuted photoreceptors are shifted towards blue wavelengths ( $\sim 430 - 440 \text{ nm } \lambda_{\text{max}}$ ). It thus appears that pearlsides have combined the properties of rod photoreceptors (high sensitivity) and cone photopigments (tolerance to higher light intensities and rapid pigment recovery) to optimize vision during twilight hours (de Busserolles et al. 2017).

Nocturnal fishes often show reduced activity during the day (Helfman 1986). Their visual systems may therefore be adapted to both dim- and bright-light conditions, as is the case for two reef-dwelling nocturnal families, the cardinalfishes (Luehrmann et al. 2019) and the holocentrids (de Busserolles et al. 2021). Holocentrids have large eyes, and their single *RH1* is expressed in rods that are arranged in multiple banks stacked on top of one another – an adaptation usually found in deep-sea fishes (de Busserolles et al. 2020, 2021). Depending on the water depth at which they occur, the different holocentrid species have rod pigments with different spectral sensitivities: shallow-dwelling species have rods tuned to green wavelengths ( $\sim 500 - 507 \text{ nm } \lambda_{\text{max}}$ ), while the photoreceptors of deeper living holocentrids are tuned to blue wavelengths ( $\sim 480 - 485 \text{ nm } \lambda_{\text{max}}$ ); species living at intermediate depths have rods with intermediate sensitivities ( $\sim 490 - 495 \text{ nm } \lambda_{\text{max}}$ ) (Munz & McFarland 1973; Yokoyama & Takenaka 2004). In addition, holocentrids retain few but large cones, which express a single blue-sensitive *SWS2A* and up to two copies of the green-sensitive *RH2* (de Busserolles et al. 2021; Musilova et al. 2019a). Having large cones and a multibank retina seems especially favorable for vision during twilight hours and at night, presumably to increase sensitivity and/or to allow colour discrimination in dim-light (de Busserolles et al. 2021).

#### ***4.1.3. Vision in Turbid Waters***

The colour of fresh and brackish waters, but also that of marine water in inshore and outer reef habitats, may differ substantially between locations and seasons due to changes in solar angle and irradiance as well as varying levels of phytoplankton (chlorophyll), dissolved organic matter, and silt in the water column (Jerlov 1976; Munz & McFarland 1977). An increasing number of fishes have been found to have adapted their visual systems to such differences in photic environments [e.g., snappers (Lythgoe et al. 1994), cichlids (Carleton & Yourick 2020), stickleback (Marques et al. 2017; Novales Flamarique 2013), killifish (Fuller et al. 2003), herring (Hill et al. 2019), Atlantic tarpon (Schweikert & Grace 2018; Taylor et al. 2011), tuna (Loew et al. 2002), and cardinalfish (Luehrmann et al. 2020)]. Cone opsin losses and red-shifted spectral sensitivities (Escobar-Camacho et al. 2017; Liu et al. 2016; Weadick et al. 2012) are common in species that live in turbid waters, presumably due to the reduced levels of UV light and shifts towards longer wavelengths, respectively. For example, amino acid site 261 of *RH1* has converged to a red-shifted phenotype (Phe261Tyr) at least 20 times independently as teleosts

transitioned from blue-shifted marine environments to red-shifted brackish or freshwater habitats (Hill et al. 2019; Musilova et al. 2019a), and the same switch has also been found between closely related freshwater species (Eaton et al. 2020). Similar scenarios involving repeated changes in tuning sites when transitioning between differently colored waters are also common in cone opsins (Lin et al. 2017; Musilova et al. 2019a; Yokoyama 2008). This illustrates the somewhat limited scope under which opsins can operate as the light environment exerts strong selective pressures, leading to convergent visual phenotypes.

Adaptations to turbid waters can also occur at the chromophore level: Cichlids that live in the relatively clear Lake Malawi and in some crater lakes of Nicaragua use more of the shorter-shifted A1-derived chromophore, while those that live in the murky large lakes of Nicaragua use increased amounts of the longer-shifted A2-derived chromophore (Härer et al. 2018; Muntz 1976; Torres-Dowdall et al. 2017). This shift is likely catalyzed by *Cyp27c1* (Enright et al. 2015; Torres-Dowdall et al. 2017). As shown recently in fishes that inhabit the Panama Canal, changes in chromophores can be dynamic and occur over short periods of time (Escobar-Camacho et al. 2019). Chromophore switches might sometimes also be tied to ontogeny such as in eels that migrate between fresh and marine waters (eels also switch the rod opsin they use; **Figure 4c**) (Archer et al. 1995; Wood & Partridge 1993). Arguably the fastest way to adapt to differences in light environments, though, is by changing opsin gene expression itself (Carleton et al. 2020).

#### ***4.1.4. Vision in Variable Light Environments***

Plasticity in the expression of visual opsin genes is remarkably widespread in teleosts and can occur over different timescales (Carleton et al. 2020). In many species, opsin gene expression is plastic during development [e.g., flounder (Savelli et al. 2018), cichlids (Carleton et al. 2008; Dalton et al. 2015; Härer et al. 2017), killifish (Fuller et al. 2005, 2010), black bream (Shand et al. 2008)]. These changes are often associated with ontogenetic habitat transitions such as for example in dottybacks, which change opsin gene expression between pelagic larvae and juvenile and adult stages on the reef (Cortesi et al. 2016). However, in some species, opsin gene expression might be more hardwired showing barely any changes with development [e.g., cod (Valen et al. 2018), salmon (Novales Flamarique 2018), surgeonfishes (Tettamanti et al. 2019)]. Shifts in the photic environment, for example from clearer waters in winter to greener algae- and phytoplankton-dominated waters during summer [damselfishes (Stieb et al. 2016)], or due to seasonal changes in temperature and daylength [medaka (Shimmura et al. 2017)], may also cause adult fishes to change gene expression. In some species, adults are even able to change opsin gene expression within weeks or days when exposed to different light conditions in laboratory experiments [e.g., damselfishes and cardinalfishes (Luehrmann et al. 2018; Stieb et al. 2016),



cichlids (Nandamuri et al. 2017), killifish (Fuller & Claricoates 2011)]. Other ways to adapt to variable photic environments are by expressing different opsin complements in different parts of the retina, or by co-expressing multiple opsins within the same photoreceptor cell [e.g., archerfish (Temple et al. 2010), cichlids (Dalton et al. 2014; Torres-Dowdall et al. 2017), flatfish (Iwanicki et al. 2017), salmon (Cheng & Novales Flamarique 2004)]. For example, the eyes of the four-eyed fish (*Anableps anableps*) are adapted for simultaneous vision above and below the water, whereby the upper part of the eye that looks down into the turbid water expresses a longer wavelength-shifted opsin complement compared to the lower part that looks into air (Owens et al. 2012).

All the examples mentioned above testify that the light environment determines what fish can see. Therefore, it may come as a surprise that, within a given envelope of light, the spectral sensitivities can vary substantially in fish, even between closely related species (Carleton et al. 2020; Marshall et al. 2015, 2018; Schweikert et al. 2018). In the next section, we will discuss different aspects of the biology of fishes that might, at least in part, explain this variation.

## **4.2. Visual Opsin Genes and Life History**

### ***4.2.1. Vision and Feeding Ecology***

Intra- and inter-specific differences in visual opsin gene expression and – by extension – spectral sensitivity may arise in response to different feeding habits, which is especially evident for the shortest- and the longest-tuned photoreceptors expressing *SWS1* and *LWS*, respectively. For example, the contrast of zooplankton against the background light is increased via the absorption or reflection of short wavelengths of light, which is thought to confer a benefit to species with UV sensitivity [e.g., cichlids (Hofmann et al. 2009; Jordan et al. 2004; O'Quin et al. 2010), damselfish (Stieb et al. 2017), zebrafish (Novales Flamarique 2016; Yoshimatsu et al. 2020), perch (Loew et al. 1993), stickleback (Rick et al. 2012)]. Changes in UV sensitivity may also occur during development and, often, fishes are sensitive to UV light during the planktonic larval stage but shift their sensitivities to longer wavelengths later in life when settling and changing diet (Job & Bellwood 2007; Siebeck & Marshall 2007; Thorpe & Douglas 1993). The rainbow trout (*Oncorhynchus mykiss*), for example, undergoes such an ontogenetic switch from UV sensitivity (when being zooplanktivorous) to blue sensitivity (when starting to feed on invertebrates and small fishes) (Browman et al. 1994; Hawryshyn et al. 1989). Expression of *LWS*, on the other hand, may benefit herbivorous fishes such as some damselfishes (Stieb et al. 2017) and blennies (Cortesi et al. 2018), as the (far-)red reflectance of chlorophyll sharply contrasts with the grey to brown colour of a rubble or sandy background (Marshall et al. 2003).

#### 4.2.2. Vision, Colour and Sex

Interestingly, both UV and red sensitivity have also been associated with colour signalling, communication, and sexual selection in both freshwater and marine fishes [reviewed in (Carleton et al. 2020; Marshall et al. 2018)]. UV vision is common in smaller teleosts that live in clear waters, while bigger fishes tend to be insensitive to shorter wavelengths of light (Marshall et al. 2018; Siebeck et al. 2006). UV-reflecting body patterns are common in these smaller species and are thought to be used to “secretly” communicate with one another, hidden away from the UV-blind predatory fish [e.g., damselfish (Siebeck et al. 2010; Stieb et al. 2017), swordtails (Cummings et al. 2003), guppies (Smith et al. 2002)]. For example, it has been shown that the Ambon damselfish (*Pomacentrus amboinensis*) uses its UV-reflecting facial markings to distinguish conspecifics from heterospecific intruders (Siebeck et al. 2010). The white stripes in the iconic anemonefishes strongly reflect in the UV (Marshall et al. 2006); and in the Barrier Reef anemonefish (*Amphiprion akindynos*), single cone photoreceptors located in a small, highly acute area of the forward looking part of the retina co-express *SWS1* and *SWS2B*, which might help in discerning a conspecific intruder from a member of their own group (Stieb et al. 2019).

Vision at longer wavelengths of light – and with it functional diversification of *LWS* – has been associated with colour-selective mating in freshwater fishes such as cichlids (Seehausen et al. 2008), guppies (Sandkam et al. 2018), and sticklebacks (Boughman 2001). Similarly, a strong association between *LWS* expression and red coloration has also been reported from marine fishes such as the wrasses (Marshall et al. 2003; Michiels et al. 2008; Phillips et al. 2016). The idea behind this “sensory drive” is that the visual system is initially shaped by a species ecology and the light environment, which in turn drives the co-evolution of colorful signals ultimately leading to the formation of new species (Cummings & Endler 2018; Endler 1992). Support for this scenario comes from cichlids from Lake Victoria (Miyagi et al. 2012; Teraï et al. 2006). In the genus *Pundamilia*, for example, a shallow living species (*P. pundamilia*) expresses a blue-shifted *LWS* opsin and the males are blue-colored, while a deeper living species (*P. nyererei*) has red-colored males and females express a red-shifted *LWS* copy, facilitating color-assortative mating (Seehausen et al. 2008). However, even in these cichlids, unambiguous evidence for sensory drive remains difficult to establish (Wright et al. 2020).

It is notable that in long-wavelength-sensitive species that prominently feature orange or red colors, such as the wrasses and guppies, the *LWS* genes have expanded substantially (Sandkam et al. 2018; Cortesi et al. 2020). Similarly, in damselfishes and salmonids, which rely on UV vision for feeding and communication, *SWS1* has been duplicated (Mitchell et al. 2020; Musilova et al. 2019a).

## 5. CONCLUSION

Visual pigments – composed of an opsin protein and a retinal chromophore – are at the core of animal vision. Phylogenetic comparative approaches and *in vitro* protein reconstructions have revealed that changes in key spectral-tuning sites of the opsin protein lead to shifts in their spectral sensitivity, permitting a direct link between opsin genotypes and visual phenotypes. The vertebrate ancestor already possessed five types of visual opsin genes (four cone opsins and a rod opsin) that were sensitive from the ultraviolet to the red light. In the most species-rich clade of vertebrates, teleost fishes, the visual opsin genes continued to proliferate and to functionally diversify. This has primarily happened through ancestral as well as many lineage-specific gene duplications. Why fishes have so many visual opsin genes is not entirely clear, but correlations can be drawn with the respective light environment, the ecology, and the coloration of a species. Based on the work from previous generations of scientists and aided by the technological advancements of the last decade, contemporary vision researchers are now at a point where they are able to move beyond correlations in their attempts to unravel the mechanistic links causing the astonishing diversity in visual opsin genes in fishes.

## SUMMARY POINTS

1. The vertebrate ancestor already possessed five types of visual opsin genes, one rod opsin (*RH1*) and four cone opsins (*SWS1*, *SWS2*, *RH2* and *LWS*). In teleost fishes, visual opsin gene copy numbers continued to expand, like in no other vertebrate lineage.
2. The evolution of visual opsin genes in teleosts is primarily driven by differences in the light environment that the various species inhabit. Differences in (feeding) ecology and coloration may also play a role in the fine-tuning of the visual sensory system.
3. Shallow-living species have opsin gene repertoires that may contain all four cone opsin types with photoreceptor peak spectral sensitivities that range from the ultraviolet (UV) to the red spectrum (350 - 600 nm  $\lambda_{\text{max}}$ ).
4. Many deeper-living species have lost the UV- and red-sensitive cone opsins (*SWS1* and *LWS*) and their photoreceptors are sensitive to the center, blue-green part of the light spectrum (~440 - 520 nm  $\lambda_{\text{max}}$ ).
5. The green-sensitive *RH2* cone opsins have by far the most dynamic evolutionary history in teleost fishes with many ancestral, lineage-, and species-specific gene duplications and losses.

6. *LWS* paralogs in characins and mormyrids are remnants of the teleost specific whole-genome duplication. A more distinct *LWS* paralog in gobies suggests that an even earlier gene duplication event had taken place.
7. An unusual example of opsin gene proliferation exists in deep-sea fishes, where *RH1* independently duplicated in at least three different lineages. The most extreme case is that of the silver spinyfin, *Diretmus argenteus*, with 38 functionally diversified *RH1* copies.
8. Many fishes seem to have more visual opsins than necessary to complete a given visual task. These “extra” visual opsins may be used at different developmental stages, different seasons (or shorter timeframes), or in different parts of the retina. They may also be the result of phylogenetic inertia or drift.

## **FUTURE ISSUES**

1. While some vision-related genes (especially the visual opsins) are well studied, others are not. Future research should focus on the entire network of genes underlying vision (Mehta et al. 2021).
2. With the exception of the zebrafish model system, little is known about the neuronal circuits that mediate visually guided behavior and light responses in teleosts beyond the photoreceptors (Baden et al. 2020). Recent technological advances such as *in vivo* calcium imaging and reverse-genetic approaches in non-model teleosts as well as sophisticated behavioral experiments will greatly facilitate comparative studies in the future.
3. How opsin gene expression is controlled remains for the most part an unknown. Single-cell RNA sequencing coupled with functional (epi)genomics and reverse genetics will provide the opportunity to elucidate these pathways going forward.
4. Visual opsins may also function as light receptors outside the eyes and this is an area that we expect will receive increased attention in the future.

## **DISCLOSURE STATEMENT**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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### **Highlight Box 1:**

#### **THE DEEP SEA: EXTREME VISUAL ADAPTATIONS TO EXTREME CONDITIONS**

Visual adaptations in the deep sea have mostly one aim: to catch more photons. Having larger eyes is only one way to do so. Some deep-sea fishes have peculiar eye morphologies including upward-looking tubular-shaped eyes that may contain accessory, sideward-looking mirror eyes without lenses. Other deep-sea fishes possess thick multibank retinæ with rod cells stacked in layers, or they may have a single layer containing modified, exceptionally long rod photoreceptors. The longer the rod outer segments, the more efficient they are at capturing photons. No wonder then that the longest rods amongst fishes are found in the deep sea. Other adaptations include the photopigments themselves. The silver spinyfin's 38 rod opsin genes, which produce a plethora of differentially tuned proteins, represents one more record among vertebrates. Yet another unique visual adaptation is present in some deep-sea dragonfishes that use red photophores under their eyes. Using a bacteriochlorophyll-derived photosensitizer inside their photoreceptors, they are able to heavily shift the spectral sensitivity of their rod photoreceptors to the far red. Since red wavelengths and red vision are extremely rare in the deep sea, red bioluminescence might serve as a private communication channel or to illuminate red-blind prey. For an in-depth review on the topic see (de Busserolles et al. 2020).

## Figure Legends:

### Figure 1

Visual sensory system of teleost fishes. (a) The majority of teleost fishes have a camera-style eye typical for vertebrates [images with permission of Valerio Tettamanti (3x) and Zuzana Musilova (2x)]. (b) The retina of vertebrates is “inverted”, that is, the photoreceptor cells are located at its outside. In fishes, photoreceptors are often arranged in regular patterns such as rows (as shown for zebrafish) or square mosaics with (as shown for medaka) and without the corner cones (as shown for the Nile tilapia, a main model species among cichlids). The photos show the single/double cone retinal mosaic of the shallow-water cichlid fish *Konia eisentrauti* (left) and the stand-alone double cones of the deep-water species *K. dikume* (right), in which the mosaic pattern has been lost; both species are native to crater lake Barombi Mbo, Cameroon [photos taken from (Musilova et al. 2019b)]. (c) Rod photoreceptor cells have longer and slimmer outer segments compared to cone cells, resulting in a longer pathway for the light to travel through, thus increasing sensitivity. Upon the light-induced activation of the chromophore, opsin proteins undergo a conformational change and initiate the phototransduction cascade, which converts the light impulse into a neuronal signal. The main components of the vertebrate phototransduction cascade are shown. (d) Top: The absorption spectra of the visual rod (dashed) and cone (solid) opsins of the Nile tilapia and their corresponding peak spectral sensitivities ( $\lambda_{\max}$ ) [adapted from (Spady et al. 2006)]. Bottom: Schematic representation of the bovine rhodopsin. The key spectral-tuning sites that are known to shift  $\lambda_{\max}$  in RH1 are highlighted in yellow (according to Musilova et al. 2019a). (e) Phylogeny of the vertebrate visual opsin genes. Lamprey = *Geotria australis*, shark = *Callorhinchus milii*, teleosts = five to eight representative opsin genes included. The five basic types of visual opsins were already present in the vertebrate ancestor.

### Figure 2

The visual opsin gene repertoire of teleost fishes. (a) Gene duplication history of visual opsin genes from the vertebrate ancestor to the percomorph fish, the most species-rich crown group of teleosts. (b) Phylogenetic hypothesis of teleost fishes at the level of orders [modified from (Betancur-R et al. 2017; Musilova et al. 2019a)], illustrating ancestral duplications in visual opsin genes. The numerous lineage-specific duplications are not shown. (c) Diversity of the rod and cone opsin genes across teleost fishes. Filled rectangles indicate the presence of a particular

visual opsin gene in a given genome (and the number of copies), while rectangles crossed out indicate absence (based on data from Chen et al. 2018; Cortesi et al. unpublished, 2015; Liu et al. 2019; Musilova & Cortesi unpublished; Musilova et al. 2019a, complemented by additional data from GenBank examined for the purpose of this review).

### Figure 3

Functional diversification of visual opsin genes in teleost fishes. (a) List of 32 key spectral-tuning amino acid sites based on Musilova et al. (2019a), Yokoyama (2008), Yokoyama & Jia (2020). Amino acid alignment positions are referenced to the bovine rhodopsin (Palczewski 2000). (b) Individual gene trees (simplified) of the teleost visual opsin genes (rod RH1 and cone SWS1, SWS2, RH2 and LWS). Changes in key-tuning sites characteristic for a particular visual opsin gene or larger subclades of teleosts are mapped on the respective branches (and are color-coded according to (a)), based on an analysis of several hundred fish genomes reported in Cortesi et al. (unpublished, 2015), Musilova & Cortesi (unpublished), and Musilova et al. (2019a) and complemented by additional data from GenBank examined for the purpose of this review.

### Figure 4

Environmental drivers of visual opsin evolution in teleost fishes. (a) Water depth, and the associated light environment, are a main predictor of the visual opsin gene repertoire of teleosts. Shallow-living species exposed to the entire light spectrum typically exhibit the full range of visual opsins including the UV-sensitive *SWS1* (shown here for the Nile tilapia), while species living in the depth, where blue light prevails, often lack the shortest- (*SWS1*) and longest-tuned (*LWS*) visual opsins but show expansions of *RH2* and *RH1* (as illustrated for cod, lanternfish, fangtooth and dragonfish) [data from (Musilova et al. 2019a)]. (b) The time of the day when a species is active is reflected in the expression patterns of visual opsin genes. The visual system of nocturnal fishes is based mostly on rods and these fishes express comparatively lower quantities of cone opsins than diurnal species (as shown here for coral fish; based on data from De Busserolles et al. 2021; Luehrmann et al. 2019; Stieb et al. 2017). Deep-sea pearlsides feature transmuted cones with a rod-like appearance but a molecular machinery of cones (as shown here for *Maurolicus muelleri*; based on data from De Busserolles et al. 2017). (c) Turbidity and the

associated shifts in the light spectrum impact the visual system of fishes. Migratory eels (*Anguilla* sp.) exhibit an ontogenetic shift in the expression of their two *RH1* copies (*RH1<sub>dso</sub>* = deep sea opsin; *RH1<sub>fwo</sub>* = freshwater opsin), whereby juveniles living in turbid freshwater habitats primarily express the longer-wavelength shifted *RH1<sub>fwo</sub>* (Zhang et al. 2000). (d) Trophic ecology determines visual opsin expression in fishes. Planktivorous and algivorous cichlids from Malawi Lake exhibit higher expression levels of the UV-sensitive opsin *SWS1* compared to benthos-feeders or fish-eaters (data from Hofmann et al. 2009), and herbivorous damselfishes (Pomacentridae) express higher levels of *LWS* than their planktivorous relatives (data from Stieb et al. 2017).



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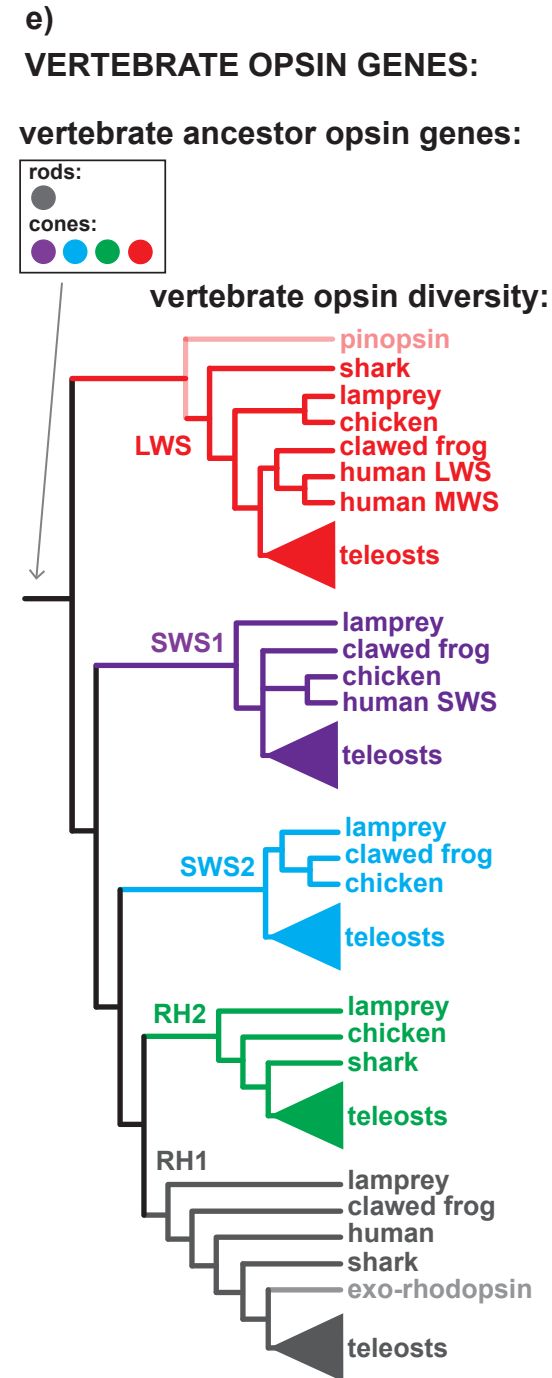
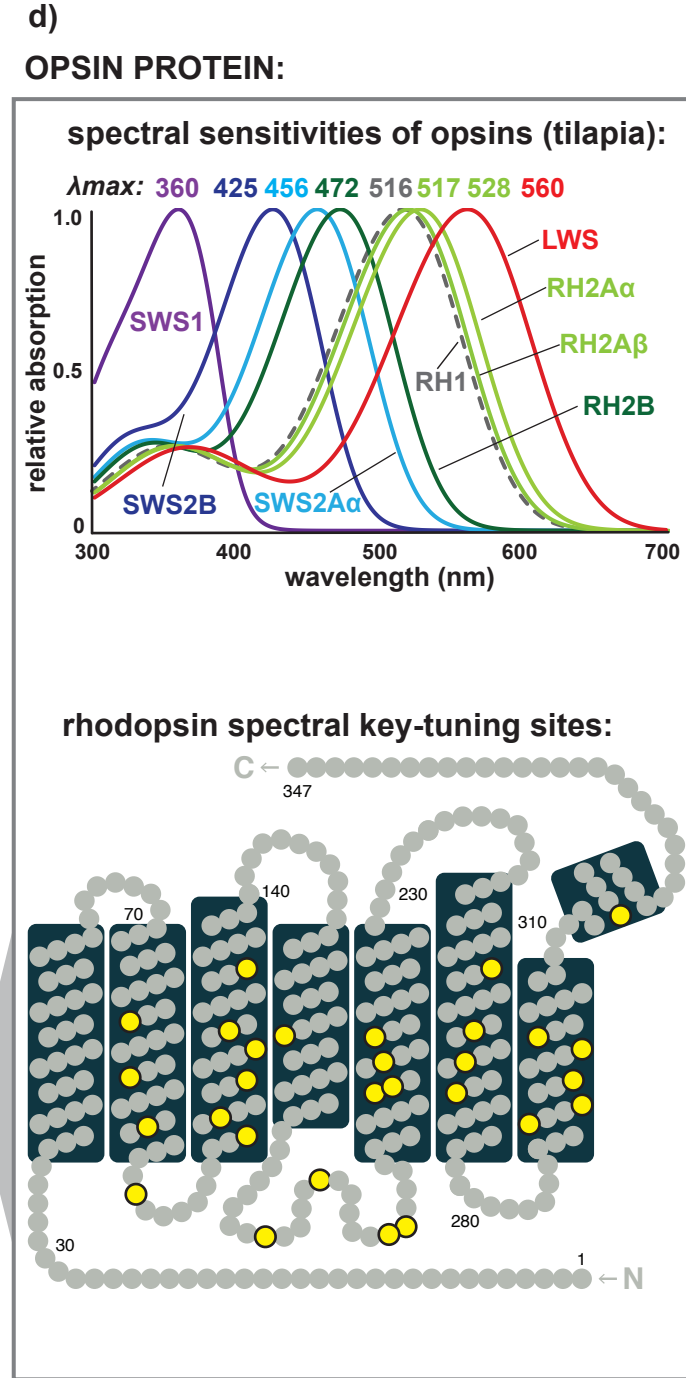
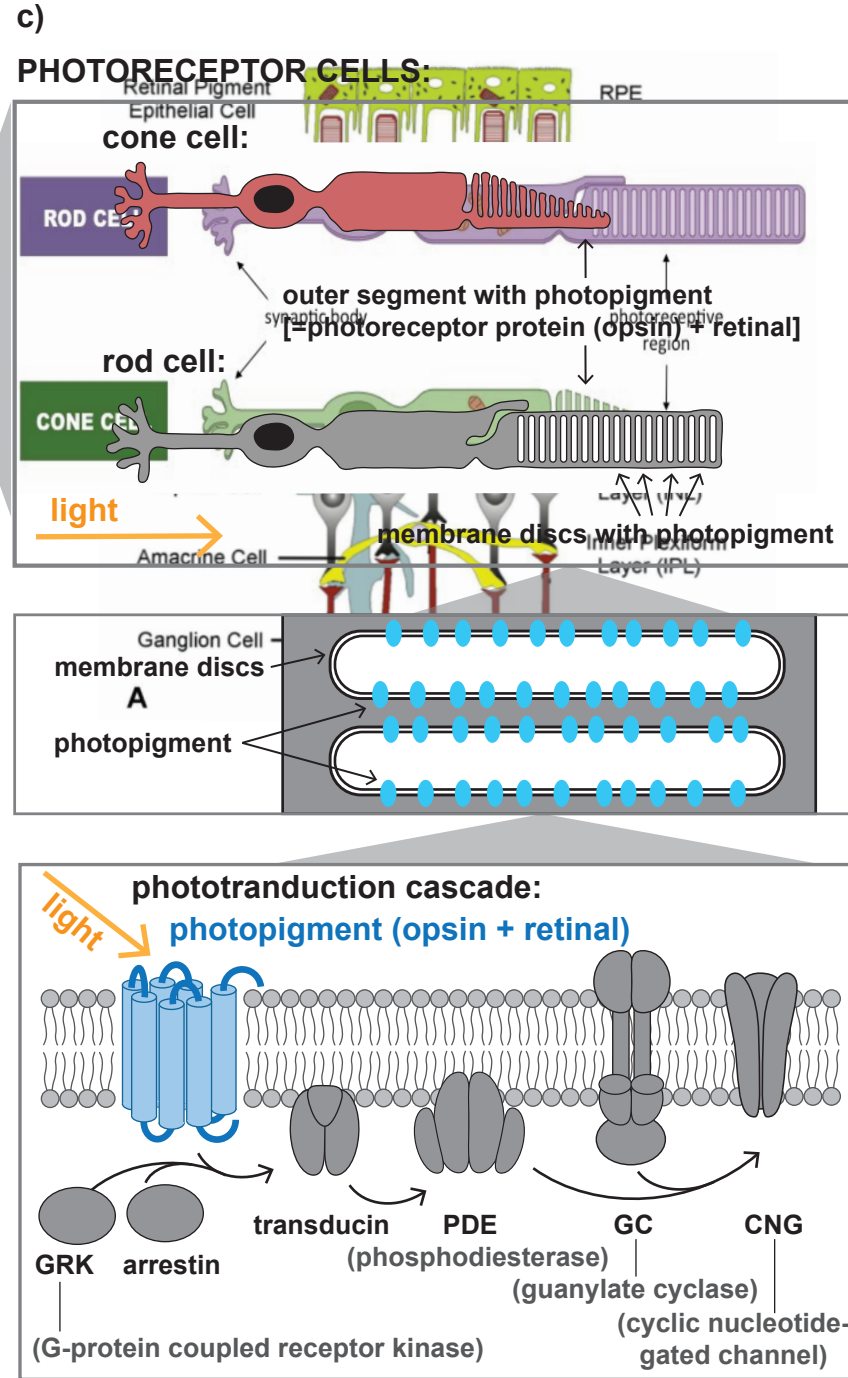
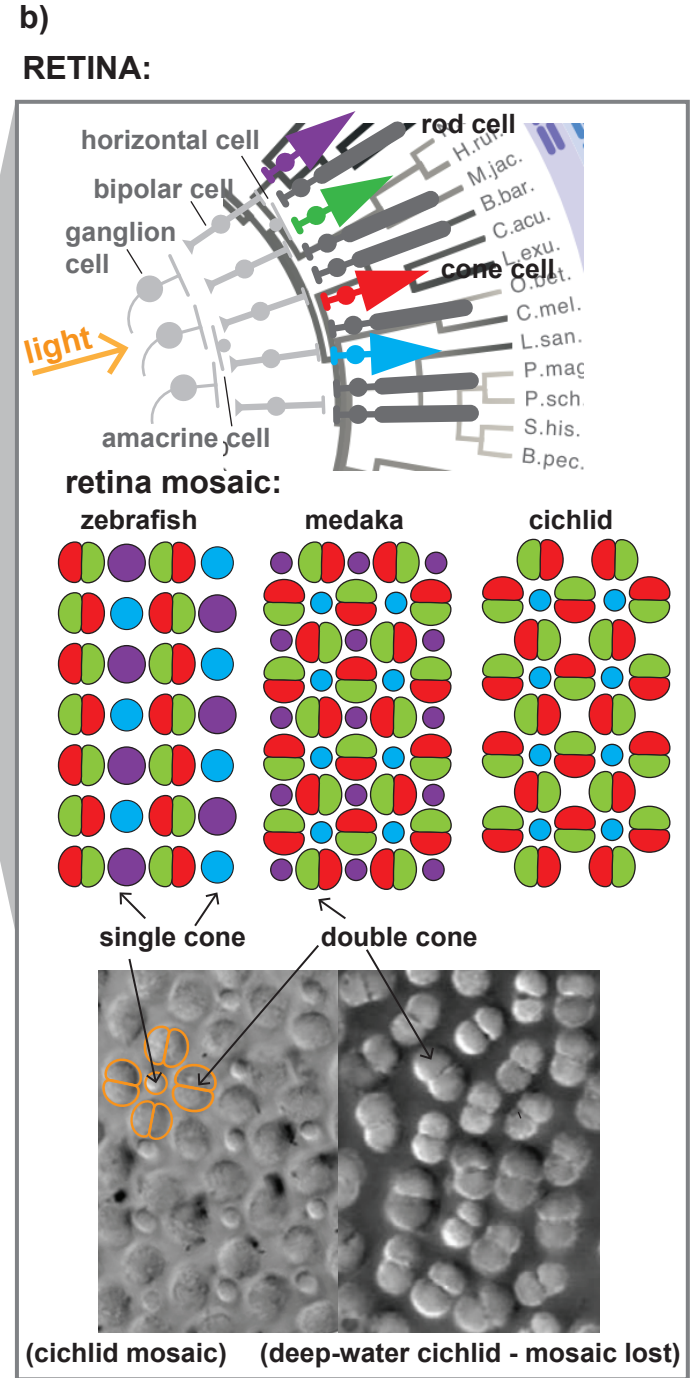
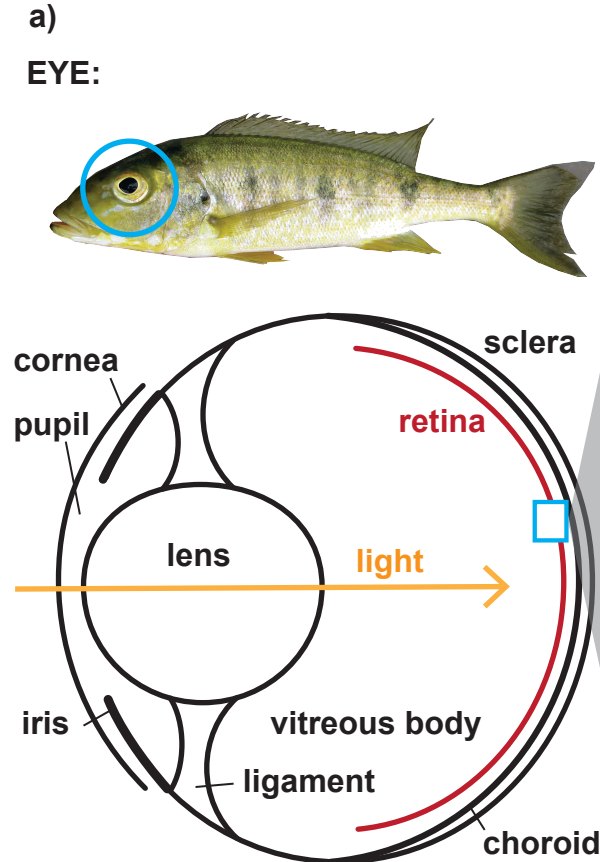


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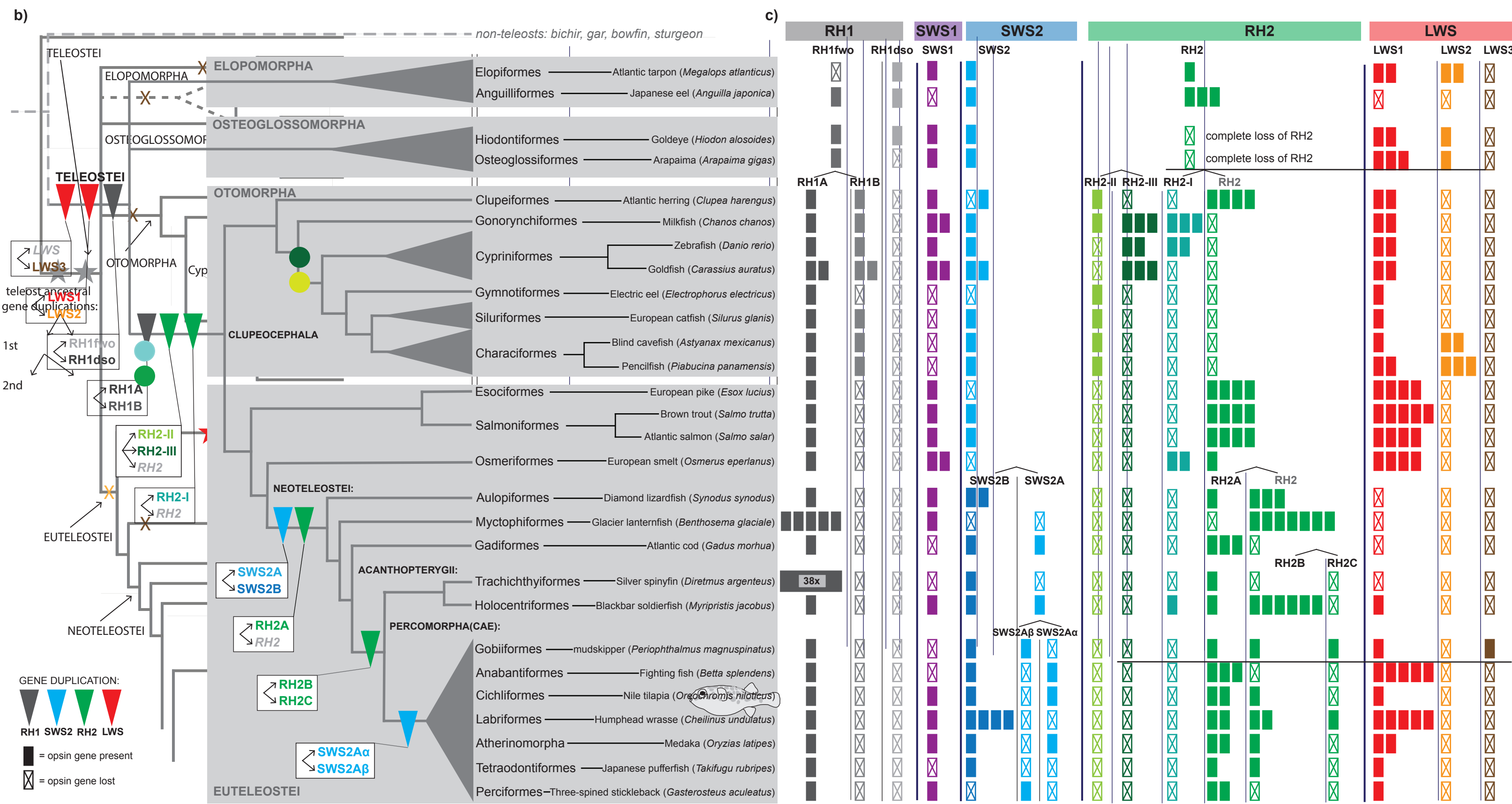
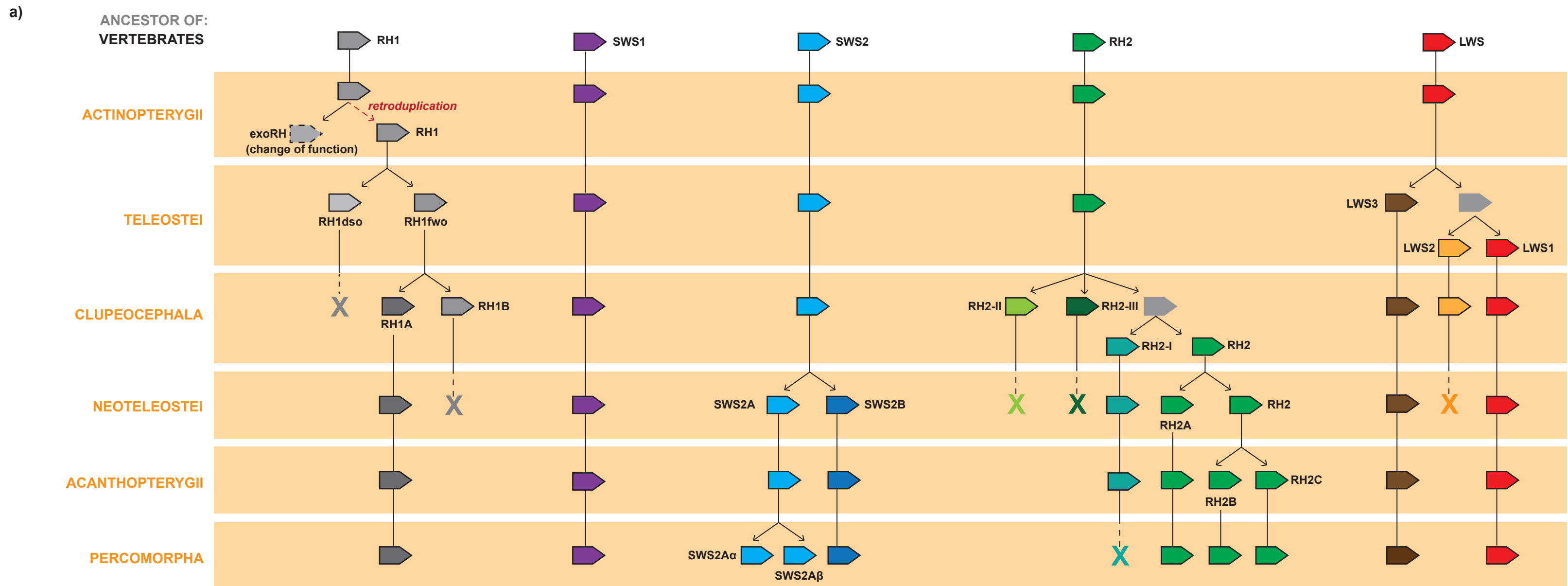
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a) KEY-TUNING SITES:

