Functional Duplication of the Short-Wavelength-Sensitive Opsin in Sea Snakes: Evidence for Reexpanded Color Sensitivity Following Ancestral Regression

Isaac H. Rossetto [1,*], Kate L. Sanders [1,*], Bruno F. Simões [1,2], Nguyen Van Cao[3], and Alastair J. Ludington [1]

[1]School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

[2]School of Biological and Marine Sciences, University of Plymouth, Plymouth, Devon, United Kingdom

[3]Department of Aquaculture Biotechnology, Institute of Oceanography, Vietnamese Academy of Science and Technology, Nha Trang, Khánh Hòa, Vietnam

*Corresponding authors: E-mails: isaac.rossetto@adelaide.edu.au; kate.sanders@adelaide.edu.au.

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Abstract

Color vision is mediated by ancient and spectrally distinct cone opsins. Yet, while there have been multiple losses of opsin genes during the evolution of tetrapods, evidence for opsin gains via functional duplication is extremely scarce. Previous studies have shown that some secondarily marine elapid snakes have acquired expanded "UV–blue" sensitivity via changes at key spectral tuning amino acid sites of the Short-Wavelength Opsin 1 (SWS1) gene. Here, we use elapid reference genomes to show that the molecular origin of this adaptation involved repeated, proximal duplications of the SWS1 gene in the fully marine *Hydrophis cyanocinctus*. This species possesses four intact SWS1 genes; two of these genes have the ancestral UV sensitivity, and two have a derived sensitivity to the longer wavelengths that dominate marine habitats. We suggest that this remarkable expansion of the opsin repertoire of sea snakes functionally compensates for the ancestral losses of two middle-wavelength opsins in the earliest (dim-light adapted) snakes. This provides a striking contrast to the evolution of opsins during ecological transitions in mammals. Like snakes, early mammals lost two cone photopigments; however, lineages such as bats and cetaceans underwent further opsin losses during their adaptation to dim-light environments.

Key words: vision, evolution, snakes, visual opsins, gene duplication.

Significance

This study of snake vision provides the second report of SWS1 opsin duplication in a tetrapod and the only evidence (to our knowledge) of a vertebrate with more than two SWS1 opsins. Spectral divergence of the gene copies suggests a reexpansion of color sensitivity in sea snakes following ancestral losses of middle-wavelength opsins in their earliest terrestrial ancestors.

The elaboration of animal vision has been attributed, at least partly, to the duplication and functional divergence of photopigment-encoding opsin genes. These "visual" opsins are G protein–coupled receptors that trigger the light signaling cascade in the rod and cone photoreceptor cells of the retina; cone-expressed opsins are activated by UV to red wavelengths in bright light conditions, while rhodopsin is highly sensitive to blue–green light and is specialized for dim-light vision. The ancestral complement of opsins in terrestrial vertebrates consists of five spectrally distinct

photopigments: a Long-Wavelength Opsin (LWS; with peak light wavelength absorbance [λ_{max}] ≈ 510–560 nm), Short-Wavelength Opsin 1 (SWS1; λ_{max} ≈ 360–440 nm), Short-Wavelength Opsin 2 (SWS2; λ_{max} ≈ 400–430 nm), Rhodopsin 1 (RH1; λ_{max} ≈ 478–510 nm), and Rhodopsin 2 (RH2; λ_{max} ≈ 450–530 nm; Yokoyama 2008; Hagen et al. 2023). However, while many terrestrial vertebrates have maintained this ancestral complement, multiple lineages in all major tetrapod clades have lost one or more opsins during transitions to low-light environments (Hagen et al. 2023).

The RH2 cone opsin was lost in the ancestral mammal following a sensory bottleneck attributed to nocturnality (Jacobs 2009). This was followed by losses of SWS2 in the common ancestor of marsupials and eutherians (Gerkema et al. 2013) and a loss of SWS1 in early monotremes (Wakefield et al. 2008). Later lineages of bats, deep-diving cetaceans, and subterranean mammals underwent further opsin losses during their secondary transitions to dim-light environments (Jacobs 2013; Emerling and Springer 2014; Sadier et al. 2018; Fasick et al. 1998; Levenson et al. 2006; Peichl and Moutairou 1998). Pseudogenization of the RH2 opsin has been reported in some nocturnal owls and the aquatically foraging penguins (Borges et al. 2015). In the earliest ancestor of living snakes, a dim-light bottleneck resulted in losses of SWS2 and RH2 (Simões et al. 2015) and further losses of SWS1 and (in some lineages) LWS in the highly fossorial scolecophidian snakes (Gower et al. 2021; Simões et al. 2015).

While gene losses are a conspicuous aspect of opsin evolution, remarkably few tetrapod lineages have been shown to have undergone reelaboration of their visual system via opsin duplication. The African bullfrog has a spectrally similar LWS opsin on each of the two sex chromosomes (Schott et al. 2022). Similarly, the fat-tailed dunnart has two RH1 copies with conserved coding regions (Cowing et al. 2008). Old World primates and Howler monkeys gained trichromacy by duplication and divergence of the LWS opsin (Jacobs et al. 1996; Hunt et al. 1998; Dulai et al. 1999), and females of many New World primate lineages gained a similar trichromacy via allelic polymorphism of the X-linked LWS opsin (Carvalho et al. 2017). The two spectrally distinct SWS1 opsins observed in semiaquatic *Helicops* snakes are likely the result of a recent gene duplication (Hauzman et al. 2021) and, before our study, represent the only report of SWS1 duplication in a tetrapod. This imbalance between opsin losses versus gains in tetrapods leaves open the question of whether functional duplication commonly compensates for ancestral gene losses in visual and other sensory systems underpinned by multigene receptor families.

This study used published reference genomes (supplementary table S1, Supplementary Material online) to examine visual opsin complements across five ecologically distinct species of elapid snakes. Elapids present an excellent system for investigating the molecular evolution of vision genes. In their descent from dim-light adapted ancestors, early snakes lost two cone opsin genes (SWS2 and RH2), rendering all living species dichromatic (Davies et al. 2009; Simões et al. 2015). However, within only the last ~25 Myr, elapids have undergone two transitions from terrestrial to spectrally complex, long-wavelength–dominated marine environments (Sanders et al. 2008; Lee et al. 2016). Consistent with the expectation that snakes possess only three opsin classes (Simões et al. 2016), we detected single copies of RH1 and LWS in all five taxa and a single SWS1 in four taxa: the terrestrial tiger snake and banded krait (*Notechis scutatus* and *Bungarus multicinctus*); the amphibious sea krait (*Laticauda laticaudata*); and the fully marine sea snake—*Hydrophis curtus*. Unexpectedly, however, we found *four* intact SWS1 opsin genes (two of these inverted) on chromosome 4 of the fully marine *Hydrophis cyanocinctus* genome (fig. 1; Li et al. 2021). Inspection of amino acid sequences (table 1; also see GenBank: OR147829–OR147836 for SWS1 exon 1 sequences) showed that these genes have diverged at spectral tuning site 86, which is highly influential in determining the peak wavelength of absorbance of the SWS1 photopigment (Fasick et al. 2002; Cowing et al. 2002; Shi and Yokoyama 2003; Yokoyama et al. 2005, 2008). Gene copies A and C have phenylalanine (F) at site 86, which is the ancestral amino acid state in terrestrial elapids and confers peak sensitivity to UV light (λ_{max} ≈ 360 nm based on microspectrophotometry [MSP]: Simões et al. 2016, 2020). Copies B and D have a tyrosine (Y) substitution, which permits violet/blue light sensitivity (λ_{max} ≈ 428 nm based on MSP: Hart et al. 2012; Simões et al. 2020). It must be noted that the copy D locus has significantly lower read coverage than copies A–C (supplementary fig. S1, Supplementary Material online), perhaps indicating that one of the copies is an artifact of genome misassembly. Average read depth confirms that at least three fully intact copies are present; however, further investigation into the assembly quality is required to resolve this.

The discovery of duplication followed by spectral divergence provides a new explanation for the detection (by Sanger sequencing) of both F and Y variants at SWS1 site 86 within some individuals of *H. cyanocinctus* and other *Hydrophis* species (Simões et al. 2020). Each of these variants was previously hypothesized to have been retained by transspecies allelic polymorphism and heterozygote advantage at a single SWS1 locus (Simões et al. 2020). Under this previous hypothesis, a long-wavelength-sensitive allele arose early in the radiation of *Hydrophis*, was fixed in some lineages, and was maintained alongside the UV-sensitive allele in *H. cyanocinctus* and at least one other "polymorphic" lineage (*Hydrophis atriceps–Hydrophis fasciatus*). Visual

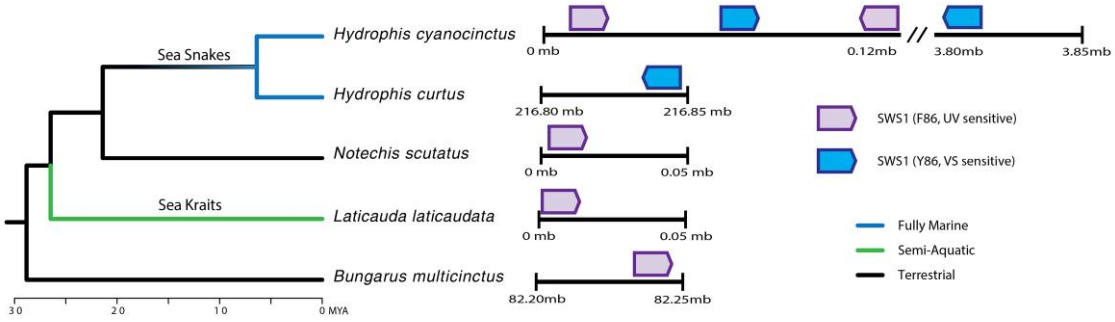

Fig. 1.——Copy number and location of Short-Wavelength-Sensitive-1 genes in elapid snake genomes. Gene labels represent distances relative to the chromosome or scaffold assembly start positions. Every copy of SWS1 in *H. cyanocinctus* and *H. curtus* was found on chromosome 4 of their respective genome assemblies (supplementary table S1, Supplementary Material online). The single *N. scutatus* gene was found on scaffold ULFQ01013886 (GenBank: GCA_900518725.1), the *L. laticaudata* gene on scaffold BHFT01024328.1 (GenBank: GCA_004320025), and the *B. multicinctus* gene on scaffold 8 (GenBank: GCA_023653725.1) of their respective genome assemblies (supplementary table S1, Supplementary Material online). Right-pointing gene arrows indicate a 5′-3′orientation while left-pointing gene arrows indicate reverse complementation. Genes are colored according to the assumed light wavelength sensitivities of their encoded opsins as predicted from spectral tuning site substitutions (table 1). This phylogeny is adapted from Sanders et al. 2008.

capabilities under this scenario would be modified only in the heterozygous portion of the population, whereas opsin duplications are expected to confer expanded spectral sensitivity in all individuals that carry functionally divergent gene copies. The discovery of multiple SWS1 copies in *H. cyanocinctus* also implies a very different pathway to adaptive change. The split between *H. cyanocinctus* and *H. curtus* was estimated to have occurred between 2 and 7 Ma based on mitochondrial and (low variation) nuclear sequences and fossil calibration dates (Sanders el al 2013; Lee at al. 2016). A very approximate split of 4.5 Myr for *H. cyanocinctus* and *H. curtus* and their sequence divergence of 8% for the full SWS1 gene provides a substitution rate of 0.89% per lineage per million years for this gene in sea snakes. Based on this rate and the pairwise divergences between gene copies in *H. cyanocinctus* (0.9% between B and C, and 2.1% between A and D), we date these duplications within the last 1 Myr. However, this should be treated as a tentative, preliminary estimate given the challenges of applying molecular clocks to recent divergences (Ho et al. 2005). Copy D in *H. cyanocinctus* and the single gene in *H. curtus* are positionally homologous (supplementary table S1, Supplementary Material online) when accounting for the interchromosomal inversion between these species (Li et al. 2021). Copy D may therefore be the ancestral gene and copies A–C its duplicates (supplementary fig. S2, Supplementary Material online). Additional high-quality genomes are needed to locate gains and losses of SWS1 in *Hydrophis*, including whether the inverted SWS1 present in *H. curtus* represents the ancestral form of this gene or a persisting duplicate. Understanding the phylogenetic distribution and spectral sensitivity of retained SWS1 copies in the ecologically diverse *Hydrophis* clade will also be key to understanding selection pressures. *Hydrophis curtus* is a day-active, generalist predator, whereas *H. cyanocinctus* is a nocturnal/crepuscular specialist on elongate crevice-sheltering fishes (Simões et al. 2020). It is likely, however, that various nonexclusive factors explain opsin gains and losses in sea snakes.

All sea snakes experience highly variable light intensities and wavelengths as they forage in benthic habitats (at depths of a few to 250 m; Crowe-Riddell et al. 2019) and periodically surface to breathe air. It is plausible that photoreceptors containing the blue light–sensitive (Y variant) SWS1 are activated during foraging dives in blue light–dominated benthic waters, and UV-sensitive (F variant) SWS1 are activated upon return to the UV spectra–rich surface to breathe. Alternatively, the presence of SWS1 opsins with distinct peak absorbances might permit heightened color discrimination to distinguish predators, prey, and/or potential mates against colorful marine backgrounds. This trichromacy could be achieved via the simultaneous activation of the three cone-type photopigments in *H. cyanocinctus*: LWS opsin ($\lambda_{max} \approx 560$ nm; Simões et al. 2020), UV-sensitive SWS1 opsin ($\lambda_{max} \approx 360$ nm; table 1), and violet/blue-sensitive SWS1 opsin ($\lambda_{max} \approx 428$ nm; table 1). If this were the case, the gain of two violet/blue-sensitive SWS1 opsins could partially restore the color discrimination lost with the pseudogenization of the SWS2 opsin ($\lambda_{max} \approx$ 400–450 nm; Yokoyama 2008) in early snakes (Simões et al. 2015). In stark contrast, other tetrapods have undergone further opsin degradation following secondary transitions to aquatic environments (Fasick et al. 1998; Levenson et al. 2006; Peichl and Moutairou 1998). Neuroanatomical and behavioral studies will ultimately be needed to determine the capacity for trichromacy in *H. cyanocinctus*.

Table 1
Amino Acids at Key Spectral Tuning Sites Within SWS1. Spectral Tuning Site Locations Are Based on an Annotated Vertebrate Reference Sequence (GenBank = NM_174567)

Species	46	49	52	86	90	93	97	113	114	116	118	265	λ_{max} (nm)
H. cyanocinctus (copy A)	L	F	T	**F**	A	V	S	E	A	L	T	Y	~360
H. cyanocinctus (copy B)	L	F	T	**Y**	A	V	S	E	A	L	T	Y	~428
H. cyanocinctus (copy C)	L	F	T	**F**	A	V	S	E	A	L	T	Y	~360
H. cyanocinctus (copy D)	L	F	T	**Y**	A	V	S	E	A	L	T	Y	~428
H. curtus	L	F	T	**Y**	A	V	S	E	A	L	T	Y	~428
N. scutatus	L	F	T	**F**	A	V	S	E	A	L	T	Y	~360
L. laticaudata	L	F	T	**F**	A	V	S	E	A	L	T	Y	~360
B. multicinctus	L	F	T	**F**	A	A	S	E	A	L	T	Y	~360

Letters in bold represent the amino acid residues of the highly influential spectral tuning site 86.

Increased transcriptomic expression of multiple SWS1 genes might also result in higher concentrations (Loehlin et al. 2016) of retinal photopigment, maximizing photon capture efficiency in low-light conditions. Further benefit would be conferred if flexible expression enabled functionally distinct SWS1 genes to be used during particular activity periods or life stages. The topology of photoreceptor cells may also correspond with the underwater visual field, with UV-attuned opsins expressed in ventral photoreceptor populations and blue-attuned opsins expressed in dorsal populations. Regional opsin expression would therefore match the incoming light wavelengths of distinct backgrounds. This would maximize perception of the photic environment, enabling snakes to better detect predators, prey, and potential mates throughout the water column.

Duplicated opsins would hold no functional significance if only a single copy is translated into an opsin protein. Retinal expression data is therefore required to confirm transcription of one of each F86/Y86 spectral variant before assuming complex adaptive functions. Moreover, given the recent origin of the SWS1 duplications in *H. cyanocinctus*, it is possible that only some of these genes are maintained by purifying selection. The sequence conservation of all four copies suggests that the duplicates are retained, but future studies will be needed to confirm this. The molecular origins of the duplications also remain uncertain; however, the inversion of some gene copies is characteristic of secondary rearrangement following unequal sister chromatid exchange (Reece et al. 2015).

We suggest that the duplication of SWS1 in sea snakes has effectively reelaborated the extended color sensitivity that was lost with the deletion of two middle-wavelength opsins in early snakes (Simões et al. 2015). This presents a striking contrast to the extensive losses of opsins during successive dim-light transitions in mammals. Genomic, neuroanatomical, and behavioral investigations are now required to identify the origins, mechanisms, and functional advantages of the opsin duplications discovered in sea snakes.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online (http://www.gbe.oxfordjournals.org/).

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Data Availability

Refer to supplementary table S1, Supplementary Material online for genome accession information.

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