

# Visual Pigment Evolution in Reptiles

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## Introductory article

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Online posting date: 22<sup>nd</sup> June 2017

**The long history and great ecological and morphological diversity of reptiles (all amniotes except mammals and birds) is matched by their visual system diversity. Although less known than in other amniotes, visual pigments have been studied in all extant reptile orders except Sphenodontia. There have been no additions to the five visual pigments present in the ancestral vertebrate, although there have been multiple independent losses. Crocodylians retain three visual pigments, many lizards as well as Testudines four or five and snakes one to three. Adaptive pigment evolution includes tuning site amino acid substitutions and switches between chromophore types that together generate ultraviolet to infrared spectral sensitivity. Reptiles present some of the best evidences of evolutionary rod–cone and cone–rod transmutation with, for example typically cone visual pigments expressed in rod-like photoreceptors. Reptile visual pigments show evidence of substantial adaptive evolution, at least some of which is associated with major ecological shifts.**

## Introduction

Vision provides sensory information fundamental for the survival of most animals, having a profound impact on behaviours such as mating and feeding. Before the development of modern molecular genetics, many studies of vertebrate vision (Walls, 1942) used anatomical methods to investigate the presence and attributes of particular photoreceptors, the visual cells in the retina responsible for detecting light. Historical studies also investigated the biochemistry and physiology of visual pigments contained in the outer segments of these photoreceptors (Crescitelli, 1977). In vertebrates, two photoreceptor types populate the retina: cones

and rods (Walls, 1942). Cone photoreceptors generally mediate bright light (photopic) vision (Buser and Imbert, 1992). Retinae often contain different cone types that together can contain multiple visual pigments, generally some combination of two short-wavelength sensitive visual pigments (SWS1 and SWS2), a long-wavelength pigment (LWS) and a medium-wavelength visual pigment (rhodopsin RH2). Because these visual pigments have different spectral sensitivities, variable populations of cones are able to provide photopic colour discrimination. Rod photoreceptors are generally of a single type in any given retina and their outer segments contain the pigment rhodopsin 1 (RH1), used for dim-light (scotopic) vision. Both rods and cones contribute to vision in low light (mesopic: e.g. twilight) conditions (**Figure 1**). The visual pigments (in this article written in capitals, i.e. SWS1, RH1, etc) are encoded in opsin/rhodopsin genes (written here in lower case italics, i.e. *sws1*, *rh1*, etc.). The ancestral vertebrate is thought to have had five visual pigments and thus five visual opsin genes: *rh1*, *rh2*, *sws1*, *sws2* and *lws* (**Figure 1**) (Cronin *et al.*, 2014).

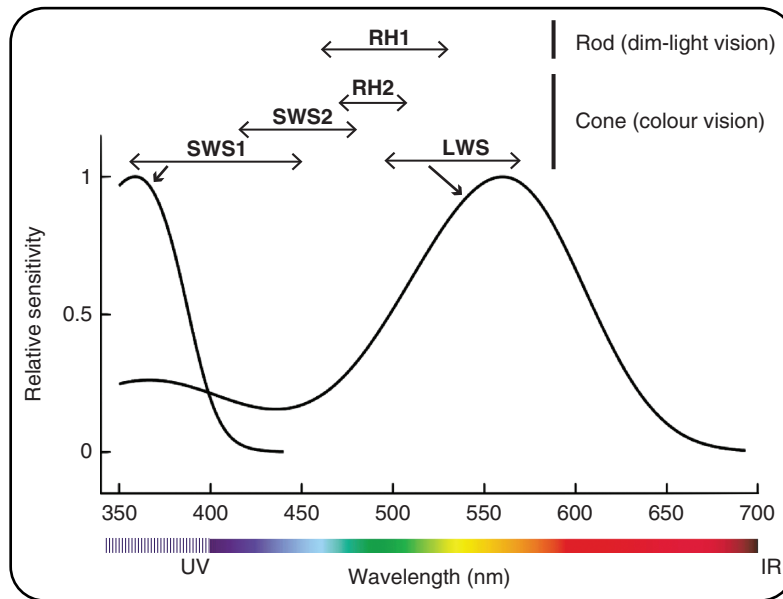
Visual pigment molecules comprise a membrane protein, an opsin with seven transmembrane domains, bound to a photosensitive chromophore by a protonated Schiff base linkage (**Figure 2**). Different visual pigments together are able to detect a wide range of light wavelengths between 360 and 560 nm. Depending on the amino acid sequence of the opsin and the type of chromophore, each pigment type has a particular peak wavelength of absorbance ( $\lambda_{\max}$ ), known as visual pigment spectral tuning (Yokoyama, 2008). Spectral tuning of visual pigments is dictated by the interaction between amino acids within the binding pocket of the opsin protein and the chromophore located within this pocket (Kochendoerfer *et al.*, 1999), which affects the energy necessary to activate isomerisation of the chromophore (Lin *et al.*, 1998). Thus, amino acids at particular sites can have a powerful effect on pigment  $\lambda_{\max}$ . For example, a change between a phenylalanine (F) and a tyrosine (Y) at site 86 (F86Y) in SWS1 results in a change in  $\lambda_{\max}$  from 359 nm (ultraviolet, UV, peak sensitivity) to 438 nm (blue-light peak sensitivity) (Fasick and Robinson, 1998). Studies have identified key tuning amino acid sites in vertebrates using site-directed mutagenesis (artificial substitutions of amino acids) and *in vitro* expression (Yokoyama, 2008; Yokoyama *et al.*, 2014). This allows visual pigment  $\lambda_{\max}$  to be predicted from amino acid sequences of corresponding visual opsin genes (**Figure 2**). However,  $\lambda_{\max}$  prediction is somewhat controversial because there are synergetic interactions among spectral site amino acids and the effects of

**eLS subject area:** Evolution & Diversity of Life

### How to cite:

Simões, Bruno F and Gower, David J (June 2017) Visual Pigment Evolution in Reptiles. In: eLS. John Wiley & Sons, Ltd: Chichester.

DOI: 10.1002/9780470015902.a0026519



**Figure 1** Vertebrate visual pigments and corresponding ranges of light sensitivities ( $\lambda_{\max}$ ).

substitutions at such tuning sites are not entirely consistent across vertebrate taxa (Hauser *et al.*, 2014). Furthermore, in some vertebrate groups, changes in spectral tuning are also achieved through the replacement of an A<sub>1</sub> chromophore (11-*cis*-retinal) with A<sub>2</sub> (11-*cis*-3,4-dehydroretinal), which long-wavelength shifts the  $\lambda_{\max}$  by up to 20 nm (Hárosi, 1994; Enright *et al.*, 2015). **See also: Visual Pigment Genes: Evolution**

Visual pigment spectral tuning has been studied using a variety of methods, including opsin gene cDNA (complementary deoxyribonucleic acid) sequencing, microspectrophotometry (MSP), site-directed mutagenesis and *in vitro* expression (see Bowmaker and Hunt, 2006; Hunt *et al.*, 2009; Davies *et al.*, 2009; Simões *et al.*, 2015, 2016a,b and references therein). All these methods have advantages and limitations, for example MSP directly measures  $\lambda_{\max}$  in photoreceptor outer segments but without identifying visual pigments (a 420-nm sensitive pigment can be SWS2 or a Y86 SWS1), site-directed mutagenesis is laborious, opsin gene sequencing does not identify which pigments are present in particular photoreceptor types, and predicting  $\lambda_{\max}$  from opsin sequences is limited by current knowledge about effects of amino acid changes on spectral tuning.

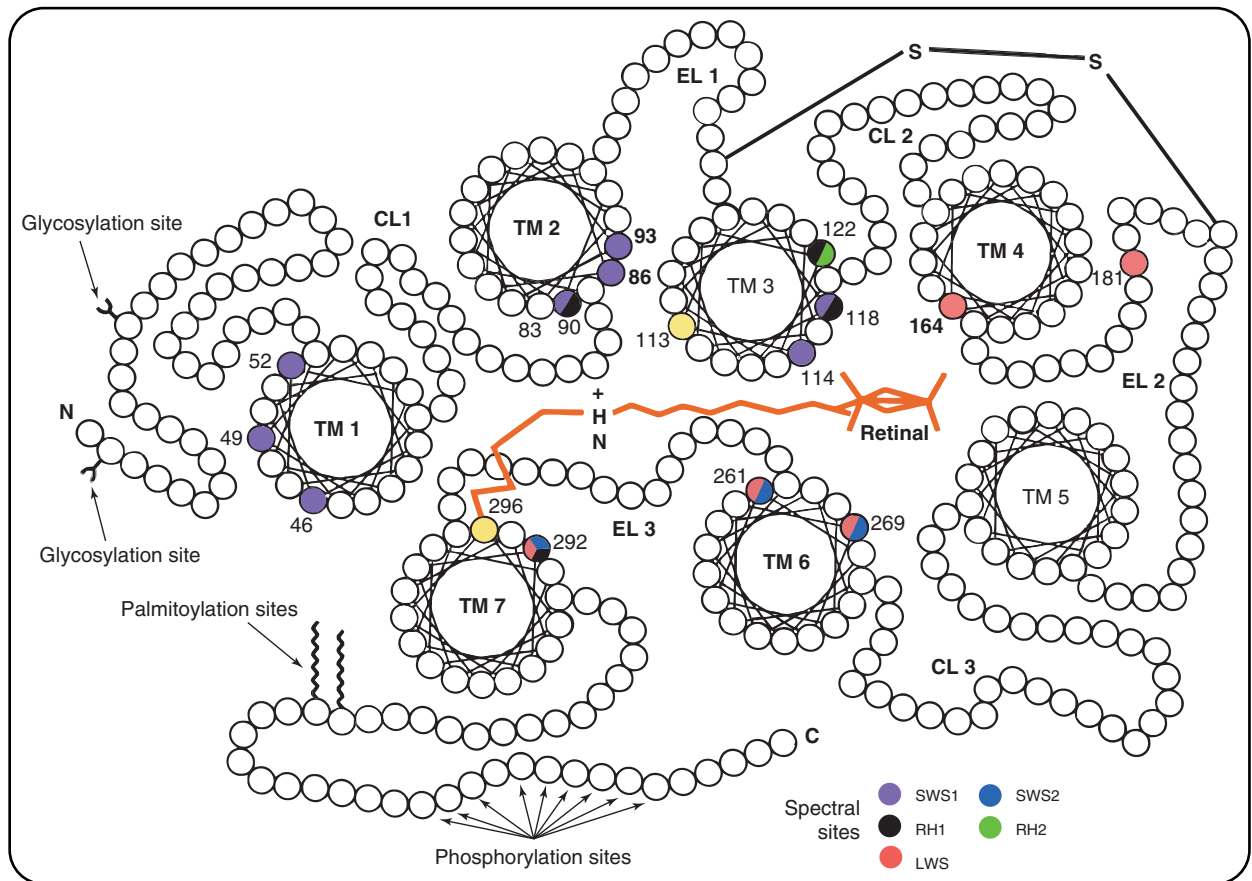
In many vertebrate groups, such as fish, frogs, some lizards, monotremes and marsupials, cone photoreceptors contain an oil droplet (Walls, 1942). These droplets can be clear or coloured. Their function remains somewhat obscure, but they may decrease photon capture, shift peak sensitivity to longer wavelengths and/or narrow the spectral bandwidth of visual pigments present in the photoreceptor outer segments (Loew *et al.*, 2002). This may improve colour vision by reducing glare, chromatic aberration and unwanted short-wavelength sensitivity (Walls, 1942). Transparent oil droplets may be involved in the detection of polarised light (Horváth and Varju, 2004).

To some, Reptilia is the formal name for the least inclusive monophyletic group (clade) that includes all living amniotes except mammals, that is it includes birds, which are more closely related to crocodylians than to any other living group. Some other workers (Modesto and Anderson, 2004) prefer to term this clade Sauropsida. Here, we use 'reptiles' in the more traditional, informal sense of all living amniotes except birds and mammals. Thus, in the sense used here, reptiles do not form a natural (monophyletic) group but are instead a paraphyletic assemblage (**Figure 3**). The more than 10,450 living species of reptiles (<http://www.reptile-database.org>; August 2016) are the product of approximately 275 million years of evolution (Shedlock and Edwards, 2009). There are four major lineages (Orders) of living reptiles, the crocodiles, alligators and gavials (Crocodylia); the turtles, terrapins and tortoises (Testudines); the lizards and snakes (Squamata) and the tuatara (Rhynchocephalia). Phylogenetic relationships and visual pigment complements among sauropsids are shown in **Figure 3**.

## Non-lepidosaurian Reptiles (Crocodiles, Turtles)

Testudines have approximately 345 living species classified in 99 genera and 14 families (<http://www.reptile-database.org>; August 2016). **See also: Reptilia (Reptiles)**

The visual cells in Testudines have oil droplets with varying colours including clear, orange, pale-green and red (Ohtsuka, 1985a). The visual pigments of some freshwater and marine species have been studied using MSP. The rod pigment  $\lambda_{\max}$  (518–520 nm) is higher than that hypothesised for the ancestral vertebrate (500 nm: Yokoyama, 2008). In *Trachemys scripta*,

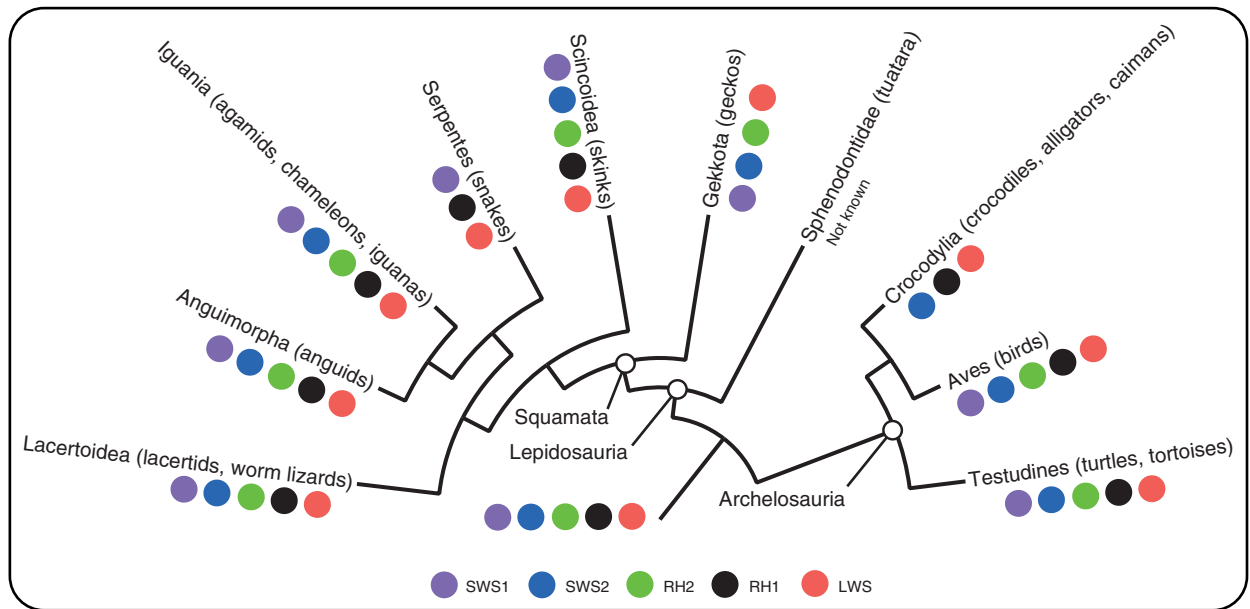


**Figure 2** Two-dimensional diagram illustrating the seven transmembrane helices (TM) and extra loop (EL) and intracellular loop (CL), showing the arrangement of the helices around the chromophore, shown in orange. Although the helices are of different lengths, for simplicity, each helix is shown with only the central 18 amino acids. The amino acid residues with major impact on the spectral tuning ( $\lambda_{\max}$ ) are shown, and numbering is based on bovine rod opsin.

four visual pigments were found in cones with a UV-sensitive SWS1 (372 nm), blue-sensitive SWS2 (458 nm), RH2 (515 nm) and LWS with  $\lambda_{\max}$  of 620 nm (Loew and Govardovskii, 2001). In this species, visual pigment  $\lambda_{\max}$  is correlated with the colour and light transmission properties of the type of oil droplet present in the corresponding photoreceptor (Ohtsuka, 1985a). In the freshwater *Pseudemys* and *Geoclemys*, three cone opsins were detected with  $\lambda_{\max}$  of 440, 530/540 and 620 nm (Ohtsuka, 1985b). The marine *Chelonia* has cone pigments with lower  $\lambda_{\max}$  of 440, 502 and 562 nm (Liebman and Granda, 1971). Unpublished analysis of visual opsin genes in the *Chrysemys* genome (Figure 4) suggests the presence of four cone opsins, and analysis of their amino acids suggests 360 nm SWS1 UV sensitivity and a 555-nm-sensitive LWS pigment. Differences between the freshwater and the marine turtles as well as differences between opsin gene data prediction and MSP measurement of  $\lambda_{\max}$  are associated with an  $A_2$  chromophore detected in at least in two species (Ohtsuka, 1985b). The absence of SWS1 in *Pseudemys* and *Geoclemys* needs to be confirmed by more direct molecular methods given that  $\lambda_{\max}$  values among different opsins overlap, thus complicating the identification of visual pigments by MSP.

However, the genomes of the painted turtle (*Chrysemys picta*) and green sea turtle (*Chelonia mydas*) have five visual opsin genes including *sws1* and *rh1* (Figure 4), and they therefore may be tetrachromatic. Given the complex system of oil droplets, use of different chromophores, the number and variety of cones and up to five visual pigments (Ohtsuka, 1985b), Testudines have among the most complex photoreceptor systems in tetrapode vertebrates.

Crocodylia comprises 25 extant species classified in 9 genera (<http://www.reptile-database.org>; August 2016). Crocodylians lost oil droplets during their evolution but retain both rods and cones (Walls, 1942). The four visual pigments detected by MSP (Sillman *et al.*, 1991) in cones of the American alligator (*Alligator mississippiensis*) (Sillman *et al.*, 1991) have  $A_1$ -based chromophores with  $\lambda_{\max}$  of 444, 503, 535 and 566 nm. The rods express a 501-nm-sensitive RH1. However, only three visual opsin genes, *sws2*, *lws* and *rh1*, can be identified by examination of three high-coverage crocodylian genomes: *A. mississippiensis*, *Crocodylus porosus* and *Gavialis gangeticus* (Figure 4). This suggests loss of SWS1 and UV-sensitive vision, with SWS2 mediating light detection at short wavelengths. It also suggests



**Figure 3** Phylogenetic relationships among sauropsids ('reptiles' and birds) (based on Modesto and Anderson, 2004 and Pyron *et al.*, 2013), showing visual pigment complements.

that RH1 may be expressed in cones and rods, challenging the current paradigm that RH1 is used only for nocturnal vision. Visual pigment identity and the mechanisms responsible for long-wavelength vision (566 nm) remain unclear. Spectral sites in the opsin amino acid sequences of *A. mississippiensis* suggest LWS opsin  $\lambda_{\max}$  of 534 nm, almost identical to the 535-nm visual pigment detected by MSP.

MSP has also been used to assess spectral sensitivity of saltwater (*C. porosus*) and freshwater crocodiles (*Crocodylus johnstoni*) (Nagloo *et al.*, 2016). Retinal stereology of these two species revealed five distinct photoreceptor types consisting of three single cones, one double cone and a rod. The three single cones contain violet- (424 and 426 nm), green- (502 and 510 nm) and red (546 and 554 nm)-sensitive pigments in the saltwater and freshwater crocodiles, respectively, indicating potential trichromacy in these two species. The visual pigments of both members of the double cones in both species have the same  $\lambda_{\max}$  as the red sensitive single cone, and the rod pigment has a  $\lambda_{\max}$  at 503 and 510 nm in saltwater and freshwater crocodiles, respectively. The visual pigments with longer wavelength sensitivity in freshwater crocodiles suggest that this species is well adapted to the longer wavelength (red) shifted freshwater environments. However, given the small amount of MSP and opsin data available for crocodylians, further studies are necessary to understand transitions between freshwater and marine habitats in these archosaurs.

## Squamates – Lizards

Squamata diverged from its closest living relative Rhynchocephalia (tuataras) approximately 260MY

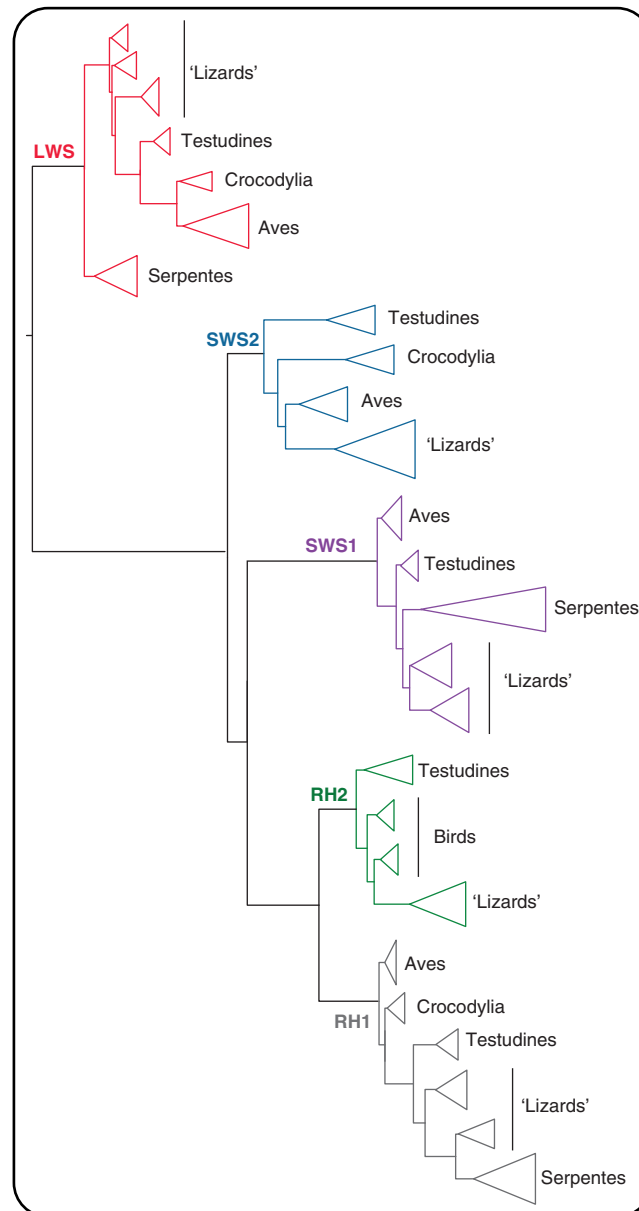
(<http://www.timetree.org>). Not much is known about the visual system of the tuataras, only that the retina is dominated mostly by cones (Meyer-Rochow *et al.*, 2005). What visual pigments are expressed in tuatara cones is unknown.

Squamata has approximately 10,070 extant species (<http://www.reptile-database.org>; August 2016), constituting ~20% of all living vertebrates. Retinal photoreceptor diversity in the order varies greatly among major lineages (Figure 3), including retinæ having only cones, only rods or duplex retinæ with rods and up to four types of cones (Walls, 1942). Despite this diversity, there have been few studies focusing on squamates compared with other major vertebrate groups such as fish, birds or mammals. Squamata includes lizards and snakes. The lineages commonly called 'lizards' are not a natural group, being paraphyletic with respect to snakes (Figure 3; Pyron *et al.*, 2013). **See also: Sauria (Lizards)**

The vision anatomist Gordon Walls' (1942) studies of squamate eyes prompted him to formulate his 'transmutation' hypothesis. This argued that rods could re-evolve from cones in lineages that had lost cones, and *vice versa*, as a consequence of ecological shifts. Walls found his best evidence for this hypothesis among some lineages of highly diurnal and nocturnal geckos and snakes.

Dibamidae, the sister group to all other living squamates, comprises two extant genera and 23 species. These limbless, burrowers have only cone photoreceptors and lack oil droplets (Walls, 1942), their visual pigments are not known.

The infraorder Gekkota includes the highly visual geckos (6 extant families) and the semiburrowing pygopodids (1 family). The retinal photoreceptors of diurnal geckos comprise only cones, whereas rods (possibly transmuted cones) have been observed in crepuscular and nocturnal species (Crescitelli, 1977). Visual pigments have been studied in geckos using MSP, showing



**Figure 4** Phylogenetic tree of the visual pigments in reptiles and birds.

the presence of three visual pigments with  $A_1$  chromophores: a UV-absorbing pigment ( $\lambda_{\max}$  of 362–364 nm), a ‘blue’-sensitive visual pigment ( $\lambda_{\max}$  452–475 nm) and a ‘green’-sensitive pigment ( $\lambda_{\max}$  521–542 nm). No visual pigments with a  $\lambda_{\max}$  characteristic of typical RH1 have been found in the species studied thus far (Ellingson *et al.*, 1995; Loew *et al.*, 1996).

Only one scincoid species, the diurnal *Platysaurus broadleyi*, has been studied by MSP. This species has only cone-populated retinas with colourless to green oil droplets. Four visual pigment classes with  $A_1$  chromophores were found with  $\lambda_{\max}$  in the UV (363 nm), blue (451 nm), green (492 nm) and long wavelengths (570 nm). The presence of UV-sensitive photoreceptors

and colourless oil droplets is consistent with the ability to see UV reflective throats in this species, used in male–male interactions (Fleishman *et al.*, 2011). Opsin sequencing detected five visual pigments in the scincoids *Melanoseps occidentalis* and *Feylinia* sp. (Simões *et al.*, 2015) suggesting that one visual pigment may be lost in *P. broadleyi* or that MSP failed to detect all visual pigments in this species. In *Melanoseps* and *Feylinia*, the spectral tuning amino acid residues suggest ca. 493 nm RH1, similar to the 492 nm found for one of the cone pigments in *Platysaurus* by MSP. Given the similar range in  $\lambda_{\max}$  of RH2 and RH1 (**Figure 1**), we cannot discount that MSP was incapable of distinguishing between the  $\lambda_{\max}$  of both pigments. The



retinal anatomy of *Melanoseps* and *Feylinia* is not known, but if only cones are present in these species (as in *Platysaurus*), it may suggest that photoreceptor transmutation occurred also within Scincoidea.

The visual pigments of two species of lacertoid lizards, *Podarcis muralis* and *Zootoca vivipara*, have been studied by MSP (Martin *et al.*, 2015). Both species have a UV-sensitive visual pigment (367 and 358 nm, respectively), a short-wavelength (blue) pigment (457 and 437 nm) and a medium-wavelength pigment (492 and 497 nm). *P. muralis* has  $A_1$  chromophores and therefore a 562-nm-long wavelength sensitivity. However, in *Z. vivipara*, the LWS opsin is combined with both  $A_1$  and  $A_2$  chromophores producing both a 544-nm-sensitive pigment and one sensitive to infrared with  $\lambda_{\max}$  of 617 nm (Martin *et al.*, 2015). The use of both UV sensitive and infrared vision likely enables *Z. vivipara* to finely discriminate small variations in throat colouration in this species (Martin *et al.*, 2015). Among other lacertoids, opsin sequencing suggests that *Takydromus* and the amphisbaenians (worm lizards) retain all five ancestral vertebrate visual pigments (Simões *et al.*, 2015). This is particularly surprising for amphisbaenians given that species in this group are generally highly fossorial. The anguimorph *Ophiodes striatus* also has five visual pigments and a burrowing ecology (Simões *et al.*, 2015).

The use of both  $A_1$  and  $A_2$  chromophores is observed in the all-cone retinas of iguanian lizards among chameleons (genera *Chamaeleo* and *Furcifer*), where the LWS pigments are red (550–555 nm) and infrared sensitive (610–615 nm). The SWS1 pigment has a  $\lambda_{\max}$  between 375 and 385 nm, the SWS2 pigments between 440 and 450 nm and a medium-wavelength pigment between 480 and 505 nm (Bowmaker *et al.*, 2005). In *Chamaeleo* and *Furcifer*, the visual pigments are associated with clear ( $\lambda_{\text{cut}}$  350 nm), transparent ( $\lambda_{\text{cut}}$  390 nm) and pale ( $\lambda_{\text{cut}}$  410–455 nm) to dense-yellow ( $\lambda_{\text{cut}}$  490 nm) oil droplets. It has been suggested that the fine-tuning between the colouration of the oil droplet and the associated visual pigment  $\lambda_{\max}$  improves spectral sensitivity by reducing spectral overlap between spectrally adjacent cones (Govardovskii, 1983).

Among other iguanians, *Crotaphytus* lizards possess four  $A_1$ -based visual pigments with 359-, 459-, 481- and 558-nm peak sensitivities. No rod pigment (RH1) was found by MSP in this species. New World anole (*Anolis*) lizards have been studied extensively by MSP, and four spectral classes of visual pigments have been detected. Most anoles have a UV-sensitive pigment with  $\lambda_{\max}$  between 365 and 367 nm, a short-wavelength pigment (448–462 nm), a medium-wavelength pigment (490–503 nm) and a long-wavelength pigment (560–568 nm). In *Anolis carolinensis*, an  $A_2$ -based chromophore has been detected that shifts sensitivity of the LWS pigment to the infrared ( $\lambda_{\max}$  625 nm) (Loew *et al.*, 2002). These visual pigments were identified by opsin sequencing as encoded by *sws1*, *sws2*, *rh2* and *lws* opsin genes, respectively (Alföldi *et al.*, 2011). However, a functional *rh1* gene is also present in the *Anolis* genome (Figure 4) with a  $\lambda_{\max}$  of 515 nm (Kawamura and Yokoyama, 1997) suggesting that typically scotopic pigments may be expressed in a small number of photoreceptors in the all-cone retina of *Anolis*. *Uta stansburiana* also has five visual pigments, as detected by Su *et al.* (2006).

## Squamates – Snakes

There are more than 3620 extant species of snakes (<http://www.reptile-database.org>; August 2016), with a substantial diversity of ecological niches (and therefore photic environments), including highly fossorial, arboreal, gliding and fully marine species. Snakes fascinated vision biologist Gordon Walls (Walls, 1942) and snake systematist Garth Underwood (Underwood, 1970) because of their high diversity of photoreceptor types and complements. This diversity includes tiny eyes with rod-only retinas in ‘blind’, ‘worm’ and ‘thread’ snakes (Scoleophidia) and large eyes with all-cone retinas in some highly visual, diurnal colubroid snakes with up to four cone types. The origin of snakes is contentious, with advocates of both a marine and terrestrial, burrowing origin. Evidence from retinal anatomy and visual pigments is not conclusive but is consistent with the ancestral snake living in dim-light conditions (Simões *et al.*, 2015; Hsiang *et al.*, 2015). **See also: Serpentes (Snakes)**

All living snakes lack oil droplets, these being lost in stem snakes early in their evolution, possibly in the same time frame as two of the visual pigments (RH2 and SWS2) present in other squamate lizards were lost (Simões *et al.*, 2015, text above). These same two pigments are absent in placental mammals, perhaps as a result of a nocturnal phase early in their evolution (Gerkema *et al.*, 2013). The most extreme burrowers among snakes (Scoleophidia) and the fossorial *Anilius scytale* lack cones and have lost two further visual pigments (SWS1 and LWS) rendering them rod monochromats with an RH1  $\lambda_{\max}$  of 493–497 nm (Simões *et al.*, 2015). The loss of all cone opsins (SWS1, SWS2, Rh2 and LWS) is also found in other extreme burrowers among amphibians (Kos *et al.*, 2001; Mohun *et al.*, 2010) and mammals (Emerling and Springer, 2014). However, some other fossorial or semifossorial snakes have retained typical cone opsins, for example *Xenopeltis unicolor* has SWS1 (361 nm) LWS (560 nm) and RH1 (497 nm) pigments (Davies *et al.*, 2009). The semiaquatic *Thamnophis sirtalis* has an all-cone retina expressing three visual pigments with  $\lambda_{\max}$  of 360 nm, 482 and 554 nm (Sillman *et al.*, 1997). These pigments have been identified by opsin gene sequencing as SWS1, RH1 and LWS (Simões *et al.*, 2016a) indicating that rod-to-cone transmutation may have occurred in this species (because RH1 is expressed in an apparently all-cone retina). The transmutation of rods to cone-like rods in *Thamnophis* has been confirmed for the all-cone retina of *Thamnophis proximus* in a ground-breaking study including microscopy, MSP, opsin gene sequencing and *in vitro* expression (Schott *et al.*, 2016).

The elapid sea snakes *Hydrophis (Lapemis) curtus* and *Hydrophis (Acalyptophis) peronii* appear to have adapted to their aquatic environment by evolving a blue-shifted SWS1 pigment ( $\lambda_{\max}$  of 428–429 nm) (Hart *et al.*, 2012; Simões *et al.*, 2016a). Hart *et al.* (2012) through MSP also detected a 496-nm-sensitive pigment in the supposed all-cone retinas of these two sea snake species, suggesting that the RH1 detected by opsin gene sequencing is expressed in transmuted, cone-like rods (Simões *et al.*, 2016a). Visual opsin gene sequencing is consistent with the understanding that multiple transmutations from cones to rods

(or, at least, to rod-like cones) and *vice versa* have happened several times in the evolution of snakes (Simões *et al.*, 2016a).

The set of RH1, SWS1 and LWS visual pigments present in the ancestral snake has been retained in most living snakes, including vipers, colubrids, boas and pythons. For example, *Boa* and *Python* have pigments with  $\lambda_{\max}$  of 357/360 (SWS1), 494/495 (RH1) and 549/551 nm (LWS), respectively, as determined by MSP (Sillman *et al.*, 1999, 2001). Opsin gene sequencing across many major lineages of snakes suggests that although there have not been many opsin losses and no duplications, evolution of the visual system of this reptile lineage may have been exceptional in comparison with other major lineages of tetrapod vertebrates (Simões *et al.*, 2016b). Many snakes have UV-sensitive ( $\lambda_{\max}$  ~360 nm) SWS1 visual pigments but mutations at spectral sites in some species are not typical of pigments with UV sensitivity. Although many of the combinations of SWS1 tuning site mutations in snakes are not found in other vertebrates for which pigment  $\lambda_{\max}$  has been measured (preventing  $\lambda_{\max}$  prediction), the suspicion that several snakes are not UV sensitive is supported by the discovery of UV-blocking lenses in several diurnal colubrids with likely high visual acuity (such as the vine snake *Ahaetulla nasuta* and gliding snake *Chrysopelea ornata*) (Simões *et al.*, 2016b). In addition, the LWS pigment  $\lambda_{\max}$  has shifted to middle wavelengths (~536 nm) in multiple (especially colubrid) snakes. In *Helicops angulatus*, a polymorphic spectral tuning SWS1 site may allow some kind of trichromacy or a wider range of spectral sensitivities in this species (Simões *et al.*, 2016b).

Adaptation of the snake visual system is further evident in results of analyses of selective pressures across the visual opsin genes. Visual opsin genes of snakes are generally under purifying selection, several amino acids at sites with functional importance (particularly in transmembrane domains) appear to be under adaptive evolution and patterns of variation in selection are partly explained by ecology as well as retinal anatomy. Snakes are known to have a well-developed vomeronasal organ and chemosensory system, but vision is also likely to play an important role in snake biology and evolution given that at least some visual opsin gene evolution is adaptive and related to spectral tuning (Simões *et al.*, 2016b). The first snake visual opsin gene sequences were published only in 2009. Although snakes have now been subjected to more thorough evolutionary analyses of visual pigments than other reptiles, modern studies of snake visual pigment function and evolution are in their infancy and much remains to be discovered.

## Concluding Remarks

The visual pigments of birds resemble those of reptiles in that the ancestral vertebrate opsin gene complement does not seem to have undergone duplications, unlike in some mammals and actinopterygian and chondrichthyan fishes (Davies *et al.*, 2012). The majority of birds most closely resemble some nonsnake squamates and testudines in that they retain the five ancestral vertebrate visual pigments and have a duplex retina with photoreceptor oil droplets (Davies *et al.*, 2012). While most reptiles analysed so far have retained UV-sensitive vision, bird evolution has witnessed multiple switches between UV- and blue-sensitive SWS1

(Hunt *et al.*, 2009; Borges *et al.*, 2015). The mechanism underpinning UV-blue transitions in birds (Phenylalanine or Serine at site 86 (Hunt *et al.*, 2009)) appears to be convergent with that in some squamates.

Reptile visual pigments are generally less studied than those of birds, mammals and fish, especially from a molecular genetics perspective. What we know thus far, along with background information on reptile retinal diversity (Walls, 1942; Underwood, 1970), suggests that there is much of interest still to learn. The main limitations currently are the small sample of taxa for which there are multiple sources of evidence, chiefly photoreceptor complement, visual pigment complement (determined through opsin gene sequences), precise  $\lambda_{\max}$  for pigments (from MSP and/or *in vitro* expression) and accurate mapping of pigments to photoreceptors. High-throughput sequencing technologies will increasingly generate whole-genome and eye transcriptome data for reptiles, which will provide exciting opportunities for new discoveries and more thorough tests of existing hypotheses.

## References

- Alföldi J, Di Palma F, Grabherr M, *et al.* (2011) The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature* **477**: 587–591.
- Borges R, Khan I, Johnson WE, *et al.* (2015) Gene loss, adaptive evolution and the co-evolution of plumage coloration genes with opsins in birds. *BMC Genomics* **16**: 675.
- Bowmaker JK, Loew ER and Ott M (2005) The cone photoreceptors and visual pigments of chameleons. *Journal of Comparative Physiology A* **191**: 925–932.
- Bowmaker J and Hunt D (2006) Evolution of vertebrate visual pigments. *Current Biology* **16**: R484–R489.
- Buser P and Imbert M (1992) *Vision*. Cambridge, MA: The MIT Press.
- Crescitelli F (1977) The visual pigments of geckos and other vertebrates. In: Crescitelli F (ed) *Handbook of Sensory Physiology*, pp. 391–450. Berlin: Springer.
- Cronin TW, Johnsen S, Marshall NJ and Warrant EJ (2014) Visual pigments and photoreceptors. In: *Visual Ecology*, pp. 37–65. Princeton, NJ: Princeton University Press.
- Davies WL, Cowing JA, Bowmaker JK, *et al.* (2009) Shedding light on serpent sight: the visual pigments of henophidian snakes. *Journal of Neuroscience* **29**: 7519–7525.
- Davies WIL, Collin SP and Hunt DM (2012) Molecular ecology and adaptation of visual photopigments in craniates. *Molecular Ecology* **21** (13): 3121–3158.
- Ellingson JM, Fleishman LJ and Loew ER (1995) Visual pigments and spectral sensitivity of the diurnal gecko *Gonatodes albogularis*. *Journal of Comparative Physiology A* **177**: 559–567.
- Emerling CA and Springer MS (2014) Eyes underground: regression of visual protein networks in subterranean mammals. *Molecular Phylogenetics and Evolution* **78**: 260–270.
- Enright JM, Toomey MB, Sato S-Y, *et al.* (2015) Cyp27c1 red-shifts the spectral sensitivity of photoreceptors by converting Vitamin A1 into A2. *Current Biology* **25** (23): 3048–3057.
- Fasick JI and Robinson PR (1998) Mechanism of spectral tuning in the dolphin visual pigments. *Biochemistry* **37**: 433–438.
- Fleishman LJ, Loew ER and Whiting MJ (2011) High sensitivity to short wavelengths in a lizard and implications for understanding

- the evolution of visual systems in lizards. *Proceedings of the Royal Society B* **278**: 2891–2899.
- Gerkema MP, Davies WIL, Foster RG, *et al.* (2013) The nocturnal bottleneck and the evolution of activity patterns in mammals. *Proceedings of the Royal Society B* **280**: 20130508.
- Govardovskii VI (1983) On the role of oil drops in colour vision. *Vision Research* **23**: 1739–1740.
- Hart NS, Coimbra JP, Collin SP, *et al.* (2012) Photoreceptor types, visual pigments, and topographic specializations in the retinae of hydrophiid sea snakes. *Journal of Comparative Neurology* **520**: 1246–1261.
- Hárosi FI (1994) An analysis of two spectral properties of vertebrate visual pigments. *Vision Research* **34**: 1359–1367.
- Hauser FE, van Hazel I and Chang BSW (2014) Spectral tuning in vertebrate short wavelength-sensitive 1 (SWS1) visual pigments: can wavelength sensitivity be inferred from sequence data?. *Journal of Experimental Zoology B: Molecular and Developmental Evolution* **322**: 529–539.
- Horváth G and Varju D (2004) *Polarized Light in Animal Vision*. Berlin: Springer.
- Hsiang AY, Field DJ, Webster TH, *et al.* (2015) The origin of snakes: revealing the ecology, behavior, and evolutionary history of early snakes using genomics, phenomics, and the fossil record. *BMC Evolutionary Biology* **15**: 87.
- Hunt DM, Carvalho LS, Cowing JA and Davies WL (2009) Evolution and spectral tuning of visual pigments in birds and mammals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364** (1531): 2941–2955. <http://doi.org/10.1098/rstb.2009.0044>
- Kawamura S and Yokoyama S (1997) Expression of visual and nonvisual opsins in American chameleon. *Vision Research* **37**: 1867.
- Kochendoerfer G, Lin S, Sakmar T, *et al.* (1999) How color visual pigments are tuned. *Trends in Biochemical Sciences* **24**: 300–305.
- Kos M, Bulog B, Szel A, *et al.* (2001) Immunocytochemical demonstration of visual pigments in the degenerate retinal and pineal photoreceptors of the blind cave salamander (*Proteus anguinus*). *Cell and Tissue Research* **303**: 15–25.
- Liebman PA and Granda AM (1971) Microspectrophotometric measurements of visual pigments in two species of turtle, *Pseudemys scripta* and *Chelonia mydas*. *Vision Research* **11**: 105–114.
- Lin SW, Kochendoerfer GG, Carroll KS, *et al.* (1998) Mechanisms of spectral tuning in blue cone visual pigments: Visible and Raman Spectroscopy of blue-shifted Rhodopsin mutants. *Journal of Biological Chemistry* **273**: 24583.
- Loew ER and Govardovskii VI (2001) Photoreceptors and visual pigments in the red-eared turtle, *Trachemys scripta elegans*. *Visual Neuroscience* **18**: 753–757.
- Loew ER, Govardovskii VI, Rohlich P, *et al.* (1996) Microspectrophotometric and immunocytochemical identification of ultraviolet photoreceptors in geckos. *Visual Neuroscience* **13**: 247–256.
- Loew ER, Fleishman LJ, Foster RG, *et al.* (2002) Visual pigments and oil droplets in diurnal lizards: a comparative study of Caribbean anoles. *Journal of Experimental Biology* **205**: 927–938.
- Martin M, Le Galliard J-F, Meylan S, *et al.* (2015) The importance of ultraviolet and near-infrared sensitivity for visual discrimination in two species of lacertid lizards. *Journal of Experimental Biology* **218**: 458–465.
- Meyer-Rochow VB, Wohlfahrt S and Ahnelt PK (2005) Photoreceptor cell types in the retina of the tuatara (*Sphenodon punctatus*) have cone characteristics. *Micron* **36**: 423–428.
- Modesto SP and Anderson JS (2004) The phylogenetic definition of reptilia. *Systematic Biology* **53**: 815–821.
- Mohun SM, Davies WL, Bowmaker JK, *et al.* (2010) Identification and characterization of visual pigments in caecilians (Amphibia: Gymnophiona), an order of limbless vertebrates with rudimentary eyes. *Journal of Experimental Biology* **213**: 3586–3592.
- Nagloo N, Collin SP, Hemmi JM and Hart NS (2016) Spatial resolving power and spectral sensitivity of the saltwater crocodile, *Crocodylus porosus*, and the freshwater crocodile, *Crocodylus johnstoni*. *Journal of Experimental Biology* **219**: 1394–1404. The Company of Biologists Ltd.
- Ohtsuka T (1985a) Relation of spectral types to oil droplets in cones of turtle retina. *Science* **229**: 874–877.
- Ohtsuka T (1985b) Spectral sensitivities of seven morphological types of photoreceptors in the retina of the turtle, *Geoclemys reevesii*. *Journal of Comparative Neurology* **237**: 145–154.
- Pyrón R, Burbrink FT and Wiens JJ (2013) A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* **13**: 93.
- Schott RK, Müller J, Yang CGY, *et al.* (2016) Evolutionary transformation of rod photoreceptors in the all-cone retina of a diurnal garter snake. *Proceedings of the National Academy of Sciences of the United States of America* **113** (2): 356–361.
- Shedlock AM and Edwards SV (2009) Amniotes (Amniota). In: Blair Hedges S and Kumar S (eds) *The Timetree of Life*, pp. 375–379. New York: Oxford University Press.
- Sillman AJ, Ronan SJ and Loew ER (1991) Histology and microspectrophotometry of the photoreceptors of a crocodilian, *Alligator mississippiensis*. *Proceedings of the Royal Society B* **243**: 93–98.
- Sillman AJ, Govardovskii VI, Rohlich P, *et al.* (1997) The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*): a microspectrophotometric, scanning electron microscopic and immunocytochemical study. *Journal of Comparative Physiology A* **181**: 89.
- Sillman AJ, Carver JK and Loew ER (1999) The photoreceptors and visual pigments in the retina of a boid snake, the ball python (*Python regius*). *Journal of Experimental Biology* **202**: 1931–1938.
- Sillman AJ, Johnson JL and Loew ER (2001) Retinal photoreceptors and visual pigments in *Boa constrictor imperator*. *Journal of Experimental Zoology* **290**: 359–365.
- Simões BF, Sampaio FL, Jared C, *et al.* (2015) Visual system evolution and the nature of the ancestral snake. *Journal of Evolutionary Biology* **28**: 1309–1320.
- Simões BF, Sampaio FL, Loew ER, *et al.* (2016a) Multiple rod-cone and cone-rod photoreceptor transmutations in snakes: evidence from visual opsin gene expression. *Proceedings of the Royal Society B* **283** (1823pii: 20152624).
- Simões BF, Sampaio FL, Douglas RH, *et al.* (2016b) Visual pigments, ocular filters and the evolution of snake vision. *Molecular Biology and Evolution* **33**: 2483–2495.
- Su C-Y, Luo D-G, Terakita A, *et al.* (2006) Parietal-eye phototransduction components and their potential evolutionary implications. *Science* **311**: 1617–1621.
- Underwood G (1970) The eye. In: Gans C and Parsons TS (eds) *Biology of the Reptilia: Morphology B*, vol. 2, pp. 1–97. New York: Academic Press.
- Walls GL (1942) *The Vertebrate Eye and Its Adaptive Radiation*. New York: Fafner Publishing Company.
- Yokoyama S (2008) Evolution of dim-light and color vision pigments. *Annual Review of Genomics and Human Genetics* **9**: 259–282.



Yokoyama S, Xing J, Liu Y, *et al.* (2014) Epistatic adaptive evolution of human color vision. *PLoS Genetics* **10**: e1004884.

Schwab IR (2012) *Evolution's Witness: How Eyes Evolved*. Oxford: Oxford University Press.

Vitt LJ and Caldwell JP (2014) *Herpetology. An Introductory Biology of Amphibians and Reptiles*, 4th edn. San Diego, CA: Academic Press.

## Further Reading

Reuter T and Peichl L (2008) Structure and Function of the Retina in Aquatic Tetrapods. In: Thewissen JGM and Sirpa Nummela (eds) *Sensory Evolution on the Threshold: Adaption in Secondarily Aquatic Vertebrates*, pp. 149–172. Berkeley, CA: University of California Press.