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Multiple rod–cone and cone–rod photoreceptor transmutations in snakes: evidence from visual opsin gene expression

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In 1934, Gordon Walls forwarded his radical theory of retinal photoreceptor ‘transmutation’. This proposed that rods and cones used for scotopic and photopic vision, respectively, were not fixed but could evolve into each other via a series of morphologically distinguishable intermediates. Walls’ prime evidence came from series of diurnal and nocturnal geckos and snakes that appeared to have pure-cone or pure-rod retinas (in forms that Walls believed evolved from ancestors with the reverse complement) or which possessed intermediate photoreceptor cells. Walls was limited in testing his theory because the precise identity of visual pigments present in photoreceptors was then unknown. Subsequent molecular research has hitherto neglected this topic but presents new opportunities. We identify three visual opsin genes, *rh1*, *sws1* and *lws*, in retinal mRNA of an ecologically and taxonomically diverse sample of snakes central to Walls’ theory. We conclude that photoreceptors with superficially rod- or cone-like morphology are not limited to containing scotopic or photopic opsins, respectively. Walls’ theory is essentially correct, and more research is needed to identify the patterns, processes and functional implications of transmutation. Future research will help to clarify the fundamental properties and physiology of photoreceptors adapted to function in different light levels.

1. Introduction

In a landmark paper, Walls [1] argued that the two generally recognized categories of light-sensitive vertebrate retinal cells (photoreceptors), rods and cones, are not intransmutable but could evolve into one another via a series of intermediate morphotypes. The selective pressure for this ‘transmutation’ would arise from ecological shifts, such as diurnal to crepuscular activity. Thus, Walls [1,2] suggested that rods could re-evolve from cones in lineages that had lost cones, and *vice versa*. This radical theory challenged the orthodox view (that implied a more fixed dichotomy) that had been in place since Schultze [3] suggested the concept of two categories of vertebrate photoreceptors. Vertebrate rods probably originated by evolving from cones, with the two types being present in the ancestral jawed vertebrate more than 400 Ma [4], and persisting as discrete types other than in cases of potential transmutation.

Although Walls’ theory was radical, it did not challenge the fundamental aspect of the duplicity (or duplexity) theory of vision, which recognizes a functional

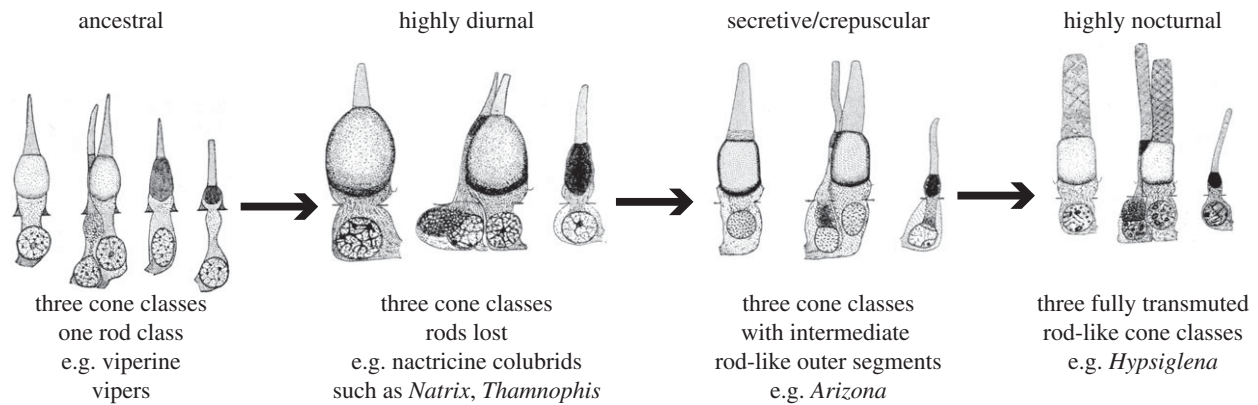


Figure 1. Retinal photoreceptor complements of colubroid snakes, providing evidence of transmutation as conceived by Walls [2]. Four different morphotypes are illustrated, each showing the different classes of photoreceptors present in the retina of a single species of snake. Arrows indicate the transformation series envisaged by Walls. Photoreceptor images reproduced from Walls [2] with permission from Cranbrook Institute of Science.

divergence between dim- (scotopic) and bright-light (photopic) photoreception associated with rods and cones, respectively (e.g. [5]). However, the transmutation theory does prompt fundamental questions about what the essential properties of photoreceptors adapted for scotopic and photopic vision are, how these properties effect key functions, and how they evolve.

Walls [1,2] found his evidence for transmutation in studies of the retinal anatomy of squamate reptiles, especially geckos and snakes, work that was reinforced by subsequent surveys by Underwood [6–8]. Walls [1] outlined two key pieces of evidence to support the hypothesis that evolutionary transformation occurred between the putatively homologous photoreceptor types. First, the morphological correspondence of the photoreceptor complements of pure-cone and pure-rod squamate retinas; and, second, the presence of morphologically intermediate photoreceptor complements among living squamates. The ‘best’ series of morphological intermediates identified by Walls [1] spanned diurnal, crepuscular/secretive and fully nocturnal North American colubroid snakes (figure 1).

Since Walls’ work, much has been learned about the molecular biology of vertebrate vision. In general, this has supported the view that there are two main morphological and physiological classes of photoreceptors. Photoreceptors with typically cone-shaped outer segments mediate photopic vision; they express *rh2*, *sws1*, *sws2* and/or *lws* opsin genes (here we denote opsin genes in lower case italics and opsins in upper case, e.g. *rh1* and RH1), and they have low sensitivity to light. Photoreceptors with rod-shaped outer segments mediate scotopic vision, express the *rh1* opsin (generally known as rod rhodopsin) gene and have high sensitivity (e.g. [4,9]). Typically, cones and rods are also neurally connected to ganglion cells with different levels of convergence so as to underpin high and low acuity spatial vision, respectively. Typically, too, the kinetics of cone phototransduction and regeneration are faster than that of rods. A typical vertebrate duplex retina contains one type or class of rod and two or more classes of cone photoreceptors, the latter differentiated primarily by size and whether they are single or double cells (e.g. [2]).

Cones and rods were originally defined primarily on (and named for) contrasting morphologies of their outer segments (the specialized, visual pigment-containing part of the cell). To this definition, subsequent microscopic and biochemical evaluations recognized additional, generally correlated characters such as the form of the footpieces (synaptic pedicels) and outer segment membrane discs (table 1). Although the most readily determined identifying characteristic of rods and cones is outer

segment shape (and size), the work of Walls and others identified cases in which this character lacks a binary distribution of character states, and the correlations between rod- and cone-like outer segments and other features are not complete. Some authors have therefore preferred a physiological definition; for example, Lamb [4,9] considered rods to be photoreceptors capable of detecting individual photons. Clearly, care is needed in using and interpreting the terms ‘rod’ and ‘cone’.

Walls’ transmutation theory has been scrutinized only with respect to geckos. It has been demonstrated that gecko photoreceptors have outer segment discs and pigments of the type typically found in cones, and they express opsin genes generally associated with photopic vision, irrespective of whether they are nocturnal species with a superficially pure-rod retina or diurnal species with a superficially pure-cone retina [5,13–16]. Since Walls’ [1,2] and Underwood’s [6–8] influential works, most research on vertebrate vision has been carried out on fishes, birds and mammals, and there have been very few considerations of potential instances of transmutation in snakes or the reporting of evidence that addresses this, with five exceptions noted here.

- (i) Considering outer segment shape, the colubrine colubrid snake *Telescopus fallax* has one class of single cone, a double cone and possibly a single rod. However, Munk & Rasmussen [17] reported that the ‘rods’ have a slightly tapering (cone-like) outer segment and those inspected had a continuity (typical of cones) between the outer segment’s discs and plasma membrane. Munk & Rasmussen [17] thus considered these superficially rod-like cells to be transmuted or ‘secondary rods’ (i.e. rod-like cones). Walls [18] reported ‘visual purple’ (rod rhodopsin—an RH1-based visual pigment with a vitamin A₁-derived chromophore) in *T. fallax* based on visual inspection, but Munk & Rasmussen pointed out [17] that the true identity (opsin class) of the photopigments remained unknown.
- (ii) The nocturnal dipsadine colubrid *Hypsiglena* was considered by Walls [1] to have a pure-rod retina comprised entirely of highly transmuted (rod-like) cones, and E.R.L. (reported in [19,20]) detected likely photopic (typical cone) visual pigments identified from their spectral sensitivities by microspectrophotometry (MSP).
- (iii) Chang and co-workers [21,22] have studied the diurnal natrixine colubrid *Thamnophis proximus* in detail. External cellular anatomy identifies one class of double and

Table 1. Some general differences between typical amniote rod and cone photoreceptors. (Opsin genes marked with an asterisk are not known to occur in snakes [10–12].)

	rods	cones
shape of outer segment	rod-like (cylindrical)	cone-like (distally tapering)
size of outer segment	longer	shorter
footpieces (synaptic pedicels)	small, oligosynaptic	large, polysynaptic
visual opsin genes expressed	<i>rh1</i>	<i>rh2*</i> , <i>sws1</i> , <i>sws2*</i> , <i>lws</i>
other phototransduction molecules	rod isoforms	cone isoforms
outer segment discs	individualized	partly continuous with outer plasma membrane
outer segment incisure	present	absent
oil droplets	absent	sometimes present (not in snakes)

three classes of single cones. However, at least some of the smallest single ‘cones’ have rod-like outer segment discs, have different ellipsoids from the other single photoreceptors and express *rh1*, and thus can be interpreted as transmuted (cone-like) rods.

- (iv) Applying MSP to sea snakes, Hart *et al.* [23] found three visual pigment classes present in photoreceptor populations comprised entirely of cells with cone-like outer segments. One of these pigments with a rod rhodopsin-like wavelength of peak absorbance (λ_{\max}) at ca 500 nm occurs in small single cones with relatively slender ellipsoids: Hart *et al.* [23] suggested these cells might be cone-like (transmuted) rods.
- (v) In the reportedly pure-cone retina of the diurnal colubrine colubrid *Coluber (Masticophis) flagellum*, Macedonia *et al.* [24] found three visual pigment classes by MSP with λ_{\max} values of 558, 362 and 458 nm. Based on typical λ_{\max} values, we suggest the former two are likely LWS- and SWS1-based visual pigments, respectively. The λ_{\max} of the 458 nm pigment suggests either an SWS2- or RH2-based pigment but because neither of these have been found in snakes [10–12] we consider it more likely to be an RH1-based pigment (with a lower λ_{\max} value than is typical for vertebrate RH1-based pigments).

Although Walls [1,2] focused on morphological aspects of transmutation, he considered the ‘most essential difference’ between scotopic and photopic photoreceptors to be the presence of rod rhodopsin (RH1-based pigment) in dark-adapted rods and its absence in cones. Walls [1,2] failed to find rod rhodopsin by visual inspection in a range of nocturnal colubroid snakes and he and Kühne [25] also failed to find it in diurnal colubroids, and this was taken as key support for Walls’ [1] theory that nocturnal colubroids with pure-rod retinas had evolved from diurnal colubroids with pure-cone retinas. This perceived lack of rod rhodopsin in at least some nocturnal colubroids was also influential in Underwood’s [6–8] acceptance of Walls’ theory. Crescitelli [5, p. 332] bemoaned the lack of information on the identity of snake visual pigments, and the reported lack of rod rhodopsin in nocturnal colubroids has not been tested subsequently by surveys of opsin genes expressed in snake retinas. Apart from Chang and co-workers’ discovery of *rh1* expression in cone-like rods in *T. proximus* (see above), the identity of the visual pigment genes in snakes with potentially transmuted photoreceptors remains unknown.

Here, we report the visual opsin genes expressed in the eyes of a phylogenetically and ecologically diverse assemblage of colubroid snakes, including taxa that, based on anatomical evidence, have (transmuted) rod-like cones or cone-like rods. All sampled species thought to lack morphological rods are found to express the typical rod visual opsin gene *rh1*, and all sampled species thought to lack cones express typical cone opsin genes *sws1* and *lws*. We conclude that some degree of rod-to-cone and cone-to-rod photoreceptor transmutation has occurred multiple times in snakes.

2. Material and methods

(a) Taxon sampling

Taxon sampling for opsin gene data focuses on colubroid caenophidian snakes (Caenophidia = ‘higher snakes’) selected on the basis of previous studies of photoreceptor anatomy. We sampled five species believed to have all-cone retinas (potentially having transmuted, cone-like rods) and two species believed to have all-rod retinas (with putatively transmuted, rod-like cones) (table 2). We also sampled one species of a colubrid genus (*Lampropeltis*) that has more typical rods and cones, and one species (*Arizona elegans*) considered [1] to have photoreceptors intermediate between cones and rods. These latter two taxa formed part of Walls’ [1] morphological transformation series proposed as key evidence for his transmutation theory, with the nocturnal *Hypsiglena* and *Phyllorhynchus* (both sampled here) viewed as exemplar end-members with highly transmuted (extremely rod-like) cones (figure 1).

As far as is known, the last common ancestor of caenophidians possessed typical rods and cones, as determined by the morphology of photoreceptor outer segments (e.g. [6,8,26]), such that the potentially transmuted photoreceptors in the sampled taxa are derived. In addition, our sampling covers independent origins of key retinal types. Thus, based on what we know about snake phylogeny (e.g. [27]; figure 2) and retinal photoreceptor complements (e.g. [8]), the superficially all-cone condition of hydrophiine elapids and of natricine colubrids are not homologous, and neither are the superficially all-rod complements of particular dipsadine and colubrine colubrids.

MSP sampling was restricted to single individuals of four species: *A. elegans*, *Rhinocheilus lecontei*, *Hypsiglena torquata* and *Lampropeltis getula*, all obtained from a commercial supplier. One of these sampled species (*A. elegans*) is the same as one of those sampled for opsin genes, and two others (*H. torquata* and *L. getula*) are congeneric with those sampled for opsin genes. The nocturnal or crepuscular colubrine colubrid *R. lecontei*, not sampled here for opsin genes, was considered by Walls ([1];

Table 2. Previously published details of the nine species surveyed here for visual opsin genes. (These are all colubroid caenophidian snakes (see figure 2a for phylogenetic relationships). Photoreceptor types are based primarily on morphology of outer (and to a lesser degree, inner) segments; double cones (or rods) are counted as a single photoreceptor type; ‘intermediates’ are cones with somewhat rod-like outer segments [1]. Presence or absence of rod rhodopsin as reported here was determined by looking for a reddish tinge in freshly exposed, dark-adapted retinas (rather than by, for example, surveying for expression of *rh1*). Some data on photoreceptors and pigments are for congeners rather than the exact same species studied here. Values for λ_{\max} (peak absorbance) are approximate in some cases, though all are direct MSP measurements. For voucher specimen details, see the electronic supplementary material, table S1.)

family	subfamily	genus and species	common name	ecology	types of retinal photoreceptors	reported visual pigment λ_{\max} (nm) and presence/absence of rod rhodopsin
Elapidae	Hydrophiinae	<i>Hydrophis peronii</i>	horned sea snake	aquatic, diurnal	four cones [23]	430, 496, 555 [23]
Elapidae	Hydrophiinae	<i>Notechis scutatus</i>	tiger snake	terrestrial, diurnal	three cones [6]	
Colubridae	Natricinae	<i>Thamnophis sirtalis</i>	common garter snake	semi-aquatic, diurnal	four cones [19]	360, 482, 554 [19]
Colubridae	Natricinae	<i>Natrix maura</i>	viperine water snake	semi-aquatic, diurnal	three cones [6]	no rod rhodopsin [25]
Colubridae	Dipsadinae	<i>Hypsiglena jani</i>	Texas night snake	terrestrial, nocturnal	three rods [1]	365, 500, 535 [20] no rod rhodopsin [18]
Colubridae	Colubrinae	<i>Arizona elegans</i>	glossy snake	terrestrial, nocturnal	three intermediates [1]	
Colubridae	Colubrinae	<i>Phyllorhynchus decurtatus</i>	spotted leaf-nosed snake	terrestrial, nocturnal	three rods [1]	no rod rhodopsin [18]
Colubridae	Colubrinae	<i>Lampropeltis californiae</i>	California kingsnake	terrestrial, diurnal	two cones, one rod [1]	no rod rhodopsin [18]
Colubridae	Colubrinae	<i>Telescopus fallax</i>	cat snake	terrestrial, nocturnal	two cones, one cone-like rod [17]	rod rhodopsin [18]

see [6]) to have transmuted, rod-like cones intermediate between those of *Arizona* and *Hypsiglena/Phyllorhynchus*.

(b) Molecular procedures

Extraction of mRNA and gDNA, cDNA synthesis, opsin gene amplification and sequencing, MSP and voucher barcoding was done using methods reported by Simões *et al.* [12] and detailed in the electronic supplementary material.

(c) Visual pigment λ_{\max} estimation

To some extent it is possible to predict peak absorbance (λ_{\max}) of vertebrate visual pigments from the amino acid sequences of their constituent opsins. Predictions are possible because of correlations between known amino acid sequences of opsins and λ_{\max} of corresponding pigments, measured directly in photoreceptors or for pigments for which opsins have been cloned and regenerated *in vitro*. We made λ_{\max} predictions by assessing combinations of amino acids at known sites of key importance for determining λ_{\max} (‘spectral tuning’ sites), assuming a vitamin A₁ chromophore (A₂ chromophores have not been reported in snakes) and comparing these with tuning data for other vertebrates ([28] and references cited therein). The prediction of λ_{\max} based on selected (spectral) amino acid sites is somewhat controversial because additional spectral sites may exist and different mechanisms might occur across different vertebrate groups [29]. We were unable to make confident λ_{\max} predictions in cases with spectral tuning amino acids (or combinations thereof) not reported in other vertebrates, or where they occur in other vertebrates but in pigments for which λ_{\max} has not been measured.

(d) Phylogenetics

New cDNA opsin gene sequences were aligned with published amniote opsin sequences; we included all squamate taxa for which full or near-full length opsin sequences were available plus representatives of other major amniote lineages (electronic supplementary material, table S1). Alignment was undertaken with MAFFT [30] (algorithm: auto; gap penalty: 3; off-set value: 0.1) and inspected by eye for errors (e.g. obvious misalignments; unexpected stop codons). A single alignment of all opsin sequences was generated. JMODELTEST v. 2 [31] was used to ascertain the best-fit model of sequence evolution (using a maximum-likelihood (ML)-optimized base tree and a nearest neighbour interchange + subtree pruning and regrafting best-tree search). Based on these results, we implemented a GTR + G + I model in ML and Bayesian inference (BI) phylogenetic analyses. RAXML v. 8 [32] and MRBAYES v. 3.1.2 [33] were used to perform the ML and BI analyses, respectively. The RAXML analysis used Majority Rule bootstrapping [34], randomized maximum parsimony starting trees and a fast hill-climbing algorithm. The *lws* opsin gene sequences were used as a monophyletic outgroup to root trees, following [35]. The BI analyses were run for 1 000 000 generations with chains sampled every 100 generations (after 25% of trees were discarded as burn-in), random starting trees, and four chains (three hot and one cold), and convergence in topology was assumed when the standard deviation of split frequencies fell to below 0.01.

3. Results

We amplified and sequenced three visual opsin genes from eye cDNA in all but one of the sampled snakes. Phylogenetic

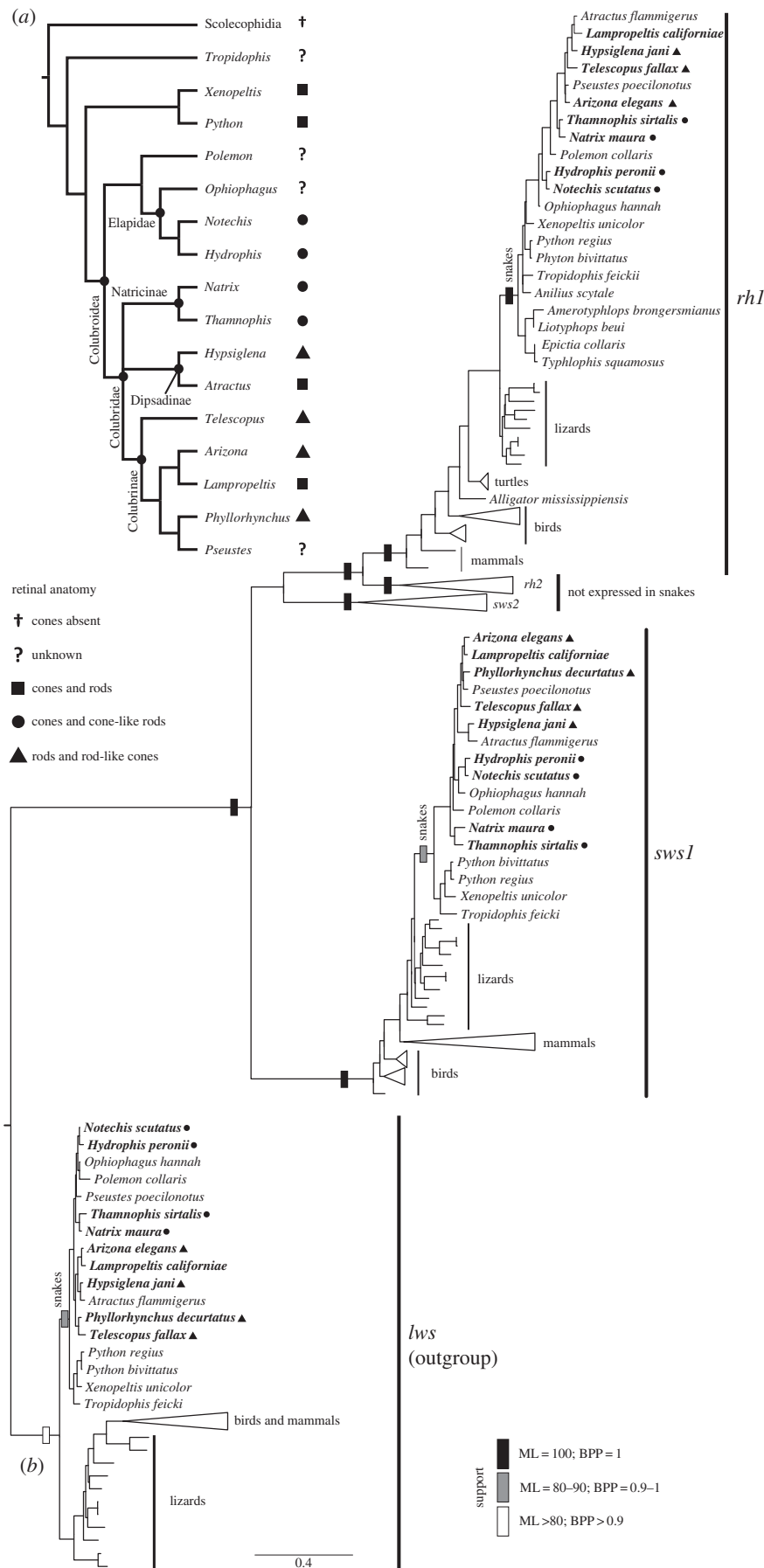


Figure 2. (a) Phylogenetic relationships among those snakes for which visual opsin gene sequence data are available (relationships based on [27]). (b) ML phylogenetic tree of visual opsins showing that *rh1*, *sws1* and *lws* are present in the sampled snakes, whether or not they have superficially all-cone or all-rod retinas. Taxa in bold are those newly sequenced for this study. Support levels for clades are based on ML bootstrap and Bayesian posterior probability values.

Table 3. Peak absorbance (λ_{\max} in nm) of visual pigments in four species of colubrine colubrid snakes, as determined by MSP. (Standard deviations and number of cells measured are also reported (see also the electronic supplementary material, figure S1). Opsin-based pigment identification is based on opsin gene identity (see text; figure 2) for conspecifics or congeners or (in the case of *R. lecontei*) on similarity of λ_{\max} values to those of other colubrines for which genetic data are available.)

species	opsin-based pigment		
	RH1	SWS1	LWS
<i>Lampropeltis getula</i>	493 \pm 2 (n = 9)	c.370 (n = 1) ^a	538 \pm 2 (n = 15)
<i>Arizona elegans</i>	484 \pm 2 (n = 9)	366 (n = 2)	538 \pm 1 (n = 21)
<i>Rhinocheilus lecontei</i>	487 \pm 2 (n = 9)	372 \pm 1 (n = 4)	539 \pm 1 (n = 19)
<i>Hypsiglena torquata</i>	500 \pm 2 (n = 12)	371 \pm 2 (n = 8)	534 \pm 2 (n = 23)

^aAbsorbance was measured for six cells, but no readings passed selection criteria; the 370 nm value reported here is an estimate based on noisy data.

results (figure 2; very similar for both ML and Bayesian analyses) confirm gene identity as *rh1*, *sws1* and *lws*. For *Phyllorhynchus decurtatus*, we were able to amplify only *sws1* and *lws*, while in all the other species we were able to amplify all three opsin genes.

Of the three pigment classes detected in the superficially pure-cone retina of *Hydrophis peronii* by Hart *et al.* [23] using MSP, the 496 nm pigment can now be attributed to an RH1 opsin with confidence. This is because the three opsin genes we amplified from this species included *rh1* with an amino acid sequence predicted (based on amino acids at 'known' spectral tuning sites) to have λ_{\max} = 493 nm (electronic supplementary material, table S2).

Using MSP, Sillman *et al.* [19] detected three pigment classes in *Thamnophis sirtalis*, with λ_{\max} values of 360, 554 and 482 nm. The former two can now be attributed to SWS1- and LWS-based pigments with great confidence given a close match between the measured λ_{\max} values and those predicted from amino acid sequences defined by our DNA sequences of *sws1* (predicted λ_{\max} 357 nm) and *lws* (555 nm) (electronic supplementary material, tables S3 and S4). The amino acid sequence of *T. sirtalis rh1* does not allow a prediction of λ_{\max} in this case (electronic supplementary material, table S2), but by elimination the 482 nm pigment measured by Sillman *et al.* [19] using MSP must be RH1-based. The expression of *rh1* in *T. sirtalis* supports Sillman *et al.*'s [19] suggestion that small single photoreceptors containing an RH1-based pigment are transmuted (cone-like) rods.

Based on amino acid sequences, the λ_{\max} of the *Hypsiglena jani* visual pigments are predicted to be 493 nm (*rh1*), 358 nm (*sws1*) and 536 nm (*lws*) (electronic supplementary material, tables S2, S3 and S4). These closely match our MSP λ_{\max} data for three pigments of *H. torquata* (ca 500, ca 371 and ca 534 nm; table 3; electronic supplementary material, figure S1; the slightly different values reported by Sillman *et al.* [20] are explained by the use of different templates). Thus, our data provide genetic identities for these pigments and support Sillman *et al.*'s [20] suspicion that some of the extremely rod-like outer segments contain typical cone pigments.

We lack genetic data for the visual opsin complement of *R. lecontei*, but our MSP data identify three pigments with λ_{\max} values (table 3; electronic supplementary material, figure S1) consistent with their being based on RH1, SWS1 and LWS opsins, as in other snakes discovered to have three pigments. Our genetic (figure 2; electronic supplementary material,

tables S2,S3 and S4) and MSP (table 3; electronic supplementary material, figure S1) data agree in identifying *rh1*, *sws1* and *lws* in both *A. elegans* and *Lampropeltis* spp. despite the cone outer segments of the former being (like those of *R. lecontei*) somewhat more rod-like [1].

4. Discussion

Our discovery of the typical rod visual opsin gene *rh1* in snakes lacking photoreceptors with classically rod-like outer segments and of typically cone opsin genes (*sws1*, *lws*) in snakes lacking classically cone-like photoreceptor outer segments provides new, molecular support for Walls' [1,2] hypothesis of photoreceptor transmutation. Based on what we know about opsin gene expression, photoreceptor anatomy and snake phylogeny (figure 2), within snakes, cone-like rods (still expressing *rh1*) have evolved at least twice independently (in diurnal elapids and natricine colubrids) and rod-like cones (still expressing *lws* and *sws1*) have also evolved at least twice independently (in nocturnal dipsadine colubrids and colubrine colubrids). There is no evidence that transmutation in snakes occurs beyond colubroids, and the ancestral snake probably had *rh1*, *sws1* and *lws* expressed in retinas comprising typical rods and cones (see also [5,10,36]). Although transmutation has occurred, it did not happen in snakes precisely as envisaged by Walls (and as shown in figure 1).

Kühne's and Walls' failures to find rod (RH1) rhodopsin in some nocturnal colubrids are not faithful indicators for a lack of this pigment (see also [5]). Historically, 'rhodopsin' was identified by a reddish tinge in freshly exposed, dark-adapted retinas that decays on exposure to light (e.g. [18]) and/or by the then accepted understanding that only rod rhodopsin (and not 'photopsins') could be reliably extracted from retinas (e.g. [37]). It was partly on the basis of a perceived lack of rod rhodopsin that Underwood [6, fig. 12] considered *Hypsiglena* and *Phyllorhynchus* to lack primary rods, and interpreted all three types of photoreceptor in these taxa to be transmuted (rod-like) cones. Our discovery of three opsins in *Hypsiglena* indicates that the rod-like photoreceptors which occur in this taxon evolved from a retina containing RH1, SWS1 and LWS opsins (and thus probably having both rods and cones).

It is clear that visual opsin gene expression and photoreceptor gross morphology are somewhat decoupled in

snakes (as in geckos: [14,38]). Underwood [7] discriminated between outer segment and footpiece transmutation, and in squamates, at least, most of the features of typical cones and rods might have somewhat decoupled in their evolution (see also [17]). In this respect, squamate retinas appear to be much more plastic and evolutionarily dynamic than those of fishes, birds or mammals. Although the most dramatic known instances of photoreceptor transmutation are in geckos and snakes, there is evidence elsewhere among vertebrates of a morphological and perhaps physiological continuum between rods and cones. For example, the typically cone opsin gene *sws2* has been found to be expressed in rods as well as cones of salamanders, with similar sensitivities and photoresponse kinetics in each cell type despite different associated transducins [39]; and rods in the highly diurnal grey squirrel are somewhat cone-like in form and physiology [40–42]. Photoreceptor transmutation might involve changes in one or more of the differences between archetypal rods and cones. Studies of non-squamate vertebrates have also demonstrated that postreceptor as well as receptor mechanisms contribute to photopic and scotopic vision, as shown by changes in the cellular organization of the retinas of individual skates as a consequence of light adaptation [43].

There is no evidence in snakes of co-expression of *rh1* with *sws1* or *lws* in single photoreceptors. Thus it is unlikely that transmutation occurs only by the evolutionary degeneration of cone or rod phototransduction in photoreceptors that were ancestrally combinative. Walls [1] considered the presence of pure-cone and pure-rod retinas in squamates as an explanation for why transmutation is restricted to this major taxon, arguing that evolutionary modification of rod : cone ratios is not possible when adapting to new photic niches if only cones or rods are present ancestrally. However, contrary to Walls' interpretation, we contend that at least some seemingly simple squamate retinas instead evolved from ancestors with more typically duplex retinas. The evolutionary plasticity of typical cone and rod features (see above) challenges the notion that pure-rod or pure-cone retinas can be identified without clarification of the use of those terms. Each component of typical rods and cones (table 1) needs to be scored independently for more taxa and mapped onto phylogenies in order to better reconstruct ancestral states.

What are the physiological properties of a cone-like cell expressing *rh1* or a rod-like cell expressing *sws1* or *lws*? Relevant data are lacking almost entirely for snakes, but some patchy information is available for geckos. Rates of decay of photoproducts in the visual cycle of transmuted (rod-like) cones of geckos are intermediate between those of typical rods and cones [44], although the exact reasons for this are unknown. Nocturnal geckos with transmuted (rod-like) cones have nocturnal colour vision [45], and we predict the same occurs in nocturnal snakes with similarly rod-like cones, such as *Hypsiglena*. It might be noted that RH1-based pigments in *H. torquata* cannot be extracted using the usual solvents (E. R. Loew, unpublished data), indicating differences from typical RH1-based pigments in biochemical properties that probably influence stability and interactions with membrane lipids. Speculatively, the retention of *rh1* in highly diurnal snakes with transmuted (cone-like) rods is potentially associated with some degree of mesopic trichromacy, with the kinetics of RH1 photoreceptors in such cases not required to be fully scotopic if their main role is not high sensitivity (unnecessary in bright light). Predicting physiological

characteristics of photoreceptors and visual capabilities from photoreceptor morphology is, however, likely to be difficult in most cases (see also [5]), and a full understanding of snake vision will require both direct physiological investigation and behavioural experiments.

Despite the documentation of great diversity in photoreceptor complements and the great number of evolutionary transformations that must have occurred among them [2,8] Jacobs *et al.* [46, p. 701] were correct in their assertion that 'enthusiasm about the ophidian eye has not proved to be particularly contagious'. Not only is understanding of vertebrate vision incomplete without a consideration of snakes (and other squamates), but natural experiments carried out by evolution in this group present an opportunity to address key questions in vertebrate vision, including the nature and plasticity of the duplex retina. We anticipate that our results will prompt further research into photoreceptor transmutation. In order to test hypotheses about proximate and ultimate causes, better data on the distribution, number and direction of transmutations and their functional implications are needed. In addition, understanding the relationships between protein sequence and function is needed for other (non-opsin) phototransduction elements (analogous to understanding of relationships between visual pigment λ_{\max} and opsin sequence). Similarly, it would be helpful if the genes responsible for various morphological features typical of cones and rods, and those determining the use of vitamin A₁ and/or vitamin A₂-derived chromophores, were identified. To this end, we would encourage new studies on aspects such as behaviour, transcriptomics, MSP, electroretinography and immunohistochemistry.

Note added in proof

Since this paper was accepted, Schott *et al.* [47] have reported details of the transmuted (cone-like) rods of the garter snake *Thamnophis proximus*.

Ethics. Snakes were euthanized using approved procedures (UK Home Office Schedule 1; University of Adelaide Animal Ethics Committee). Permits (SF010002) for research and export were granted by Western Australia Government (Department of Parks and Wildlife).

Data accessibility. Opsin and 16s sequences are available in GenBank under accession numbers KU323982–KU324007 and KU323973–KU323981, respectively (electronic supplementary material, table S1).

Authors' contributions. D.J.G., D.M.H., N.S.H., J.C.P. and B.F.S. conceived, designed and coordinated the study. D.J.G., B.F.S., K.L.S. and R.N.F. collected samples. E.L. generated MSP data. B.F.S. and F.L.S. generated molecular data. B.F.S. analysed molecular data. All authors contributed to the writing.

Competing interests. We have no competing interests.

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