

## THE PHOTORECEPTORS AND VISUAL PIGMENTS IN THE RETINA OF A BOID SNAKE, THE BALL PYTHON (*PYTHON REGIUS*)

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### Summary

The photoreceptors and visual pigments of *Python regius* were studied using microspectrophotometry and scanning electron microscopy. The retina contains rods and cones, with rods constituting at least 90% of the photoreceptor population. The rods are of a single type with long, narrow outer segments and are tightly packed. The wavelength of maximum absorbance ( $\lambda_{\max}$ ) of the visual pigment in the rods is in the region of 494 nm. Two distinct types of cone are present. The most common cone, with a stout but stubby outer segment, contains a visual pigment with  $\lambda_{\max}$

at approximately 551 nm. A relatively rare cone, with a long, slender outer segment, contains an ultraviolet-sensitive visual pigment with  $\lambda_{\max}$  at approximately 360 nm. All the visual pigments have chromophores based on vitamin A<sub>1</sub>. The results are discussed in relation to the behavior of *P. regius*.

Key words: reptile, snake, *Python regius*, scanning electron microscopy, microspectrophotometry, photoreceptor, ultraviolet vision, visual pigment.

### Introduction

As a group, the snakes have proved highly adaptable and, therefore, extremely successful. With the exception of the very cold polar regions, a few islands and the deeper ocean waters, representatives of the 2300 or so living species are found in virtually every portion of the biosphere and fill virtually every ecological niche (Cadle, 1987; Ford and Burghardt, 1993; Lillywhite and Henderson, 1993). There are, for example, both diurnal and nocturnal snakes, fully aquatic sea snakes and semiaquatic freshwater snakes, some species that spend most of their time in trees and others that live in burrows, some that thrive in jungles or under thick forest canopies and others that live in deserts or savannahs. The various snakes eat a wide variety of animals, including mammals, birds, reptiles, amphibians, fishes, insects, worms, crustaceans, molluscs and eggs. Although there are no known purely herbivorous snakes, at least one species includes plants in its diet (Gasc, 1994). The hunting strategies employed to acquire these different food items are varied, with some snakes being active foragers and others ambush predators (Mushinsky, 1987).

Different snakes occupy a wide variety of habitats that undoubtedly differ greatly in the spectral quality of the light environment, and they employ patterns of behavior that may make very different demands on the visual system. It is particularly unfortunate, therefore, that very little functional information has been gathered on visual sensitivity in the Ophidia, a group of animals whose eyes are generally relatively large and well-developed, and in which vision is

thought to be one of the dominant senses (Ford and Burghardt, 1993). For example, only four published studies have addressed the issue of spectral sensitivity and/or absorbance in the snake retina. Crescitelli (1956) measured the spectral absorbance of the visual pigment extracted from the rod photoreceptors of a viperid, *Crotalus viridis helleri*. Govardovskii and Chkheidze (1989) reported absorbance spectra obtained microspectrophotometrically from the photoreceptors of two viperids, *Vipera berus* and *V. lebetina*, and two colubrids, *Malpolon monspessulanus* and *Coronella austriaca*. Jacobs et al. (1992) employed an electroretinographic technique to determine the spectral sensitivity of the retinas of two colubrid garter snakes, *Thamnophis sirtalis* and *T. marcianus*. Sillman et al. (1997) used microspectrophotometry to characterize visual pigment absorbance in the retinas of *T. sirtalis* and *T. similis*, which contain only cones, and used scanning electron microscopy and immunocytochemistry to relate specific visual pigments to morphologically distinct cone types. Sillman et al. (1997) provided the first evidence for ultraviolet sensitivity in snakes.

The fact remains that the paucity of information regarding functional aspects of the snake retina prevents us from making any generalizations with confidence. For example, none of the studies cited above was performed on the retina of a boid snake. The Boidae, the boas and the pythons, are of great interest for several reasons. First, they are considered to be primitive snakes, known from the uppermost or Maastrichtian

phase of the Late Cretaceous, approximately 65 million years ago. In contrast, the oldest known colubrid appeared during the middle Oligocene, approximately 30 million years ago, and the viperids did not appear until the early Miocene, approximately 20 million years ago (Rage, 1987). Some of the more obvious primitive characteristics of the Boidae include vestiges of the pelvic girdle and hind limbs, coronoid bones in the lower jaw, teeth on the premaxilla and retention of both the left and right common carotids (Carroll, 1988). Second, and not unrelated to the first, it is generally accepted that the boid pattern of retinal photoreceptors is ancestral to the patterns of the more advanced snakes (Crescitelli, 1972). Finally, most of the boas and all of the pythons have specialized labial thermoreceptors which play an important role in the behavior of the snake. The input from these thermoreceptors appears to be integrated with retinal information in the optic tectum (Hartline et al., 1978; Kobayashi et al., 1992, 1995). In the present study, we employ both microspectrophotometry and scanning electron microscopy to characterize the retinal photoreceptors and visual pigments of *Python regius*, a typical boid snake.

### Materials and methods

The methods employed throughout this study, including a complete description of the microspectrophotometer, have been published previously (Loew and Lythgoe, 1978; Loew, 1982; Sillman et al., 1990, 1991; Loew and Sillman, 1993), and readers should consult the previous reports for details; only essentials of the methods are given here. Ball pythons (*Python regius*), ranging in length from 76 to 106 cm, were obtained from West Coast Reptile, Inc. (Fullerton, California, USA), who shipped them by air to either UC Davis or Cornell. Treatment of all animals used in the study conformed to the guiding principles for care and use of animals in research as set forth by the National Institutes of Health and the American Physiological Society.

#### *Scanning electron microscopy*

Following a blow to the head sufficient to stun, snakes were decapitated and the brain pithead. The eyes were removed and hemisected, and the eyecups were placed in a Petri dish filled with saline (Sigma Modified Minimum Essential Medium pH 7.6; MEM). In most cases, fine forceps were then used to separate the pigmented epithelium from the retinas, after which the retinas were transferred to a vial containing 2.5% glutaraldehyde in 0.1 mol l<sup>-1</sup> phosphate buffer, pH 7.8. On occasion, the eyecups were allowed to sit for 2–4 h in saline containing 60 μmol l<sup>-1</sup> cytochalasin D (Sigma). As reported by Chiang et al. (1995), cytochalasin D appeared to weaken the adherence of the pigmented epithelium to the retina, thus making separation of the two tissues easier. In some cases, where it was intended to examine the relationship between the photoreceptors and the pigmented epithelium, the entire eyecup was placed in fixative. These slight variations in technique resulted in no discernible differences in cell structure. After overnight fixation, the tissue was washed with

buffer, dehydrated with increasing concentrations of ethyl alcohol and critical-point-dried. The tissue was then fragmented with the fine tip of either a pair of forceps or a pulled glass pipette, mounted on a specimen stub, and sputter-coated with gold. The samples were examined with an ISI (model DS130) dual-stage scanning electron microscope. Polaroid (type 55) film was used to produce both positive and negative micrographs.

Dimensions and values derived from the micrographs were not corrected for shrinkage, which we estimate as no more than 10%.

#### *Microspectrophotometry*

Following at least 2 h of dark adaptation, snakes were killed and their retinas isolated in MEM as described above. The retina was then divided into quadrants. From one of the quadrants, a small piece of retina in a drop of saline was then placed on a glass coverslip, where it was macerated with the use of two razor blades. A second, smaller coverslip was placed over the tissue and sealed to the first using silicone grease. With the aid of very dim, deep red light, the preparation was then positioned on the stage of the microspectrophotometer. Other than this brief exposure to visible light, all procedures were performed using infrared illumination and image converters.

All absorbance data were obtained using the Cornell microspectrophotometer, a computer-driven, single-beam device capable of making accurate measurements in the near ultraviolet. Over 600 photoreceptors, both rods and cones, were scanned between 750 nm and 330 nm. Most of the scans yielded data that allowed classification of the visual pigment within the outer segment as long-, middle- or short-wavelength-sensitive. A qualitative assessment, by eye, was made of the regularity of each absorbance curve, and curves that best exhibited the regularity characteristic of visual pigment absorbance curves were subjected to further analysis. These curves were analyzed using a programmed statistical method described in detail by Loew and Sillman (1993). The curve was smoothed, and the smoothed data were differentiated to provide a preliminary estimate of  $\lambda_{\max}$ . The curve was then normalized to that value and converted to a normalized frequency scale (Mansfield, 1985; MacNichol, 1986). Linear regression was performed on the data between 30 and 70% of  $\lambda_{\max}$  on the long-wavelength limb of the doubly normalized curve, and between 40 and 70% of  $\lambda_{\max}$  on the short-wavelength limb. The linear regression values were compared with accepted templates for visual pigment curves derived from the rhodopsin data of Dartnall (Wyszecki and Stiles, 1967) and the porphyropsin data of Bridges (1967). This allowed us to generate 40 estimates for  $\lambda_{\max}$  from the long-wavelength limb and 30 estimates from the short-wavelength limb. Mean  $\lambda_{\max} \pm$  standard deviation (S.D.) was then determined using the short-wavelength limb estimates, the long-wavelength limb estimates and the combined estimates. For each of these three  $\lambda_{\max}$  values, a template curve was calculated, drawn and overlaid on the original data. A decision

as to which fitted best was made by visual examination. The template fit having the lowest s.d. usually had the best visual fit. This process was repeated for each microspectrophotometer curve, after which the  $\lambda_{\max}$  values for each curve of a spectral class were averaged to yield a final estimate of mean  $\lambda_{\max} \pm$  s.d. The  $\lambda_{\max}$  value for an individual curve was included in the ultimate average for a spectral class if its calculated s.d. was less than  $\pm 7$  nm.

## Results

### Microspectrophotometry

Microspectrophotometric examination of more than 600 rods and cones revealed the presence of three different visual pigments. Analysis of the best absorbance spectra recorded from rod outer segments ( $N=55$ ) placed the wavelength of peak absorbance ( $\lambda_{\max}$ ) for that visual pigment at  $494 \pm 2$  nm. A typical rod absorbance curve is shown in Fig. 1A together with the nomogram curve for a visual pigment whose chromophore is based on vitamin A<sub>1</sub> and whose  $\lambda_{\max}$  is at 494 nm. Single cones with relatively fat and stubby outer segments were found to contain a vitamin-A<sub>1</sub>-based visual pigment with  $\lambda_{\max}$  at  $551 \pm 3$  nm ( $N=11$ ). Single cones with relatively long, thin outer segments were found to contain a vitamin-A<sub>1</sub>-based visual pigment with  $\lambda_{\max}$  at  $360 \pm 3$  nm ( $N=4$ ). Typical absorbance spectra for the long-wavelength-sensitive pigment and the ultraviolet-sensitive pigment, together with their nomogram curves, are shown in Fig. 1B,C, respectively.

Five eyes from three snakes were analyzed with the microspectrophotometer, and all yielded the same results. There were also no differences with respect to retinal quadrants or with respect to the peripheral *versus* central retina. No absorbance spectrum that could be construed as a middle-wavelength-sensitive visual pigment was ever recorded from a cone.

### Scanning electron microscopy

The photoreceptors of the ball python, and their relationship to the retinal pigmented epithelium, are shown in the micrographs of Figs 2 and 3. The retina contains both rods and cones but is heavily dominated by the former. Although the rods could be counted, and packing density easily determined by viewing cross sections of their outer segments in micrographs such as Fig. 3B, this could not be done with the cones. This is so because the outer segments of the cones are fragile and their tips generally broke off, and because the outer segments of intact cones terminate much more proximally than do the outer segments of the rods. Nevertheless, by counting cells along the edges of the tissue, in micrographs such as Fig. 2A, we were able to estimate that cones constitute approximately 10% of the photoreceptor cell population. However, although most 'edge-on' micrographs revealed the presence of at least some cones, on rare occasions we observed tissue devoid of cones (Fig. 2B).

The rods of the ball python are typical vertebrate rods in that they have relatively long, cylindrical outer segments connected to inner segments of similar diameter. Well-developed calycal

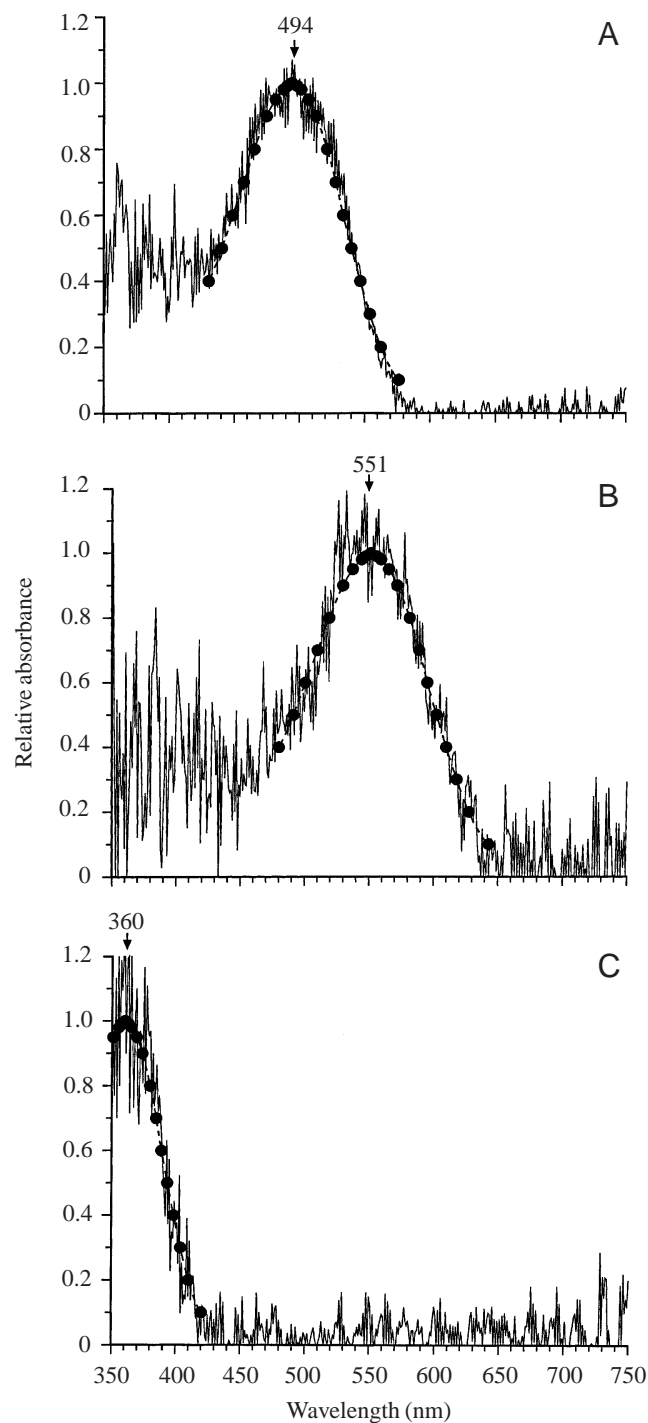


Fig. 1. Typical normalized microspectrophotometry absorbance curves obtained from the photoreceptors of *Python regius*. (A) Absorbance curve from a rod shown with the nomogram curve for a vitamin-A<sub>1</sub>-based visual pigment with peak absorbance ( $\lambda_{\max}$ ) at 494 nm (circles and smooth line). (B) Absorbance curve from a long-wavelength-sensitive cone shown with the nomogram curve for a vitamin-A<sub>1</sub>-based visual pigment with  $\lambda_{\max}$  at 551 nm (circles and smooth line). (C) Absorbance curve from an ultraviolet-sensitive cone shown with the nomogram curve for a vitamin-A<sub>1</sub>-based visual pigment with  $\lambda_{\max}$  at 360 nm (circles and smooth line). Absolute peak absorbance is 0.073, 0.062, 0.058 optical density units in A, B and C, respectively.

processes extend from the inner segments to the outer segments (open arrow in Fig. 2E). Measurement of rod outer segments that appeared to be intact (e.g. open arrow in Fig. 2C) allowed

us to determine mean outer segment length as  $27.4 \pm 2.2 \mu\text{m}$  ( $N=26$ ; range  $23.2\text{--}30.9 \mu\text{m}$ ). The outer segments are narrow, with a mean diameter of  $1.6 \pm 0.2 \mu\text{m}$  ( $N=109$ ; range

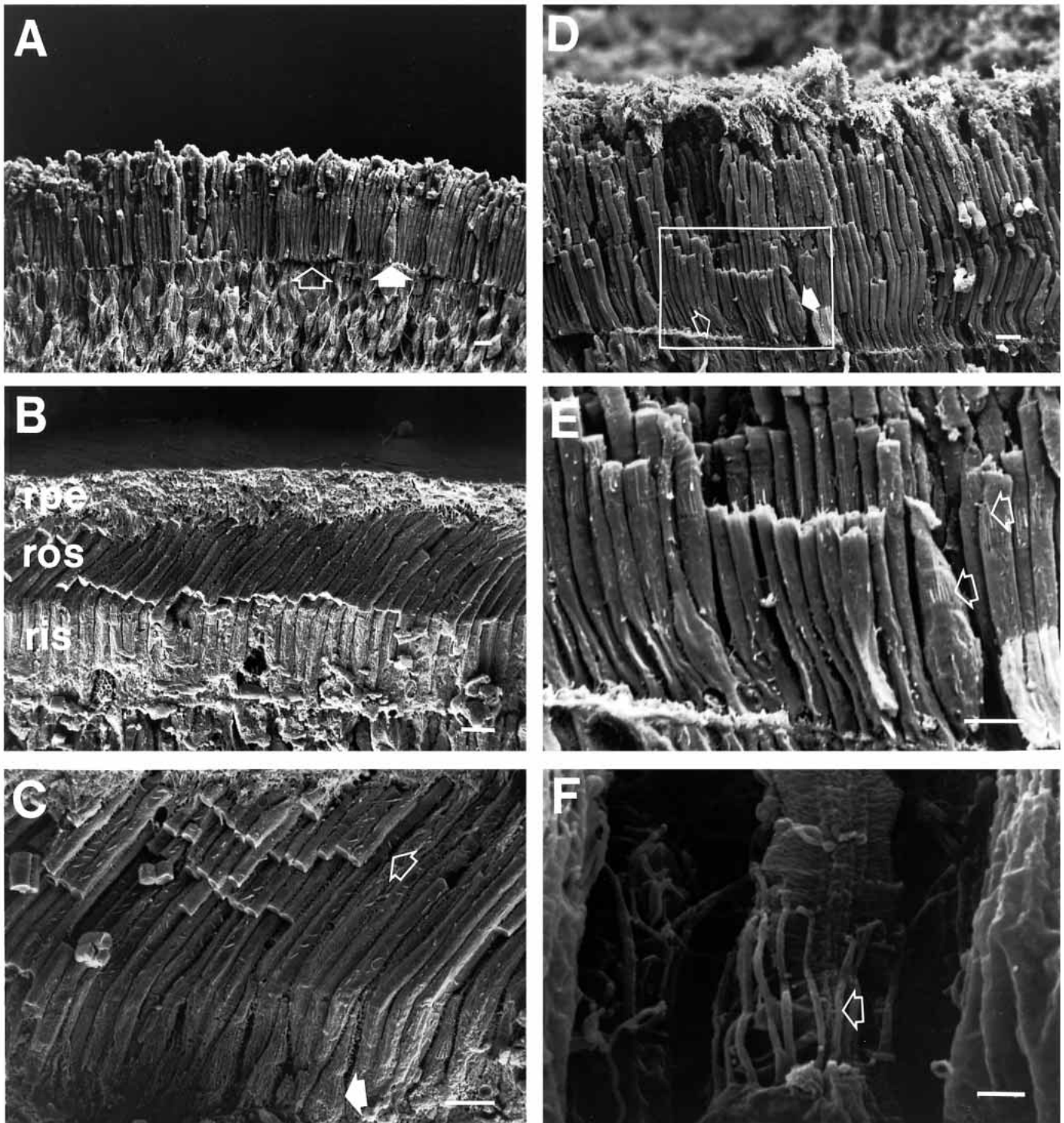


Fig. 2. Scanning electron micrographs of the photoreceptors in the retina of *Python regius*. (A) The open arrow points to the base of a rod photoreceptor, the filled arrow to the base of a cone. (B) rpe, retinal pigmented epithelium; ros, rod outer segments; ris, rod inner segments. (C) The open arrow points to an intact rod outer segment, the filled arrow to the inner segment of a typical cone. (D) The filled arrow points to a typical cone with a relatively large inner segment and robust outer segment, the open arrow to a smaller cone with a narrow, more delicate outer segment. (E) Photographic enlargement of the boxed area in D showing two distinct types of cone. The open arrows point to calycal processes. (F) The open arrow points to a calycal process on a cone outer segment. Scale bars,  $5.0 \mu\text{m}$  in A–E,  $0.5 \mu\text{m}$  in F.



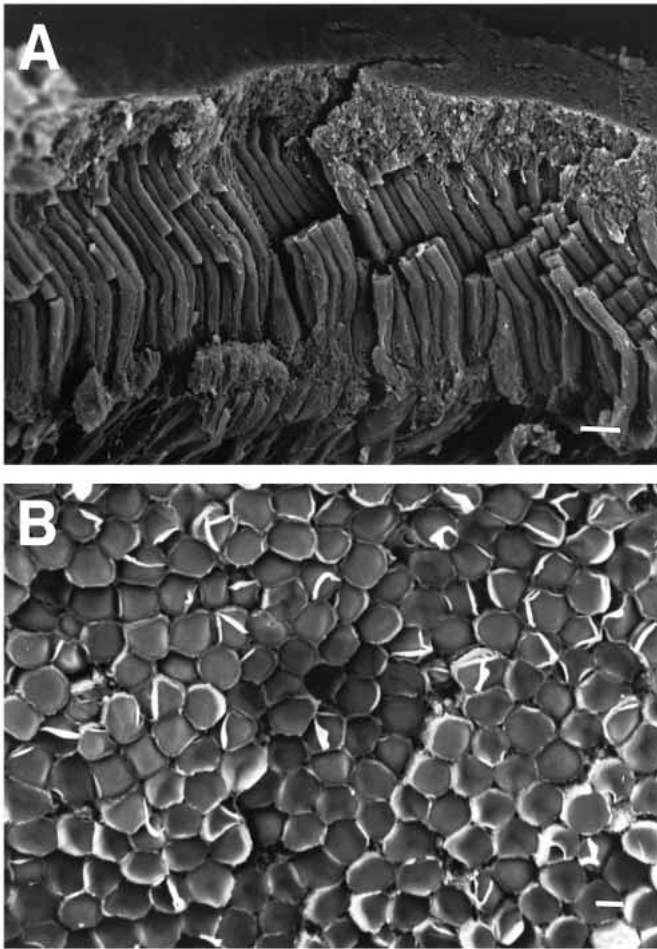


Fig. 3. Scanning electron micrographs of the photoreceptors in the retina of *Python regius*. (A) The close association between the rod photoreceptors and the retinal pigmented epithelium. (B) The tightly packed outer segments of the rods seen in cross section. Scale bars, 5.0  $\mu\text{m}$  in A, 1.0  $\mu\text{m}$  in B.

0.9–2.2  $\mu\text{m}$ ), and are rather tightly packed. Examination of six different micrographs, similar to Fig. 3B, from one ball python established rod packing density at  $457\,740 \pm 11\,517$  cells  $\text{mm}^{-2}$  (range 436 471–471 849 cells  $\text{mm}^{-2}$ ). The relationship between the rod photoreceptors and the retinal pigmented epithelium is a close one, with the processes of the retinal pigmented epithelium cells extending down between, and adhering tightly to, the outer segments of the rods (Figs 2B, 3A).

Like the rods, the cones of the ball python are accurately described as typical vertebrate photoreceptors in that they are made up of a relatively globose inner segment from which protrudes a tapering or cone-shaped outer segment. Well-developed calyces extend from the inner to the outer segment (open arrows in Fig. 2E,F). Measurement of 52 cones yielded mean values of  $2.4 \pm 0.6 \mu\text{m}$  (range 1.5–4.2  $\mu\text{m}$ ) for the outer segment diameter at the base and  $4.0 \pm 0.6 \mu\text{m}$  (range 2.4–5.2  $\mu\text{m}$ ) for the inner segment diameter at its greatest point. On rare occasions, we observed a cone that appeared to be quite distinct from the others. Fig. 2D, for example, shows

three cones of the most common variety, with relatively large inner segments and robust outer segments (filled arrow). Fig. 2D,E also shows the presence of another, more delicate, cone with a smaller inner segment extending into a relatively long, thin outer segment (open arrow).

## Discussion

### *The photoreceptor population*

The data collected in this study show that the retina of *Python regius* is duplex but is highly dominated by rods, cones constituting only approximately 10% of the photoreceptor population. The value of 10% may be a high estimate because there may have been a tendency to select preferentially regions with cones for photography, and we did find some regions entirely devoid of cones. Underwood (1970), who examined other boid retinas with the light microscope, found rod:cone ratios closer to 50:1. The rods of *P. regius*, which are very tightly packed, are of a single type with a relatively long and narrow outer segment.

The wavelength of peak absorbance ( $\lambda_{\text{max}}$ ) of the visual pigment within the rods is 494 nm, a value somewhat shorter than that reported for other snakes where rods are present. The rod pigments of the viperids *Crotalus viridis helleri* (Crescitelli, 1956), *Vipera berus* and *V. lebetina* and that of the colubrid *Coronella austriaca* all have absorbance maxima at 500 nm (Crescitelli, 1956; Govardovskii and Chkheidze, 1989). So few snakes have been studied, however, that it is not possible to say whether the shorter  $\lambda_{\text{max}}$  is unusual among snakes in general or whether it is a common characteristic of pythons. Certainly, a 6 nm shift towards the blue would have a significant effect on visual sensitivity.

In contrast to previous reports, which describe the boid retina in general as having only one type of rod and one morphologically distinct type of cone (Walls, 1942; Underwood, 1970), two distinct cone types are present in the retina of *P. regius*. The most common cone by far, as seen with both the scanning electron microscope and using microspectrophotometry, is characterized by a rather stout but stubby outer segment. This is a long-wavelength-sensitive cone containing a visual pigment with  $\lambda_{\text{max}}$  near 551 nm. Similar cone pigments, with absorbance maxima in the range 550–560 nm, have been identified in two viperid and three colubrid species (Govardovskii and Chkheidze, 1989; Sillman et al., 1997). The second cone type, again seen with both the scanning electron microscope and using microspectrophotometry, is characterized by a much thinner and longer outer segment. This cone is an ultraviolet-sensitive cone containing a visual pigment with  $\lambda_{\text{max}}$  near 360 nm. This pigment is spectrally similar to the ultraviolet-sensitive visual pigment present in one of the small single cones of the colubrid *Thamnophis sirtalis* (Sillman et al., 1997). The *T. sirtalis* retina also contains a second type of small single cone with a visual pigment absorbing maximally at 482 nm, but we found no evidence of such a pigment in the cones of *P. regius*. Since microspectrophotometry is essentially a statistical method in

which the photoreceptor population is only sampled, it is always possible that cones absorbing maximally near 482 nm were simply missed. However, we scanned a large number of photoreceptors using the same instrument and techniques that easily revealed the presence of such a cone in other animals and, therefore, we are confident that we would have found a 482 nm pigment if it had been present.

The absence in *P. regius* of either a short-wavelength-sensitive cone with  $\lambda_{\max}$  near 480 nm or a middle-wavelength cone with  $\lambda_{\max}$  in the range 500–535 nm is somewhat problematical. The *cis* or  $\beta$  band of the 551 nm pigment extends the absorbance of the pigment down into the near ultraviolet. This would provide *P. regius* with significant visual sensitivity between 400 and 500 nm even in the absence of either a short- or middle-wavelength-sensitive cone. Moreover, since the absorbance of the ultraviolet-sensitive pigment overlaps that of the 551 nm pigment up to approximately 430 nm, the two cone pigments of *P. regius* could provide a mechanism for wavelength discrimination or color vision. However, since the ultraviolet-sensitive pigment does not contribute to visual sensitivity beyond 430 nm (Jacobs, 1992), discrimination of wavelengths longer than 430 nm would not be possible without the participation of a short- or middle-wavelength-sensitive visual pigment. Although *P. regius* may, in fact, be a dichromat with very limited color vision, it is intriguing to speculate that the rods, which contain a visual pigment with  $\lambda_{\max}$  at 494 nm, might function together with the two cone pigments to fill the spectral gap. Rods, of course, are the photoreceptors that mediate sensitive vision under conditions of relative darkness, where cones do not function, and are generally thought to be silent under conditions of bright light where only cones operate. However, rods and cones both function under mesopic lighting conditions, as would occur at dawn and dusk. Rods have been shown to contribute to color vision under such conditions (Reitner et al., 1991; Stabell and Stabell, 1994; Buck, 1997) and, therefore, it is not unreasonable to think that the rods of *P. regius* might serve to extend the range of wavelength discrimination of the snake.

Moreover, from morphological data, Walls (1942) speculated that cones are the ancestral type of photoreceptor and that rods developed as 'transmutations' under the pressure of natural selection. Indeed, DNA analysis strongly suggests that cone visual pigments were a very early phylogenetic development and that rod pigments evolved much later from the middle-wavelength-sensitive cone pigment (Bowmaker, 1998). If this is so, then it is not inconceivable that the rods of *P. regius* are partially transmuted cells, i.e. rods that have retained some cone characteristics. Such a photoreceptor might very well be able to function as both a highly sensitive rod and a middle-wavelength-sensitive cone. In this regard, the secondarily nocturnal colubrid *Hypsiglena ochrorhynchus* is rather provocative. This snake, considered by Walls (1942) to be a spectacular example of transmutation, has only photoreceptors that appear morphologically to be rods. However, E. R. Loew (unpublished observations) has found that *H. torquata* has an ultraviolet-sensitive visual pigment with  $\lambda_{\max}$  at approximately

365 nm, a pigment with  $\lambda_{\max}$  at approximately 500 nm and a pigment with  $\lambda_{\max}$  at approximately 535 nm, leading one to suspect that some of the rods might contain cone pigments. In fact, other reptiles (geckos and the alligator) have rods whose visual pigments exhibit some cone characteristics (Crescitelli, 1972). It would be very interesting to know the receptor potential adaptation characteristics of the rods and cones of *P. regius* but, unfortunately, they have not yet been determined for any snake.

#### *The role of the visual system*

There has been no detailed and systematic study of the behavior of *P. regius*. *P. regius* is the smallest of the African pythons, seldom more than 0.9–1.2 m long, about the size of the snakes used in this study, although specimens of 1.8 m or more have been seen. It lives in the open forests and grasslands of West and Central Africa, where it feeds primarily on gerbils, a small nocturnal rodent. It is known to inhabit burrows and other holes for the purpose of aestivation or deposition and incubation of eggs (Cansdale, 1961; Mehrtens, 1987). The nocturnal habit of its primary prey makes it necessary for *P. regius* to be very active at night. Its retina, so heavily dominated by highly sensitive rods, is ideally suited for either foraging or ambushing in a dim light environment. Its relatively long rod outer segments would be highly effective light traps, making it very unlikely that a photon entering a rod along its longitudinal plane would not be absorbed by the visual pigment. Similarly, its high rod packing density decreases the likelihood that a photon would pass between adjacent rods and remain unabsorbed. At approximately 457 000 rods mm<sup>-2</sup>, the rod packing density in the retina of *P. regius* is similar to that of other nocturnal creatures. For example, depending on the region surveyed, rod packing density has been measured in the retina of the cat *Felis domesticus* at 275 000–460 000 rods mm<sup>-2</sup> (Steinberg et al., 1973), in the North American opossum *Didelphis virginiana* at 310 000–485 000 rods mm<sup>-2</sup> (Kolb and Wang, 1985) and in the owl monkey *Aotes trivirgatus* at 216 000–478 000 rods mm<sup>-2</sup> (Ogden, 1975). Albino laboratory rats were found to have a rod packing density of 374 000 rods mm<sup>-2</sup> by Mayhew and Astle (1997) and 400 000 rods mm<sup>-2</sup> by Cone (1963), who found no variation in density over the central two-thirds of the retina. Visual resolution is a complex phenomenon that depends not only on photoreceptor packing density but also on such factors as optics, photoreceptor-to-ganglion cell convergence and photoreceptor sampling (Pettigrew et al., 1988). In fact, in the final analysis, behavioral measurements of spatial resolution are necessary to determine the visual acuity of *P. regius*. Nevertheless, the high rod packing density makes it very likely that *P. regius*, like other nocturnal animals, makes good use of its eyes to orient towards forms and, especially, moving objects such as its prey.

Observations of the way in which pythons behave under relatively bright light lend support to the idea that vision plays an important role in the ability of *P. regius* to capture prey. Such observations are relevant since *P. regius* in the wild is often encountered sunning in bright light (Cansdale, 1961).

Diurnal activity may, in fact, be common amongst pythons, since the diamond python *Morelia spilota* basks on sunny winter days and, although it feeds at night, moves about primarily during daylight hours (Slip and Shine, 1988a). Studies on captive reticulated pythons *P. reticulatus*, under lighted conditions, showed that a visual stimulus is absolutely necessary to elicit a strike although an infrared stimulus serves to guide the strike (de Cock Buning et al., 1978; de Cock Buning, 1983). Such visually dependent behavior patterns are consistent with the retina of *P. regius*. Even in the absence of a specialized region of high cone density (Walls, 1942), the rod:cone ratio of approximately 10:1 found in *P. regius* reflects a cone density of approximately 45 000 cells mm<sup>-2</sup>. This is less than the cone packing density in the central retina of the owl monkey (78 000 cones mm<sup>-2</sup>; Ogden, 1975), but substantially greater than the cone density in the area centralis of either the North American opossum (8000 cones mm<sup>-2</sup>; Kolb and Wang, 1985) or the cat (27 000 cones mm<sup>-2</sup>; Steinberg et al., 1973). Strictly on the basis of cone packing density, it would not be unreasonable to conclude that the photopic visual resolution of *P. regius* should be better than that of the cat. Jacobson et al. (1976) found the behavioral visual acuity of the cat to be approximately 9 cycles degree<sup>-1</sup>, which is nowhere near the photopic acuity of the human (approximately 60 cycles degree<sup>-1</sup>) or the wedge-tailed eagle *Aquila auda* (approximately 138 cycles degree<sup>-1</sup>) (Pettigrew et al., 1988), but should still allow formation of a very useful image.

As is the case for *P. reticulatus*, alertness and orientation to a prey item by *P. regius*, followed by an approach and, finally, an accurate strike undoubtedly involves the combined use of photoreception and thermoreception. Input from the heat-sensing and visual systems is probably very well integrated, because neurons from both these systems are known to synapse on the same dendrites in the optic tectum of *P. regius* (Kobayashi et al., 1992, 1995). In the optic tectum of rattlesnakes, the only heat-sensing snakes other than boids, there are neurons that are activated only when they receive input from both the thermoreceptors and the photoreceptors (Hartline et al., 1978). It is certainly reasonable to expect the presence of similar tectal neurons in the boids.

#### *The role of the ultraviolet-sensitive cones*

The role of the ultraviolet receptors is of particular interest. It is reasonable to assume that the ability to absorb ultraviolet light has an important adaptive value since ultraviolet light is actually damaging to retinal tissue (Jacobs, 1992; Bennett and Cuthill, 1994), but it is not clear what that adaptive value might be in most, if not all, species with ultraviolet sensitivity. Of course, the simple broadening of spectral sensitivity that comes with ultraviolet-sensitive photoreceptors would, in itself, have important adaptive value. However, since not all vertebrates, even within the same class, have ultraviolet sensitivity, it is interesting to speculate on what might make ultraviolet sensitivity especially important to an animal (Jacobs, 1992). In the rod-dominated retina of *P. regius*, where all cones are scarce, there are so few ultraviolet-sensitive photoreceptors

that it is unreasonable to think that they could do much towards creating any kind of detailed visual picture (assuming that there is no undetected region of the retina that is especially rich in ultraviolet-sensitive cones). They could, however, still serve to alert the snake to anything that creates contrast in its environment by either reflecting or absorbing ultraviolet light. Noting that some lizards leave pheromone trails that absorb ultraviolet light (Alberts, 1989), and that garter snakes also create scent trails by extruding pheromones through the skin (Ford and Low, 1984), Sillman et al. (1997) speculated that *T. sirtalis* might use its ultraviolet sensitivity to locate scent trails which it would then follow using its chemosense. This speculation can certainly be extended to *P. regius*. There is evidence that the males of at least one species of python, *M. spilota*, locate females by following scent trails (Slip and Shine, 1988b). It would be interesting to know whether the extruded substance either reflects or absorbs ultraviolet light. Another possibility is that the mammalian prey of *P. regius* leaves a scent trail with ultraviolet activity, and that the snake uses its ultraviolet sensitivity for hunting. Some rodents are known to produce urine and feces that reflect ultraviolet light (Desjardins et al., 1973; Viitala et al., 1995), and the Eurasian kestrel *Falco tinnunculus* apparently takes advantage of this to locate voles. Such a capability could be useful to *P. regius* even though its ultraviolet-sensitive cones operate during daylight but the snake feeds primarily at night. *P. regius* could locate and position itself near well-used animal paths by day so as to improve its hunting efficiency at night. Slip and Shine (1988a) found that the diamond python, an ambush predator, takes prey at night but moves about by day. They suggest that this maximizes the time the diamond python spends in the coiled ambush position and, therefore, improves hunting efficiency.

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#### References

- Alberts, A. C.** (1989). Ultraviolet visual sensitivity in desert iguanas: implications for pheromone detection. *Anim. Behav.* **38**, 129–137.
- Bennett, A. T. and Cuthill, I. C.** (1994). Ultraviolet vision in birds: what is its function? *Vision Res.* **34**, 1471–1478.
- Bowmaker, J. K.** (1998). Evolution of colour vision in vertebrates. *Eye* **12**, 541–547.
- Bridges, C. D. B.** (1967). Spectroscopic properties of porphyropsins. *Vision Res.* **7**, 349–369.
- Buck, S. L.** (1997). Influence of rod signals on hue perception: Evidence from successive scotopic contrast. *Vision Res.* **37**, 1295–1301.
- Cadle, J. E.** (1987). Geographic distribution: problems in phylogeny and zoogeography. In *Snakes: Ecology and Evolutionary Biology* (ed. R. A. Seigel, J. T. Collins and S. S. Novak), pp. 77–105. New York: Macmillan.
- Cansdale, G. S.** (1961). *West African Snakes*. New York: Longmans.
- Carroll, R. L.** (1988). *Vertebrate Paleontology and Evolution*. New York: W. H. Freeman.
- Chiang, R. K., Yao, X. Y., Takeuchi, A., Dalal, R. and Marmor,**

- M. F. (1995). Cytochalasin D reversibly weakens retinal adhesiveness. *Curr. Eye Res.* **14**, 1109–1113.
- Cone, R. A. (1963). Quantum relations of the rat electroretinogram. *J. Gen. Physiol.* **46**, 1267–1286.
- Crescitelli, F. (1956). The nature of the gecko visual pigment. *J. Gen. Physiol.* **40**, 217–231.
- Crescitelli, F. (1972). The visual cells and visual pigments of the vertebrate eye. In *Photochemistry of Vision, Handbook of Sensory Physiology*, vol. VII/1 (ed. H. J. A. Dartnall), pp. 245–363. New York: Springer-Verlag.
- de Cock Buning, T. (1983). Thermal sensitivity as a specialization for prey capture and feeding in snakes. *Am. Zool.* **23**, 363–375.
- de Cock Buning, T., Poelmann, R. E. and Dullemeijer, P. (1978). Feeding behaviour and the morphology of the thermoreceptors in *Python reticulatus*. *Neth. J. Zool.* **28**, 62–93.
- Desjardins, C., Maruniak, J. A. and Bronson, F. H. (1973). Social rank in house mice: differentiation revealed by ultraviolet visualization of urinary marking patterns. *Science* **182**, 939–941.
- Ford, N. B. and Burghardt, G. M. (1993). Perceptual mechanisms and the behavioral ecology of snakes. In *Snakes: Ecology and Behavior* (ed. R. A. Seigel and J. T. Collins), pp. 117–164. San Francisco: McGraw-Hill.
- Ford, N. B. and Low, J. R., Jr (1984). Sex pheromone source location by garter snakes. A mechanism for detection of direction in non-volatile trails. *J. Chem. Ecol.* **10**, 1193–1199.
- Gasc, J.-P. (1994). Predation and nutrition. In *Snakes: A Natural History* (ed. R. Bauchot), pp. 108–121. New York: Sterling.
- Govardovskii, V. I. and Chkheidze, N. I. (1989). Retinal photoreceptors and visual pigments in certain snakes. *Biol. Abstr.* **90**, 1036.
- Hartline, P. H., Kass, L. and Loop, M. S. (1978). Merging of modalities in the optic tectum: infrared and visual integration in rattlesnakes. *Science* **199**, 1225–1229.
- Jacobs, G. H. (1992). Ultraviolet vision in vertebrates. *Am. Zool.* **32**, 544–554.
- Jacobs, G. H., Fenwick, J. A., Crognale, M. A. and Deegan II, J. F. (1992). The all-cone retina of the garter snake: spectral mechanisms and photopigment. *J. Comp. Physiol. A* **170**, 701–707.
- Jacobson, S. G., Franklin, K. B. J. and McDonald, W. I. (1976). Visual acuity of the cat. *Vision Res.* **16**, 1141–1143.
- Kobayashi, S., Amemiya, F., Kishida, R., Goris, R. C., Kusunoki, T. and Ito, H. (1995). Somatosensory and visual correlation in the optic tectum of a python, *Python regius*: a horseradish peroxidase and Golgi study. *Neurosci. Res.* **22**, 315–323.
- Kobayashi, S., Kishida, R., Goris, R. C., Yoshimoto, M. and Ito, H. (1992). Visual and infrared input to the same dendrite in the tectum opticum of the python, *Python regius*: electron-microscopic evidence. *Brain Res.* **597**, 350–352.
- Kolb, H. and Wang, H. H. (1985). The distribution of photoreceptor, dopaminergic amacrine cells and ganglion cells in the retina of the North American opossum (*Didelphis virginiana*). *Vision Res.* **25**, 1207–1221.
- Lillywhite, H. B. and Henderson, R. W. (1993). Behavioral and functional ecology of arboreal snakes. In *Snakes: Ecology and Behavior* (ed. R. A. Seigel and J. T. Collins), pp. 1–48. San Francisco: McGraw-Hill.
- Loew, E. R. (1982). A field-portable microspectrophotometer. In *Methods in Enzymology*, vol. 81H (ed. L. Packer), pp. 647–654. New York: Academic Press.
- Loew, E. R. and Lythgoe, J. N. (1978). The ecology of cone pigments in teleost fishes. *Vision Res.* **18**, 715–722.
- Loew, E. R. and Sillman, A. J. (1993). Age-related changes in the visual pigments of the white sturgeon (*Acipenser transmontanus*). *Can. J. Zool.* **71**, 1552–1557.
- MacNichol, E. F., Jr (1986). A unifying presentation of photopigment spectra. *Vision Res.* **26**, 1543–1556.
- Mansfield, R. J. W. (1985). Primate photopigments and cone mechanisms. In *The Visual System* (ed. A. Fein and J. S. Levine), pp. 89–106. Liss, New York.
- Mayhew, T. M. and Astle, D. (1997). Photoreceptor number and outer segment disk membrane surface area in the retina of the rat: stereological data for whole organ and average photoreceptor cell. *J. Neurocytol.* **26**, 53–61.
- Mehrtens, J. M. (1987). *Living Snakes of the World in Color*. New York: Sterling.
- Mushinsky, H. R. (1987). Foraging ecology. In *Snakes: Ecology and Evolutionary Biology* (ed. R. A. Seigel, J. T. Collins and S. S. Novak), pp. 302–334. New York: Macmillan.
- Ogden, T. E. (1975). The receptor mosaic of the *Aotes trivirgatus*: distribution of rods and cones. *J. Comp. Neurol.* **163**, 193–202.
- Pettigrew, J. D., Dreher, B., Hopkins, C. S., McCall, M. J. and Brown, M. (1988). Peak density and distribution of ganglion cells in the retina of microchiropteran bats: implications for visual acuity. *Brain Behav. Evol.* **32**, 39–56.
- Rage, J.-C. (1987). Fossil history. In *Snakes: Ecology and Evolutionary Biology* (ed. R. A. Seigel, J. T. Collins and S. S. Novak), pp. 51–76. New York: Macmillan.
- Reitner, A., Sharpe, L. T. and Zrenner, E. (1991). Is colour vision possible with only rods and blue-sensitive cones? *Nature* **352**, 798–800.
- Sillman, A. J., Govardovskii, V. I., Röhlich, P., Southard, J. A. and Loew, E. R. (1997). The photoreceptors and visual pigments of the garter snake (*Thamnophis sirtalis*): a microspectrophotometric, scanning electron microscopic and immunocytochemical study. *J. Comp. Physiol. A* **181**, 89–101.
- Sillman, A. J., Ronan, S. J. and Loew, E. R. (1991). Histology and microspectrophotometry of the photoreceptors of a crocodilian, *Alligator mississippiensis*. *Proc. R. Soc. Lond. Ser. B* **243**, 93–98.
- Sillman, A. J., Spanfelner, M. D. and Loew, E. R. (1990). The photoreceptors and visual pigments in the retina of the white sturgeon, *Acipenser transmontanus*. *Can. J. Zool.* **68**, 1544–1551.
- Slip, D. J. and Shine, R. (1988a). Feeding habits of the diamond python, *Morelia s. spilota*: ambush predation by a boid snake. *J. Herpetol.* **22**, 323–330.
- Slip, D. J. and Shine, R. (1988b). The reproductive biology and mating system of diamond pythons, *Morelia spilota* (Serpentes: Boidae). *Herpetologica* **44**, 396–404.
- Stabell, U. and Stabell, B. (1994). Mechanisms of chromatic rod vision in scotopic illumination. *Vision Res.* **34**, 1019–1027.
- Steinberg, R. H., Reid, M. and Lacy, P. L. (1973). The distribution of rods and cones in the retina of the cat (*Felis domesticus*). *J. Comp. Neurol.* **148**, 229–248.
- Underwood, G. (1970). The eye. In *Biology of the Reptilia, Morphology B*, vol. 2 (ed. C. Gans), pp. 1–97. New York: Academic Press.
- Viitala, J., Korpimäki, E., Palokangas, P. and Kolvula, M. (1995). Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* **373**, 425–427.
- Walls, G. L. (1942). *The Vertebrate Eye and Its Adaptive Radiation*. Bloomfield Hills, Michigan: Cranbrook Press.
- Wyszecki, G. and Stiles, W. S. (1967). *Color Science*. New York: Wiley.