High sensitivity to short wavelengths in a lizard and implications for understanding the evolution of visual systems in lizards

Leo J. Fleishman¹, Ellis R. Loew² and Martin J. Whiting³,*

¹Department of Biological Sciences, Union College, Schenectady, NY 12308, USA
²Department of Biomedical Sciences, Cornell University, Ithaca, NY 14853, USA
³Department of Biological Sciences, Macquarie University, New South Wales 2109, Australia

Progress in developing animal communication theory is frequently constrained by a poor understanding of sensory systems. For example, while lizards have been the focus of numerous studies in visual signalling, we only have data on the spectral sensitivities of a few species clustered in two major clades (Iguania and Gekkota). Using electroretinography and microspectrophotometry, we studied the visual system of the cordylid lizard Platysaurus broadleyi because it represents an unstudied clade (Scinciformata) with respect to visual systems and because UV signals feature prominently in its social behaviour. The retina possessed four classes of single and one class of double cones. Sensitivity in the ultraviolet region (UV) was approximately three times higher than previously reported for other lizards. We found more colourless oil droplets (associated with UV-sensitive (UVS) and short wavelength-sensitive (SWS) photoreceptors), suggesting that the increased sensitivity was owing to the presence of more UVS photoreceptors. Using the Vorobyev–Osorio colour discrimination model, we demonstrated that an increase in the number of UVS photoreceptors significantly enhances a lizard’s ability to discriminate conspecific male throat colours. Visual systems in diurnal lizards appear to be broadly conserved, but data from additional clades are needed to confirm this.

Keywords: vision; pigments; colour; lizard; ultraviolet; photoreceptor

1. INTRODUCTION

Over the past several decades, comparative vision scientists have gained a better understanding of the functional and adaptive properties of colour vision systems by studying them in relation to habitat light and the important visual tasks that each species must perform. Studies on fishes and other aquatic organisms, for example, have suggested a strong relationship between habitat light and visual system features [1]. However, among terrestrial animal groups, colour vision systems are broadly conserved and ancestral properties are at least as important as ecology and visual task in visual system evolution [1–5]. Lizards are an important model system for understanding the role of visual ecology, phylogeny and behaviour on the structure of visual systems [6–9]. They are diverse in ecology and habitat and many species rely heavily on vision as their primary sensory modality. Extensive work on the phylogeny of many groups of lizards now makes it possible to relate physiological relationships to evolutionary history. Importantly, recent molecular studies have revised our understanding of higher level relationships among lizard clades [10,11], providing a new framework with which to examine the evolution of lizard visual systems.

Here, we present a study of the visual physiology and retinal anatomy of the Augrabies flat lizard, Platysaurus broadleyi (figure 1a), from South Africa. This is an extremely interesting species to study for two reasons. First, modest variations in the ultraviolet (UV)/violet throat colour of males play a major role in male–male social interactions [12,13], but nothing is known about the species’ visual perception in this wavelength range. Second, studies of lizard photoreceptors and retinal physiology are limited to a few major clades. Platysaurus broadleyi belongs to a major group of lizards whose visual system has not been studied, and thus has the potential to offer insights into visual system evolution in lizards as a whole.

In male–male interactions, P. broadleyi often use an elaborate display in which their UV/violet throat patch is prominently displayed (figure 1b). The spectral shape of the throat colour is an important predictor of the outcome of male–male fights, and altering male colours strongly influences the course of male–male contests [12,13]. A number of lizard species have been shown to exhibit UV colour patches that may be used as signals (e.g. [9,14–22]) and in a few cases, it has been shown that a high level of UV reflectance influences mate preference [23,24]. However, P. broadleyi is the only species where it has been demonstrated that modest among-individual variations in the shape of the spectral reflectance curve in the UV region have a strong influence on conspecific behaviour [13].

The ability of an animal to make fine-scale discriminations among similar colours is limited by the spectral sensitivity, and relative density of each class of photoreceptor in its retina [25,26]. We were therefore very interested in determining whether the retina of P. broadleyi exhibited any specializations for the detection and discrimination of subtle differences in UV coloration.
Platysaurus broadleyi is an extremely colourful and sexually dimorphic lizard found in the Northern Cape Province of South Africa [32]. Males rely on visual signals at a distance but also use chemical cues when in close quarters [33]. Eight adult males and eight adult females were the subject of this study. The lizards were wild-caught, but were part of a long-term (3–4 years) captive group in Johannesburg before being shipped to the USA. They were housed in large communal cages and exposed to a 12 L:12 D cycle with a combination of fluorescent, incandescent and UV-emitting mercury vapour lamps that mimicked natural sunlight. They were provided with water ad libitum and vitamin-coated crickets on a daily basis.

**2. MATERIAL AND METHODS**

(a) **Study animal and husbandry**

Platysaurus broadleyi is an extremely colourful and sexually dimorphic lizard found in the Northern Cape Province of South Africa [32]. Males rely on visual signals at a distance but also use chemical cues when in close quarters [33]. Eight adult males and eight adult females were the subject of this study. The lizards were wild-caught, but were part of a long-term (3–4 years) captive group in Johannesburg before being shipped to the USA. They were housed in large communal cages and exposed to a 12 L:12 D cycle with a combination of fluorescent, incandescent and UV-emitting mercury vapour lamps that mimicked natural sunlight. They were provided with water ad libitum and vitamin-coated crickets on a daily basis.

(b) **Electroretinography**

Prior to recording, each lizard was anaesthetized and immobilized with an injection of 0.01 mg g⁻¹ nembutal and 0.03 mg g⁻¹ tubocurarine chloride, and then respired with a small rodent respirator through a tracheal tube. After local application of 2 per cent xylcaine gel (Astra), an indifferent platinum wire electrode was inserted through a small slit in the skin of the neck. The eyelid of one eye was held open with forceps and the cornea was swabbed with a small amount of clear 2 per cent xylcaine gel. The active electrode, mounted on a three-axis micromanipulator, was then brought into contact with the eye. The active electrode consisted of a 0.5 mm diameter steel tube mounted on the end of a sheath holding two 400 µm diameter fused silica optical fibres. Light stimuli and adapting lights were delivered through this pair of fibre optics. Because of the positioning in contact with the cornea, and because light emerging from them diverges quickly, the light from the fibres could not be focused by the animal’s lens and diffusely illuminated a broad region of the retina. Light from each of the paired fibres illuminated a nearly identical region. A wire soldered to the active electrode passed the electrical response from the eye to an A-M Systems AC amplifier, where the signal was recorded differentially between the active and inactive electrodes. The signal was filtered (1 Hz high pass, 50 Hz low pass) and passed to an analogue-to-digital conversion system (Powerlab SCOPE software) and to a Macintosh computer. The output from the eye was signal-averaged 32 times. The amplitude of the ERG b-wave was used to quantify response.

One of the two optical fibres leading to the eye transmitted a constant low intensity-adapting stimulus (ca = 1 µmol m⁻² s⁻¹ irradiance measured at the surface of the eye with a Li-Cor quantum irradiance metre) from a tungsten lamp. Chromatic stimuli were delivered through the other fibre optic. Stimuli consisted of 12 ms flashes of monochromatic light, with a 1 s interval between each flash.

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Figure 1. (a) A typical male Platysaurus broadleyi form Augrabies Falls National Park. (b) A male displaying to a rival during the breeding season. Note the expanded throat which is UV-reflective and signals fighting ability.
Light for the chromatic stimulus flashes was created with a 300 W xenon arc lamp focused onto the entrance slit of monochromator, which produced a 20 nm half-intensity bandwidth stimulus. Intensity of the chromatic stimulus was controlled with a continuously variable optical density wedge. The relationship between wedge position and the intensity for each wavelength was determined in advance using an Ocean Optics USB2000 spectroradiometer (calibrated for wavelength with an Ocean Optics HG-1 mercury/argon calibration lamp; for irradiance with a Li-Cor 1800-02 irradiance/radiance standard calibration lamp).

At each test wavelength, four stimulus intensities were employed in sequence from darkest to brightest. The actual range of intensities varied depending on the wavelength, and was adjusted in order to achieve as wide a range of responses as possible. We recorded responses from stimuli ranging from 300 to 700 nm in 10 nm intervals. Prior to the presentation of each set of four intensities at a given test wavelength, the eye was stimulated with a 550 nm stimulus flash at a fixed medium intensity. The responses to the chromatic test stimuli were divided by the response to the 550 nm standard stimulus given just prior to the test stimulus set, which controlled for changes in response sensitivity of the retina over the duration of the experiment.

For each wavelength, we plotted log stimulus intensity versus b-wave amplitude and fitted the data with a straight line (least-squares linear regression). For each individual lizard, we examined the results and chose a criterion response that fell within the range of responses seen for all wavelengths. We then calculated the stimulus intensity for each wavelength that would elicit the criterion response. We plotted wavelength versus the reciprocal of stimulus intensity at criterion. We then normalized the data relative to a maximum sensitivity of 1.0. These data were plotted on a semi-log scale to produce a relative spectral sensitivity curve for each individual.

The same procedure was carried out on three individual *A. sagrei* in order to confirm that unique results observed for *P. broadleyi* were not an artefact of differences in methodology from earlier studies. Spectral sensitivity of *A. sagrei* has been measured earlier using slightly different methods (ERG flash at criterion. We then normalized the data relative to a maximum sensitivity of 1.0. These data were plotted on a semi-log scale to produce a relative spectral sensitivity curve for each individual.

The model determines the distance in perceptual space between two cover slips and transferred to the stage of the MSP microscope at 40X. The samples came from different retinal regions but we did not attempt to identify the retinal position of each sample. A supercircuits INC PC-33C colour camera was used for video imaging. Images were captured as JPG files and oil droplets were counted from these images. In order to facilitate the identification of different oil droplet classes, a program (EYEPILLOW) was used to segment images based on colour. For each image we made two counts: one starting from the bottom right and one starting from the bottom left and counts were repeated if they did not match. We did not attempt to systematically sample different regions of the retina and we cannot discount the possibility of variation across the retina in the relative abundance of different cone types. Therefore, these counts can be viewed only as an approximation of the overall abundance of oil droplets across the retina.
each cone type and its relative abundance in the retina. A distance in perceptual colour space, \( \Delta S \), is calculated in units of multiples of just noticeable difference. We assumed that colour perception is based on single cones [41], and the output from each cone type is proportional to the natural log of relative photon capture: determined by multiplying the normalized absorption spectrum by the normalized transmission spectrum of the oil droplet most commonly associated with that cell type. Methodological details and assumptions for our implementation of the model are found in the electronic supplementary material, M1.

We randomly selected pairs of throat colours (spectral reflectance) from a set of 136 males and determined the impact of changes in the relative abundance of the UVS photoreceptor on the value of \( \Delta S \) for each throat colour pair. We also determined the proportion of throat colours that could be reliably discriminated by differences in the two regions are not significantly different (paired sample \( t \)-test, \( t_5 = 3.79, p < 0.05 \)), while in A. sagrei sensitivity in the two regions are not significantly different (\( t_2 = 0.12, p > 0.05 \)).

3. RESULTS

(a) Electrotoretinography

The relative spectral sensitivity curves of the three males and three females were very similar in overall shape, but the small sample size precluded a statistical comparison. However, the range of maximum–minimum values for males and for females overlapped at all but one of the wavelengths measured (450 nm), and at this wavelength the means differed by only 0.04. We therefore combined the data for males and females. The ERG spectral sensitivity curve (figure 2a) consists of an elevated sensitivity at 360–370 nm, drops down to a local minimum at 440 nm, shows a very small peak at 450 nm and then rises smoothly to a broad plateau from 550 to 610 nm. The pattern is similar in broad outlines to that reported for a variety of other lizards [3,29] except that the UV sensitivity is considerably elevated.

In order to be certain that the high UV sensitivity was not an artefact of recording procedures (e.g. low levels of UV in our adapting stimulus), we measured spectral sensitivity of A. sagrei in our laboratory (figure 2b). This curve was consistent in shape with previous spectral sensitivity curves for A. sagrei (L. J. Fleishman 1997, unpublished data) and most other Anolis lizard curves [3].

Figure 2c shows a direct comparison of peak sensitivity of P. broadleyi and A. sagrei at a region of elevated UV sensitivity (360–370 nm) and at the SWS region (450–460 nm). These regions were chosen because they span the peak sensitivity of the UVS and SWS photoreceptors, respectively (see below). In P. broadleyi, UV sensitivity is significantly greater than SW sensitivity (paired sample \( t \)-test, \( t_5 = 3.79, p < 0.05 \)), while in A. sagrei sensitivity in the two regions are not significantly different (\( t_2 = 0.12, p > 0.05 \)).

(b) Microspectrophotometric and oil droplet measurements

The P. broadleyi retina possesses single cones with oil droplets in their inner segments and double cones that include a principal member with an oil droplet and an accessory member with a dispersed yellow pigment in its inner segment. Four classes of visual pigments were identified, which can be characterized as UVS, SWS, MWS (middle wavelength-sensitive) and LWS (long wavelength-sensitive). A representative example of each pigment is plotted in figure 3. All four were best fit by \( A_1 \) pigment templates. The pigment \( \lambda_{\text{max}} \) values (nm ± SD) were: UVS, 364 ± 1.3; SWS, 451 ± 2.1; MWS, 492 ± 2.9; and LWS single, 570 ± 2.0; LWS double, principal member, 569 ± 1.0; LWS double, accessory member, 572 ± 0.6.

Five distinct classes of oil droplets and one type of dispersed inner segment pigment were found. A representative example of four oil droplets and the dispersed pigment are plotted in figure 4. A second colourless oil droplet that exhibited low density over the entire wavelength range is not shown in the figure. Table 1 summarizes the cell types in which each oil droplet type was found.

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Figure 2. (a) Relative spectral sensitivity of P. broadleyi (n = 6). Average and standard error are shown for six individuals (three males and three females). (b) Relative spectral sensitivity of A. sagrei (n = 3) using identical procedures. Average and standard error are shown for three individuals (2 males, 1 female). (c) Average peak spectral sensitivity for broadleyi and sagrei from a UV region (360–370 nm) and from an SW region (450–460 nm) of the spectrum. Error bars are standard errors. Asterisk indicates a significant difference (pairwise t-test, \( p < 0.05 \)).
Figure 5 illustrates a light-microscopic section of the *P. broadleyi* retina when compared with a similar section from an *A. sagrei* retina. The most noticeable difference is that there appear to be more colourless oil droplets in the *P. broadleyi* sample. Table 2 summarizes the results of oil droplet counts for *P. broadleyi* along with percentages of different oil droplet classes from three species of *Anolis*. The *Anolis* samples were prepared systematically throughout each retina (excluding the temporal or central foveae) and the percentages were consistent across each retina [42]. The *P. broadleyi* data are from four nonfoveal retinal sections chosen haphazardly and it is possible that other regions of the retina might yield different results. For the samples measured, colourless oil droplets were approximately equal to 20 per cent of all oil droplets sampled. In the three *Anolis* species, colourless oil droplets represented approximately 10 per cent of the total.

(c) **Modelling throat-colour discrimination**

Figure 6 illustrates the relative quantum catch of the four single cones (with their most common oil droplet) in *P. broadleyi*, and illustrates three typical throat colour spectra roughly covering the range of observed variation. It is apparent that most of the differences in throat colour occur over the region of the spectrum where the UVS and SWS cone sensitivities overlap, and the differences over the region covered by the MWS and LWS cones are much smaller.

We were unable to accurately estimate the relative abundance of MWS and LWS single cones. However, as differences in the throat spectra mainly occur over the sensitivity range of the UVS and SWS photoreceptors, their relative abundance and spectral position largely determine the ability of *P. broadleyi* to discriminate these colours. For this reason, and because the major difference between visual sensitivity in *broadleyi* and other lizards is in the UV, in our modelling we explored the effects of altering relative UVS cone abundance. For v. 1 of our model (UVS : SWS : MWS : LWS = 1 : 1 : 1 : 1), the mean $\Delta S$ was 3.0 (s.e. = 0.19). For v. 2, with elevated UVS abundance (3 : 1 : 1 : 1), mean $\Delta S$ = 3.7 (s.e. = 0.24). For v. 3, with no UVS cone present (0 : 1 : 1 : 1), mean $\Delta S$ was 1.87 (s.e. = 0.14). The means were significantly different overall (ANOVA, $F_{2,297} = 22.4$, $p < 0.0001$), and each of the three means differed significantly (orthogonal contrasts: $p < 0.001$ for all three comparisons). The proportion of pairs with $\Delta S \geq 2.0$ were 0.63 (1 : 1 : 1 : 1), 0.71 (3 : 1 : 1 : 1) and 0.13 (0 : 1 : 1 : 1). Thus, the presence of a UVS cone, and the relatively high number of UVS cones (compared with the SWS cone for example) found in the *P. broadleyi* retina significantly increased the ability to discriminate among male throat colours.

4. **DISCUSSION**

Although *P. broadleyi* belongs to a previously unstudied major clade (Scinciformata, [11]), more closely related
Table 1. Clearly identified visual pigments found in cones of *P. broadleyi* with MSP and oil droplet types found in different cell types.

<table>
<thead>
<tr>
<th>Pigment type (cone type)</th>
<th>Number measured that met selection criteria</th>
<th>Mean $\lambda_{max}$ (s.d.)</th>
<th>Inner segment oil droplet or dispersed pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVS (single)</td>
<td>6</td>
<td>364 (1.3)</td>
<td>C2</td>
</tr>
<tr>
<td>SWS (single)</td>
<td>4</td>
<td>451 (2.1)</td>
<td>C1</td>
</tr>
<tr>
<td>MWS (single)</td>
<td>5</td>
<td>492 (2.9)</td>
<td>G</td>
</tr>
<tr>
<td>LWS (single)</td>
<td>11</td>
<td>570 (2.0)</td>
<td>Y or G</td>
</tr>
<tr>
<td>LWS (principal member of double)</td>
<td>3</td>
<td>569 (1.0)</td>
<td>Y</td>
</tr>
<tr>
<td>LWS (accessory member of double)</td>
<td>3</td>
<td>572 (0.6)</td>
<td>Dispersed pigment</td>
</tr>
</tbody>
</table>

Figure 4. Representative examples of absorption spectra from MSP recordings of four classes of inner segment oil droplets and one example of dispersed inner segment pigment (DP). Each class of oil droplet is described here, followed in parentheses by the number of recordings that met the selection criteria ($n$), the mean value for $\lambda_{mid}$ (the wavelength at which absorbance is halfway between zero and the maximum value), and the standard deviation of $\lambda_{mid}$. Names of droplet classes are based on their appearance under light microscopy and/or spectral. Oil droplet classes were: peaked Y (peaked yellow, $n = 2$, mean $\lambda_{mid} = 467$, s.d. = 2.1); Y (yellow, $n = 8$, mean $\lambda_{mid} = 476$, s.d. = 4.0); G (green, $n = 18$, mean $\lambda_{mid} = 518$, s.d. = 3.8); DP (dispersed pigment, $n = 2$, mean $\lambda_{mid} = 462$, s.d. = 10.6); C1 (colourless 1, $n = 2$, mean $\lambda_{mid} = 380$, s.d. = 0). In addition to the oil droplets and inner segment pigment plotted here, a second colourless oil droplet C2 was found that exhibited low absorption with no measurable increase across the range of wavelengths quantified.

to the Gekkota than to the Iguania, the photoreceptor make-up of its retina more closely resembles that of diurnal lizards from the Iguania than does either the nocturnal or diurnal Gekkota. This suggests that the general pattern observed here is a widespread ancestral pattern shared by diurnal lizards from multiple major lizard clades. All of these species have a combination of single cones with oil droplets in the inner segment and double cones in which the primary member contains an oil droplet and the accessory member contains a dispersed pigment. Single cones fall into the same four spectral classes: UVS, SWS, MWS and LWS, while double cones are LWS. This pattern is also similar to that described for most bird retinas, although in many species, the UVS cone is replaced with a violet-sensitive cone [4]. This supports the general conclusion that diurnal lizards share an ancestral pattern of tetrachromatic vision and that the Gekkota visual system differs because of its nocturnal ancestry, and probably does not represent a widespread pattern in the non-iguanian lizard clades. Future studies that focus on diurnal lizards from multiple clades outside the Iguania that depend less on vision and more on olfaction will be of great interest.

The ERG relative sensitivity curve arises from the summed retinal response of all photoreceptors types, weighted by their relative densities [3,29,45]. In regions of the spectrum where photoreceptor sensitivities overlap, it is difficult to discern the relative input of the different classes to the summed output. As in Anolis [7], at wavelengths greater than 470 nm, *P. broadleyi* has three cone classes that add their outputs to produce a summed ERG response. Below 470 nm, for the most part, only SWS and UV cones contribute to the response, and these have non-overlapping peaks. It is thus possible to estimate the relative density of these two cone classes in the retina from the ERG sensitivity curve. The ERG curve was determined in 10 nm intervals, and the UVS cone peaks between 360 and 370 nm, while the SWS cone peaks fell between 450 and 460 nm. We thus looked at peak values over these two wavelength ranges in order to estimate relative numbers of these two cone classes in the retina. As illustrated in figure 2b, this evidence suggests that in *P. broadleyi*, UVS cones are roughly three times as common as SWS cones, while in *A. sagrei*, the two cone types are roughly equal in abundance.

If this estimate is correct, it should be reflected in the count of colourless oil droplets. It is impossible to distinguish C1 and C2 oil droplets by visual inspection. In *A. sagrei*, we expect them to be roughly equal in numbers. If *P. broadleyi* possesses three times as many UVS cones, and therefore three times the number of C2 oil droplets, with no difference in the number of C1 oil droplets, we would expect colourless oil droplets to be twice as common in the *P. broadleyi* retina as in *A. sagrei*. This prediction is borne out (table 2). One important caveat is that the sample from *P. broadleyi* came from four retinal sections chosen haphazardly, and in some animal species there are variations in relative numbers of photoreceptors over the retina. This, however, is not the case for *Anolis*, where relative abundances of different oil droplet classes are consistent throughout the retina [42]. While we cannot be certain that our count for *P. broadleyi* is representative of the entire retina, the agreement between the colourless oil droplet count and the ERG sensitivity data provides strong circumstantial evidence that the elevated sensitivity in the UV region seen in the ERG recordings is owing to a roughly threefold increase in...
the relative abundance of UVS cones when compared with other lizard species that have been examined.

(a) The implications of having more ultraviolet-sensitive cones
Enhanced UV sensitivity might simply function to make *P. broadleyi* more sensitive (i.e. more able to detect at the limits of low light levels) to conspecific males or some other stimuli. However, the animals are found in brightly lit habitats, and it seems unlikely that absolute sensitivity to UV is an important issue. We therefore hypothesized that the apparent increase in UVS cone densities functions to enhance the ability to detect small variations in male throat colours. In order to explore this possibility, we modelled chromatic discrimination thresholds in *P. broadleyi* assuming different relative abundances of UVS cones using a variant of the model proposed in Vorobyev & Osorio [39]. We found that a threefold increase in UVS cones significantly increased the ability of the model retina to detect small differences in conspecific throat colour. This occurred because the noise in the UVS photoreceptor channel is reduced by the square root of the relative numbers of detectors contributing to each opponent channel [38,46]. It is clear that the presence of UVS cones in the retina is critical to the discrimination process, as indicated by the low number of throat colour pairs that could be discriminated reliably by a visual system consisting of only three classes of single cones (excluding the UVS cone).

The *P. broadleyi* retina is thus well adapted for the visual task of discriminating among conspecifics with subtle differences in body coloration in the short wavelength portion of the spectrum. We cannot distinguish between two possibilities that might explain the evolution of this pattern. First, elevated UVS densities may be a widespread feature of the group, which allowed *P. broadleyi* to evolve a communication system based on short wavelength variation in throat colour. Second, the communication system and visual system may have co-evolved with increased UV sensitivity evolving in concert with the evolution of the UV/violet signal. Additional species within the Cordylidae will have to be studied to distinguish among these possibilities. The strong overall similarity of the *Platysaurus* visual system with that of the distantly related Iguaninae highlights the critical importance of considering broad, conservative evolutionary patterns in the study of visual systems and visual ecology.

Experiments were carried out under Union College IACUC protocol no. 1056 and followed guidelines of Pough [47]. *Platysaurus broadleyi* were collected and shipped to
REFERENCES


