# Nocturnal colour vision in geckos

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**Nocturnal animals are said to sacrifice colour vision in favour of increased absolute sensitivity. This is true for most vertebrates that possess a dual retina with a single type of rod for colour-blind night vision and multiple types of cone for diurnal colour vision. However, among the nocturnal vertebrates, geckos are unusual because they have no rods but three cone types. Here, we show that geckos use their cones for colour vision in dim light. Two specimens of the nocturnal helmet gecko *Tarentola* (formerly *Geckonia) chazaliae* were able to discriminate blue from grey patterns by colour alone. Experiments were performed at 0.002 cd m$^{-2}$, a light intensity similar to dim moonlight. We conclude that nocturnal geckos can use cone-based colour vision at very dim light levels when humans rely on colour-blind rod vision.**

**Keywords:** colour vision; gecko; nocturnal vision; cones

## 1. INTRODUCTION

Many animals use colour as a reliable cue to detect and recognize objects such as food, hosts and mates. To have colour vision, an animal must possess at least two photo-receptor types with different absorption spectra. At night, animals usually sacrifice colour vision for enhanced sensitivity. The most important task of their eyes is to capture as many photons as possible for reliable vision. To achieve this goal, the eyes of nocturnal animals have large pupils and short focal lengths. In addition, they should sum receptor signals to improve the signal-to-noise ratio. Colour vision, by contrast, demands a comparison of signals, the opposite of summation, and thus reduces the signal-to-noise ratio, making colour vision unreliable in dim light (Vorobyev 1997). Therefore, almost all vertebrates have a dual visual system, with multiple types of cone for colour vision in bright light and a single type of rod for colour-blind, but highly sensitive, vision in dim light. Furthermore, the retinae of nocturnal species have higher rod ratios.

Most diurnal vertebrates have a cone-dominated retina with few rods, and lizards have gone to the extreme: their retinae contain only cones (Walls 1942) and provide them with excellent colour vision (Wagner 1932). Nocturnal geckos are descended from diurnal lizards and thus also have pure cone retinae, although generally without oil droplets. As an adaptation to their nocturnal lifestyle, the cones have become larger and more rod-like than those of diurnal lizards (Crescitelli 1972; Röll 2000). The eyes also have larger apertures and shorter focal lengths than those of

their diurnal relatives. This allows them to capture much of the small amount of light that is available during the night.

Despite being night active, geckos have three types of cone, sensitive to: ultraviolet (UV), blue and green light (Loew *et al.* 1996). Kröger *et al.* (1999) suggested that their lenses are probably multifocal. Multifocal optics improves the spatial resolution of colour vision, because the refracting power of the lens varies to create well-focused images on the retina for each cone type. These adaptations enhance colour vision and suggest that geckos might use colour vision even in dim light. Because nocturnal geckos have adaptations for both nocturnal vision and colour vision, we examined with behavioural methods whether the night-active helmet gecko, *Tarentola* (formerly *Geckonia) chazaliae*, has colour vision in very dim light.

## 2. METHODS

The existence of two or more spectral types of photoreceptor is not sufficient proof for colour vision. The information from the different receptor types must be preserved and compared by neurons in the retina and brain. Therefore, to be positive that an animal has colour vision, behavioural tests are necessary. A common method is to train the animal to choose between two colours, one of which can be grey. It is important to make achromatic cues, such as brightness, unreliable for the animal during such an experiment. One way of doing this is to vary the intensities of the stimuli (Jacobs 1981).

Geckos of the species *T. chazaliae* were obtained from Swedish breeders and kept in glass terraria ($70 \times 40$ cm in area and 40 cm high) with sand and several shelters, under a 12 L : 12 D cycle. During the dark phase, a light bulb provided a low light intensity of 0.002 cd m$^{-2}$ during the colour training experiment. The temperature ranged from 22–25 °C at night to 27–32 °C during the day. The geckos were fed with five to six crickets three times a week. The crickets, except for those used in the behavioural tests, were dusted in minerals and vitamins (special blend for reptiles) every other feeding. Training and testing took place in the terraria during the last 2 h of the dark phase. After behavioural tests, the geckos always received additional crickets.

Two geckos, one male and one female, were trained to associate an untreated 'tasty' cricket with the colour blue and a salted 'distasteful' cricket with grey. If the gecko snapped at the cricket that was held in the forceps decorated with the blue colour, it received the cricket as a reward. The crickets associated with grey stimuli were dipped in a saturated solution of NaCl and the geckos never ate them. Wagner (1932) used a similar method for training diurnal lizards. After every incorrect choice, one cricket was given to the gecko from the forceps with the blue colour to prevent any decline of interest. Binomial tests were performed to analyse whether choice frequencies differed significantly from chance (50%), in each single test, for each animal.

To avoid the use of achromatic cues in the experiment, the blue and grey stimuli were designed as chequered patterns, similar to those used by Osorio *et al.* (1999) for experiments on chicken colour vision, consisting of different shades of the colours (figure 1*b*). Several different patterns were used to avoid any kind of local cues for the gecko. We minimized achromatic cues by first matching blue and grey colours for the achromatic system and then adding intensity noise (see Jacobs 1981; Osorio *et al.* 1999; Kelber *et al.* 2003). We assumed that the green receptors build the basis of the achromatic system because at least 75% of the cones in the gecko retina are green-sensitive, 12–20% are blue-sensitive and less than 5% are UV-sensitive (Loew *et al.* 1996). Therefore, shades of blue and grey were matched to give the same quantum catch ($\pm 1\%$) in the green-sensitive receptors of the geckos (table 1). Eight colour pairs were matched and eight different pairs of chequered patterns were created, resulting in blue versus grey patterns similar in intensity for the green-sensitive receptor.

To account for the possibility that blue and UV receptors contribute to achromatic vision we added intensity noise. Also for that purpose, we designed 16 additional grey patterns: four grey$_{+20}$ patterns and four grey$_{+10}$ patterns that were brighter (resulting in 20 or 10% higher quantum catches in the gecko green receptor, respectively) and four grey$_{-20}$ patterns and four grey$_{-10}$ patterns that were darker (resulting in 20 or 10% lower quantum catches, respectively, in the gecko green-sensitive receptor; figure 1*c*). The four grey$_{+20}$ patterns had a higher quantum catch in the blue receptor whereas all others had equal or
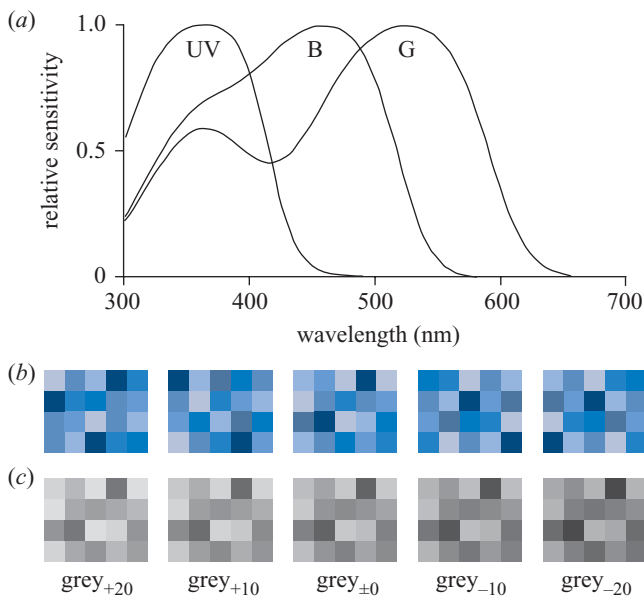
Figure 1. (*a*) Spectral sensitivities of three spectral types of cone in nocturnal geckos, in dim light. Peak sensitivities of UV, blue (B) and green-sensitive (G) cone were taken from Loew *et al.* (1996), and curves were calculated using the Stavenga–Smits–Hoenders rhodopsin template, taking into account the length of the outer segments (50 μm). (*b*) Examples of blue patterns used in the main experiment. (*c*) Examples of grey patterns of different mean intensities, with intensity decreasing from left to right. The corresponding blue pattern is situated above the grey$_{\pm 0}$ pattern.

Table 1. Relative quantum catches as calculated from equation (2.1).

(Quantum catches are shown for blue (left) and the matched grey (right) colours used in grey$_{\pm 0}$ patterns. Quantum catches in the ultraviolet ($Q_{UV}$) and blue ($Q_B$) receptors differ but those in green receptors ($Q_G$) were matched. In grey$_{+20}$ patterns, $Q_G$ was 20% higher than in grey$_{\pm 0}$ and blue patterns, and $Q_B$ was 5% higher than in blue patterns.)

| blue colour | $Q_{UV}$ | $Q_B$ | $Q_G$ | grey colour | $Q_{UV}$ | $Q_B$ | $Q_G$ |
|---|---|---|---|---|---|---|---|
| blue 1 | 12 | 135 | 280 | grey 1 | 11 | 128 | 279 |
| blue 2 | 12 | 133 | 257 | grey 2 | 11 | 117 | 255 |
| blue 3 | 12 | 113 | 203 | grey 3 | 9 | 93 | 205 |
| blue 4 | 11 | 101 | 186 | grey 4 | 9 | 85 | 184 |
| blue 5 | 11 | 98 | 159 | grey 5 | 8 | 73 | 157 |
| blue 6 | 10 | 86 | 158 | grey 6 | 8 | 73 | 157 |
| blue 7 | 9 | 79 | 143 | grey 7 | 8 | 68 | 143 |
| blue 8 | 9 | 58 | 96 | grey 8 | 7 | 46 | 93 |

lower quantum catches. One example for each group of grey patterns is shown in figure 1*b*. The cage illumination did not contain much UV light, and quantum catches in the UV receptors were generally low, so they were not considered to play a major role for achromatic vision.

The patterns were 25 × 20 mm in size, each containing 5 × 4 squares of different shades (figure 1*b*) and presented 5–10 cm in front of the gecko. All patterns were printed with coloured ink using a colour inkjet printer (Epson Stylus Photo 1270, P153A) on white copy paper.

To match the shades of blue and grey we measured the colour of the illuminating light $I(\lambda)$ and the spectral reflectances of all shades of blue and grey $S(\lambda)$ with a spectrophotometer (Ocean Optics S2000). The receptor sensitivities of *T. chazaliae* are not known but four closely related species of nocturnal geckos have been studied (Loew *et al.*
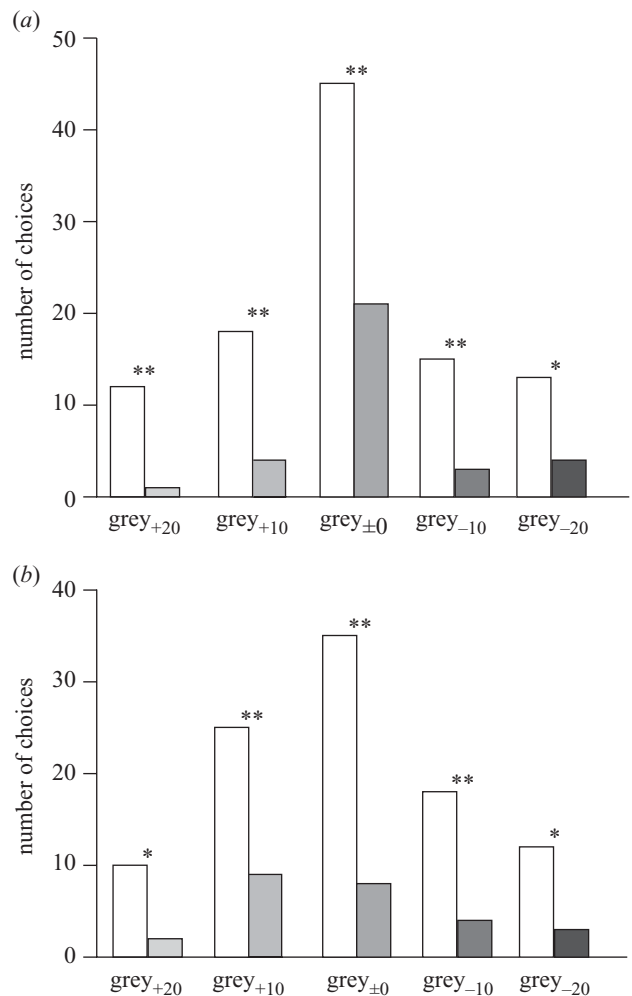
Figure 2. Choices made for the blue pattern (open bars) and the grey pattern (shaded bars) by (*a*) the male and (*b*) the female *Tarentola* (formerly *Geckonia*) *chazaliae* in the main experiment. All choice distributions differ significantly from chance. Binomial tests were used to test whether choice distributions differed from chance: $^{*}p < 0.05$, $^{**}p < 0.01$.

1996). We assumed that *T. chazaliae* has similar receptors, maximally absorbing at 365, 460 and 525 nm (figure 1*a*). The spectral sensitivity $R_i(\lambda)$ was calculated using the Stavenga–Smits–Hoenders rhodopsin template (Stavenga *et al.* 1993), and self-screening of the visual pigment in the long receptors was taken into account. The number of quanta $Q_i$ absorbed by photoreceptor type *i* when looking at a stimulus colour was then calculated using equation (2.1) (Kelber *et al.* 2002):

$$Q_i = c \int I(\lambda) S(\lambda) \left( 1 - e^{-kR_i(\lambda)l} \right) d\lambda, \qquad (2.1)$$

where *c* is a constant that depends on the absolute experimental light intensity and on the optics and physiology of the gecko eye, $I(\lambda)$ is the light intensity, $S(\lambda)$ is the reflectance of the stimulus, *k* is the absorption coefficient of vertebrate photoreceptors (0.05 μm$^{-1}$), *l* is the outer segment length (50 μm in nocturnal geckos (Röll 2000)), and $R_i(\lambda)$ is the spectral sensitivity of photoreceptor *i*.

## 3. RESULTS AND DISCUSSION

Both geckos chose the blue patterns more frequently than the grey patterns in all tests (binomial tests for each individual animal, $p < 0.05$ or $p < 0.01$; figure 2), independent of the intensity of the grey patterns. We therefore concluded that both geckos used colour to discriminate

between the two stimuli at dim moonlight intensities ($0.002$ cd m$^{-2}$) when humans are colour-blind.

Within reptiles, colour vision has been proved for some turtles and for diurnal lizards (Wagner 1932; Neumeyer 1998). Diurnal lizards have four types of cone with coloured oil droplets and thus probably tetrachromatic colour vision (Kelber *et al.* 2003). Nocturnal geckos have lost the red-senstive cone and oil droplets but they have adapted a night-active lifestyle without completely sacrificing colour vision. To our knowledge, our results provide the first behavioural proof of colour vision in a vertebrate under these dim light conditions. Geckos share this ability with nocturnal moths (Kelber *et al.* 2002). In other nocturnal vertebrates such as cats and owls (Kelber *et al.* 2003), colour vision has been proved only in bright light. However, these animals rely on colour-blind rod vision in dim light.

Obviously, as an adaptation to their nocturnal lifestyle, the eyes of geckos have become so light sensitive that photon noise does not limit colour vision in low light intensities. In addition to adaptations of the optics and receptor physiology, geckos might use neural pooling mechanisms, as has been proposed for nocturnal moths (Kelber *et al.* 2002). The colour signal results from a comparison of cone signals and thus is more robust to changes in the illumination than vision based on single cone signals (Kelber *et al.* 2003). Colour vision improves discrimination in patchy illumination and might therefore be useful for the nocturnal geckos when they search for well-camouflaged food, for partners, or when they navigate using landmarks.

Other nocturnal vertebrates that might be able to discriminate colours in dim light include toads and frogs. Instead of having a pure cone retina like the lizards, they have two types of rod (Liebman & Entine 1968) that might be used for colour vision at night.

Our results show that nocturnal geckos are able to discriminate colours in very dim light. Exactly what they use this ability for and precisely how well they see colour, remains to be investigated. Geckos are only the second animal group after a nocturnal hawkmoth (Kelber *et al.* 2002) that have been proved to see colour when we are colour-blind. However, this remarkable ability may be more common than we thought.

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