The pupils and optical systems of gecko eyes

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The nocturnal helmet gecko, *Tarentola chazaliae,* discriminates colors in dim moonlight when humans are color blind. The sensitivity of the helmet gecko eye has been calculated to be 350 times higher than human cone vision at the color vision threshold. The optics and the large cones of the gecko are important reasons why they can use color vision at low light intensities. Using photorefractometry and an adapted laboratory Hartmann–Shack wavefront sensor of high resolution, we also show that the optical system of the helmet gecko has distinct concentric zones of different refractive powers, a so-called multifocal optical system. The intraspecific variation is large but in most of the individuals studied the zones differed by 15 diopters. This is of the same magnitude as needed to focus light of the wavelength range to which gecko photoreceptors are most sensitive. We compare the optical system of the helmet gecko to that of the diurnal day gecko, *Phelsuma madagascariensis grandis*. The optical system of the day gecko shows no signs of distinct concentric zones and is thereby monofocal.

Introduction

During the evolution of the diurnal lizards, their eyes have lost the typical vertebrate duplex retina with both rods and cones and are instead left only with different types of single and double cones (Röll, 2000; Underwood, 1970; Walls, 1942). However, at some point in evolution a group of lizards, the geckos, turned to a nocturnal lifestyle. In response to the demands of nocturnal vision without rods, the cones of nocturnal geckos have become much larger and more light-sensitive than those of their diurnal relatives (Röll, 2000). Nocturnal geckos have retained three different photopigments sensitive to UV, blue, and green (Loew, 1994) and their eyes are sensitive enough to obtain color information at night (Roth & Kelber, 2004). At intensities corresponding to dim moonlight (0.002 cd m^{-2}), the nocturnal helmet geckos, *Tarentola chazaliae,* could discriminate colors in a behavioral dual choice experiment. At these dim light intensities, their pupils are round and fully opened. In this study, we investigated the pupil, the dimensions of the eye, and the cone dimensions of helmet geckos and used these data to calculate the optical sensitivity with the aim of understanding the adaptations that allow the animals to see colors under dim light conditions.

Eyes adapted for vision at night, such as the eyes of nocturnal geckos, with a large pupil and a short posterior nodal distance (here also called focal length, f), are especially affected by longitudinal chromatic aberration. As a result, light of short wavelengths is refracted more strongly and thus focused closer to the lens than light of long wavelengths. If this is not corrected for in an eye adapted for nocturnal vision, the retinal image is severely blurred. Multifocal optical systems with distinct concentric zones of different refractive powers have been suggested to correct for some of the defocus on the retina caused by chromatic aberration (Kröger, Campbell, Fernald, & Wagner, 1999). Kröger et al. have shown that the eyes of the nocturnal gecko, *Homopholis wahlbergi,* have multifocal optical system. We were interested to know whether the differences between zones of different refractive power match the range of wavelengths the nocturnal geckos are sensitive to.

In addition, the light-adapted pupils in nocturnal geckos are different variations of vertical slit pupils. Apart from the effectiveness in shutting out light during the day, the mode of constriction of slit pupils has been suggested to

be of advantage in multifocal eyes, since it allows for all refractive zones of the optical system to be functional at all states of pupil constriction (Kröger et al., 1999; Malmström & Kröger, 2006). We investigated the pupil dynamics and the multifocal optical system of helmet geckos to see whether the light-adapted pupil allows for all concentric zones of the optical system to refract incoming light.

Some geckos reverted again to a diurnal lifestyle. As a result, the cones of diurnal geckos are small (Röll, 2000) since large photoreceptors are costly and not necessary when light is abundant. In addition, diurnal geckos have small circular pupils and small eyes relatively to body size (Werner, 1969). Since their pupils are small relative to the focal lengths of their eyes, they are less affected by chromatic aberration. Accordingly, previous photorefractometric results show monofocal optical systems in the day gecko, *Phelsuma madagascariensis* (Kröger et al., 1999). We compared the optical system of the day gecko, *Phelsuma madagascariensis grandis,* to that of the nocturnal helmet gecko to determine the differences between both species.

One fast method to investigate the optical state of camera-type eyes is photorefractometry. The method makes it possible to study the refractive power in the eyes of living non-cooperative animals from some distance (Schaeffel, Farkas, & Howland, 1987). However, quantitative measurements on the eyes of terrestrial vertebrates are complicated. The refractive power of the cornea is too high to be ignored and lens measurements alone are not sufficient to describe the optical system.

The Hartmann–Shack wavefront sensor has been the most common in-vivo measurement method for ocular wavefront aberrations in human eyes during the last 15 years (Liang, Grimm, Goelz, & Bille, 1994). We have developed a Hartmann–Shack wavefront sensor to obtain quantitative results from gecko optical systems. To allow studies on small eyes, this sensor has a higher resolution than most sensors used on human eyes. However, a common problem occurring in studies of unharmed animals, i.e., limited control of focus and gaze direction (Harmening, Vobig, Walter, & Wagner, 2007; Huxlin, Yoon, Nagy, Porter, & Williams, 2004), might still affect the wavefront analysis.

Material and methods

Animals

We studied two day geckos, *Phelsuma madagascariensis grandis* (Figure 1a, denoted by DG1 and DG2), and eight nocturnal helmet geckos, *Tarentola chazaliae* (Figure 1b, denoted by NG1–NG8). Most day geckos are endemic to Madagascar and the day geckos used in this
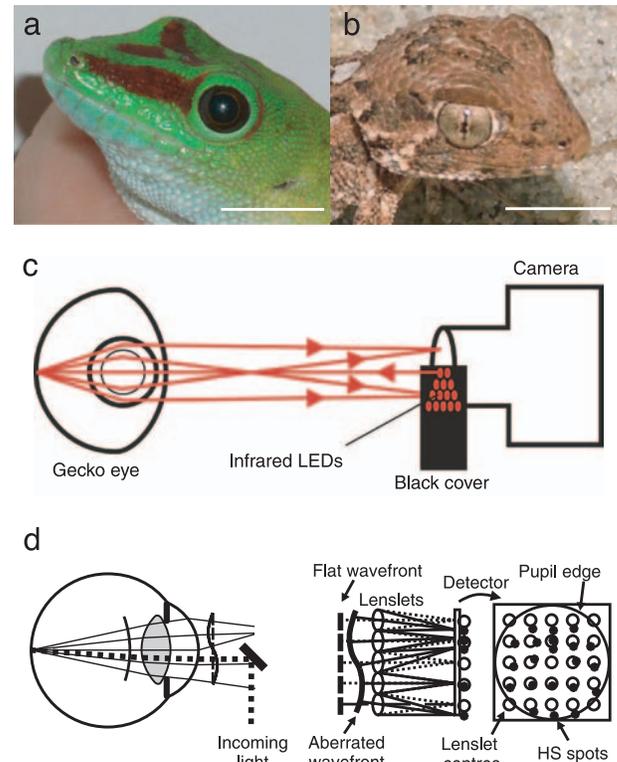

Figure 1. (a) Two day geckos, *Phelsuma madagascariensis grandis,* and (b) eight nocturnal helmet geckos, *Tarentola chazaliae,* were used in our study. Scale bars, 1 cm. (c) The photorefractometry method was used to qualitatively determine the optical system of the gecko eye. (d) In order to perform quantitative measurements, we developed an adapted Hartmann–Shack wavefront sensor (for details, see text).

study were borrowed from a respected pet shop, Tropikhuset in Malmö, Sweden, and from a zoo, Tropikariet in Helsingborg, Sweden. The helmet geckos are night-active and inhabit the coast of Morocco (Henkel & Schmidt, 2003). The helmet geckos for this study were obtained from different Swedish breeders and kept in glass terraria (minimum ground area of 60×40 cm and 40 cm high) with sand, water, and several shelters, under a 12L:12D cycle. All experiments have been approved by the Swedish animal welfare agency (M160-04).

During photorefractometry and Hartmann–Shack experiments, the helmet geckos were restrained in a box, with two openings in the front for ocular measurements. The two day geckos were hand-held by an assistant because of their slender body shape. All measurements were performed in darkness on both eyes of the awake, unharmed geckos. Measurements on the helmet geckos were performed during late subjective night. There was no difference in the results when we did the same experiments during subjective daytime. The day geckos were examined during their normal daytime but were kept in darkness for at least 30 min before the measurements to achieve as large pupils as possible.

Pupil dynamics

The pupil sizes of the helmet gecko (NG6) and two helmet geckos that were not used in the other experiments (NG7 and NG8) were measured under different illuminations. Pictures were taken with a digital camera (Sony, DSC-F707) and the diameter, A, and areas of the pupils were determined with the program ImageJ 1.33.

Optical sensitivity of the helmet gecko eye

Three helmet geckos (NG2, NG3, NG6) were used to obtain the eye dimensions. Directly after decapitation, the heads of the geckos were frozen before they were sectioned horizontally in a cryostat. Pictures were taken every 70 μm to find the largest eye dimensions for the calculation of the posterior nodal distance (f).

Pieces of retina from another helmet gecko were fixated for transmission electron microscopy with 2% paraformaldehyde, 2% glutaraldehyde in cacodylate buffer overnight followed by post fixation in 1% osmium tetroxide, dehydration, and embedding in Epon. From the histological sections, we obtained the diameter d and the length l of the cone outer segments.

For the calculations on the optical sensitivity for white light, S_w, of helmet geckos we used the measurements on pupil diameter (A), posterior nodal distance (f), and cone length (l) and width (d) (Equation 1; Land, 1981; Land & Nilsson, 2002). The optical sensitivity S_w gives the number of photons absorbed by a photoreceptor when looking at an extended source of white light:

$$S_w = (\pi/4)^2 A^2 (d/f)^2 (kl/(2.3 + kl)), \tag{1}$$

where k is the absorption coefficient of the receptor. A typical vertebrate cone absorption coefficient k of 0.035 was taken from Warrant and Nilsson (1998).

Photorefractometry

To qualitatively examine whether the gecko eyes have multifocal optical systems, photorefractometric pictures were taken (Malkki & Kröger, 2005; Schaeffel et al., 1987). In short, infrared light illuminates the eye and the outgoing light reflected from the retina is focused by the eye's optical system and recorded by a video camera (Figure 1c; Sony, DCR-TRV 730E). Typical frames were extracted from short video sequences with computer and Adobe Premiere 6.0 software. Since the lower half of the camera objective was covered, only light from myopic parts of the lower half of the pupil (light focused in front of the camera) entered the camera and generated a bright zone. Light from myopic parts of the upper half of the pupil was blocked by the cover in front of the camera that

the region thus appeared dark (Figure 1c). Bright and dark rings in the photometric images of the pupils thereby qualitatively indicate distinct zones of different refractive powers in the optical system of the eye, with bright rings in the upper half being hyperopic relative to dark rings.

Hartmann–Shack wavefront sensor

For measurements with a Hartmann–Shack wavefront sensor, one sends a narrow beam of monochromatic light into the eye and measures the wavefront of the reflected light as it propagates back from the retina through the optical system of the eye (Figure 1d). The wavefront that exits the eye is a surface perpendicular to the rays. The shape of the wavefront describes the total optical system of the eye with respect to the monochromatic light coming in parallel to the optical axis. The wavefront should ideally be flat in a monofocal, emmetropic eye but is deformed in the presence of optical errors, and the deformations indicate the relative optical path length differences (Figures 2a and 2b).

A Hartmann–Shack sensor measures the shape of this wavefront by an array of small lenses (often called lenslet array) where each lenslet focuses a part of the wavefront into a spot on the detector. The displacement of the spot relative to the optical axis of the lenslet is proportional to the tilt of this part of the wavefront. For thorough descriptions, see Atchinson (2005), Liang et al. (1994), Lundström (2007), and Prieto, Vargas-Martín, Goelz, and Artal (2000).

To assess the optical properties of the small gecko eyes in detail, a high-resolution Hartmann–Shack sensor was specially developed at the Royal Institute of Technology in Stockholm, Sweden. This sensor is described in detail in Buschbeck (2007) and Manneberg (2005). It uses 100 × 100 lenslets of 250 × 250 μm, with focal lengths of 18 mm. The entrance pupil of the eye is imaged onto the array with a magnification of 6.25, which means that the wavefront over a pupil of 3 mm in diameter is sampled with more than 4400 spots (Figures 2c and 2d).

The spot pattern is captured with a large-chip CCD (Pantera TF6M8 from Dalsa, 3072 × 2048 pixels of 12 × 12 μm). A pupil camera, together with infrared illumination, was used to place the eye in the correct location as well as to align the direction of gaze of the gecko to the measurement axis of the sensor. The light source was a fiber-coupled laser diode of 655-nm wavelength, which is outside the sensitivity spectra of all gecko photoreceptors (Crescitelli, Dartnall, & Loew, 1977). The tip of the fiber was imaged onto the pupil of the eye via a beamsplitter and gave the light beam a diameter of 1 mm and a power of approximately 15 μW upon entrance (the exposure time was 300 ms). This is well below the safety limits for human eyes and the geckos showed no signs of discomfort during the measurements.
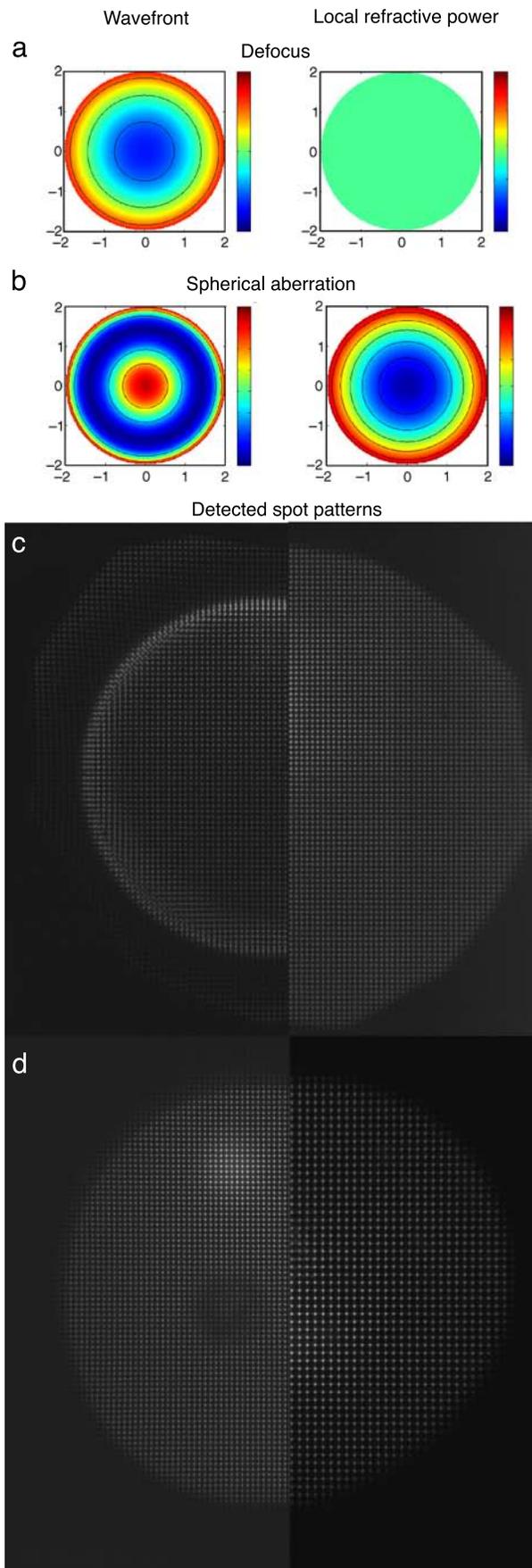
During measurements, the geckos DG1, DG2, and NG1–NG6 were placed in the focal plane of the first lens, approximately 10 mm from the aperture of the sensor. From each gecko eye (left and right), three measured spot patterns of good quality and with a gaze direction as well aligned as possible to the sensor were analyzed: the centers of the individual spots were defined by the center of gravity of the luminance distribution, a custom-written unwrapping program was used to connect each spot to the corresponding lenslet (Lundström & Unsbo, 2004), and the derivatives of Zernike polynomials up to the order of 19 were fitted to the local slopes of the wavefront with a least-squares method (American National Standard Institute, 2004).

The polynomials were fitted over a circular area with a diameter equal to the largest extent of the spot pattern and the slightly irregular shape of the pupil was then considered during the evaluation of the wavefront error.

The variation of the refractive power over the pupil was then calculated from the reconstructed wavefront. As can be seen in Figures 2a and 2b, if an eye only has a spherical refractive error (defocus) the refractive power will be constant over the pupil, whereas other optical errors will give a variation, e.g., positive spherical aberration means that the local refractive power grows larger toward the edges of the pupil. The local refractive power map is a useful tool for estimating the power of different zones in a multifocal optical system where the difference between zones is an indication of the amount of chromatic aberrations that the eye can compensate for.

Results

Pupil dynamics in the helmet gecko

At dim light intensities, the pupils of helmet geckos are round and fully opened. As the light intensity increases, the pupils constrict and change shape from round to two pairs of pinholes in a vertical line (Figure 3). The highly mobile pupil allows the pupil area to change by a factor of 100–150 in the helmet gecko, compared to 300 in the much larger nocturnal Tokay gecko, *Gekko gecko* (Denton, 1956) and only 16 in humans. During the rapid changes of light intensities at sunrise and sunset in Morocco (data

Figure 2. Examples of wavefronts and the corresponding local refractive power maps for the ideal cases of (a) pure defocus and (b) spherical aberration. (c) The measured spot pattern from two helmet geckos; NG6 (left half) and NG5 (right half), where NG6 turned out to differ from all the other geckos by having extreme transitions between zones of different refractive power. (d) The measured spot pattern from the two day geckos, where the left and right halves are consisting of DG1 and DG2, respectively. Between spots, it is approximately 40 μm.
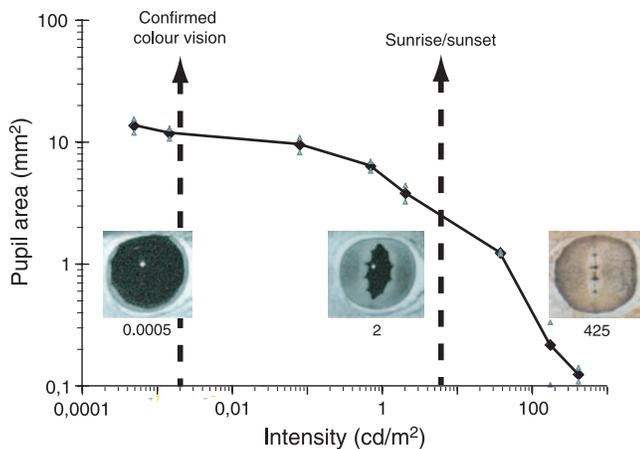
Figure 3. The pupil area and standard deviation of three nocturnal helmet geckos in different light intensities. The three pictures within the graph show the pupil sizes at certain light intensities, given in candela per square meter below each picture.

not shown), the transition from the multiple-pinhole pupil to the fully round pupil takes place within an hour. Since the helmet gecko is active mainly at night, it hunts and uses vision when the pupil is fully opened.

Optical sensitivity

Applying Gullstrand's model (for calculations, see Land & Nilsson, 2002), posterior nodal distances for three nocturnal helmet geckos were calculated. As the refractive index of the aqueous and vitreous humors, 1.336 was used (Citron & Pinto, 1973). In Gullstrand's simplified eye model, only front radius and back radius of the lens are included. However, since a multifocal optical system consists of several distinct zones of different refractive

power this model underestimates the total refractive power of the eye. Therefore, we assume that the animals have a focused image on the retina and used a refractive index of 1.58 mm ± 0.016 mm for the optical system of the three geckos. The average posterior nodal distance was calculated to be 3.5 mm ± 0.1 mm in standard deviation (Figure 4).

Calculations of the optical sensitivity, S_w, give us the relative numbers of photons absorbed by a cone looking at an extended light source (Equation 1; Table 1; Warrant & Nilsson, 1998). Even though signal summation is ignored in this calculation, the helmet gecko eye is 350 times more light-sensitive than the human eye at intensities at which both discriminate colors. At dim moonlight intensities when the helmet gecko has been confirmed to discriminate colors (0.002 cd m^{-2}; Roth & Kelber, 2004), an S_w of 28 μm^2 sr is obtained. For humans at color vision threshold (0.02 cd m^{-2}; Roth, Balkenius, & Kelber, 2008) an S_w of not more than 0.08 μm^2 sr is achieved. The optical sensitivity of single cones alone can thereby explain why nocturnal geckos are able to see colors in much dimmer light than humans.

Photorefractometry

Photorefractometric images of eyes of both day geckos examined (of which DG1 is presented in Figure 5a) suggest monofocality since no distinct concentric zones of different refractive powers are visible. This is in agreement with the photorefractometric results on a closely related species of day gecko studied by Kröger et al. (1999). All nocturnal helmet geckos in our study show multifocality, but the variation between animals is large. The variation stretches from multifocal optical systems with very strong refractive power transitions to very weak transitions. The eye of NG6, which shows an extremely
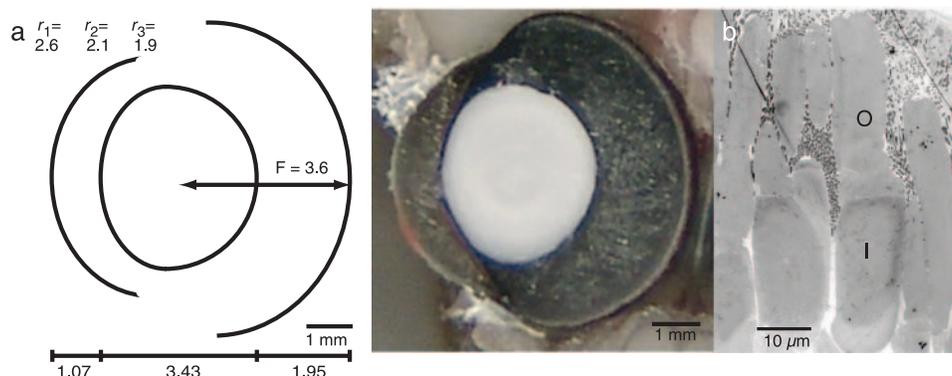

Figure 4. (a) Horizontally sectioned helmet gecko eye. Assuming a well-focused eye and using Gullstrand's model, calculation yields a post nodal distance of 3.6 mm for this specimen and a mean value of 3.5 mm ± 0.1 mm for all three helmet geckos examined. Radii of the cornea as well as the anterior and posterior lens surfaces are shown as r_1, r_2, and r_3, respectively. Scale bars, 1 mm. (b) Transmission electron micrograph image of cones in the retina of the helmet gecko with inner (I) and outer (O) segments, where the outer segments measure 30–40 μm in length and approximately 10 μm in width. Scale bar, 10 μm.

Confirmed color vision	Helmet gecko (0.002 cd m^{-2})[A]	Human (0.02 cd m^{-2})[B]	Tokay gecko
Absorption coefficient, k	0.035[C]	0.035[C]	0.035[C]
Pupil diameter, A (μm)	3900 ± 200	7000[E]	6000[G]
Focal length, f (μm)	3500 ± 100	16700[C]	6500[G]
Cone diameter, d (μm)	10 ± 2[D]	1.5[E]	10
Length of cone outer segment, l (μm)	37 ± 5[D]	30[F]	39[H]
Optical sensitivity for white light, S_w (μm^2 sr)	28	0.08	20

Table 1. The optical sensitivity, S_w in dim light was calculated using Equation 1. The mean values and standard deviations for the three helmet geckos are shown. The eye of the helmet gecko is 350 times more light-sensitive than that of humans at intensities when each of them discriminate colors. When assuming a cone diameter of 10 μm, the nocturnal Tokay gecko (*Gekko gecko*) has an S_w value of the same magnitude as the helmet gecko. *Note*: [A]Roth and Kelber (2004); [B]Roth et al. (2008); [C]Warrant and Nilsson (1998); [D]Mean value ± standard deviation of the double cones (Figure 4); [E]Wyszecki and Stiles (1982), an average of the human cones diameter in the fovea; [F]Land (1981); [G]Citron and Pinto (1973); [H]Dunn (1969).

sharp transition between zones, is shown in Figures 2c and 7. The transitions between zones of different refractive powers are indicated by broken lines in the left halves of the photorefractometric pictures (Figure 5, left column). The eyes of four other helmet geckos are presented in Figure 5 to show the variation between the animals. The eyes of NG2 are not shown but are similar to those of his sibling, NG5, which shows weak multifocality (Figure 5c). These two were also the youngest geckos used in this study (6 months at the time of measurements), which possibly contributes to the similarity in results. In all helmet geckos, the upper half of the outer zone of the optical system appears bright, indicating that it has less refractive power, i.e., it is hyperopic relative to the inner zone.

Hartmann–Shack wavefront measurements

The graphs marked as "wavefront" in Figure 5 show maps over the difference in optical path lengths for the rays passing through different parts of the pupil. If the optical system of an eye was perfect, without refractive errors and aberrations, the wavefront would be a flat surface indicating that all rays are focused perfectly on the retina. Here, the wavefronts are plotted after removing defocus (Figure 2a) and astigmatism to more clearly show the higher order aberrations, such as spherical aberration (Figure 2b) and coma. In the rightmost column of Figure 5, marked as "local refractive power," the refractive errors are included and these maps show the refractive powers of the eyes in diopters at the different locations within the pupil. Zero diopters, indicated in orange, means that the rays in this area are focused on the retina (i.e., no refractive error, the power of the optics matches the length of the eye), positive powers mean that the rays are focused in front of the retina (myopia), and negative powers denote rays focused behind the retina (hyperopia). These dioptric values are calculated from the wavefronts assuming that the center of the pupil is coinciding with the optical axis of the eye, which will give a small irregularity

in the middle of the graphs especially if the measurement was performed in a slightly oblique angle (i.e., the gecko was not looking straight into the sensor).

When looking at results presented in Figure 5, it should be kept in mind both that the values are given for the measured wavelength (655 nm) and that the level of accommodation was not controlled; if another wavelength was considered or if the gecko was accommodating during the measurements this would probably shift the local refractive powers. Therefore, only relative refractive powers will be discussed in the following sections.

The distribution of the refractive power over the pupil of the two diurnal geckos DG1 and DG2 is compared to the distribution of the nocturnal geckos NG1–NG5 in Figure 6. For this graph, the average refractive power was calculated over eight annular zones of the pupil. The center of these zones (marked as black dots in the local refractive power graphs of Figure 5) was located manually in each image, because the gaze direction of the geckos might not have been aligned with the axis of the sensor. The size of the zones is given relative to the radius of the full pupil, i.e., the first zone stretches from a radial distance of 0.1 to 0.2 of the full pupil radius, the second zone is from 0.2 to 0.3 of the full radius and so on out to the eighth zone from 0.8 to 0.9. To facilitate comparison in between animals the value of the first zone is always set to zero diopters. Each data point is the average of three measurements in the left eye of a gecko, with the error bars indicating the standard deviation. The results for the right eyes are not shown but were similar. The nocturnal helmet gecko NG6 was also studied with the wavefront sensor but showed such an abrupt change in the wavefront in both eyes that it could not be reliably quantified (Figure 2c).

The wavefront maps in Figure 5 show that the total amount of aberrations is similar in both species of geckos examined; the root-mean-square error (standard deviation) of the wavefront for the higher order aberrations was (mean value ± standard deviation over a 2-mm pupil) 0.24 ± 0.06 μm for the nocturnal geckos and 0.27 ± 0.02 μm for the diurnal geckos. A comparison of the calculated Zernike coefficients showed no significant differences,
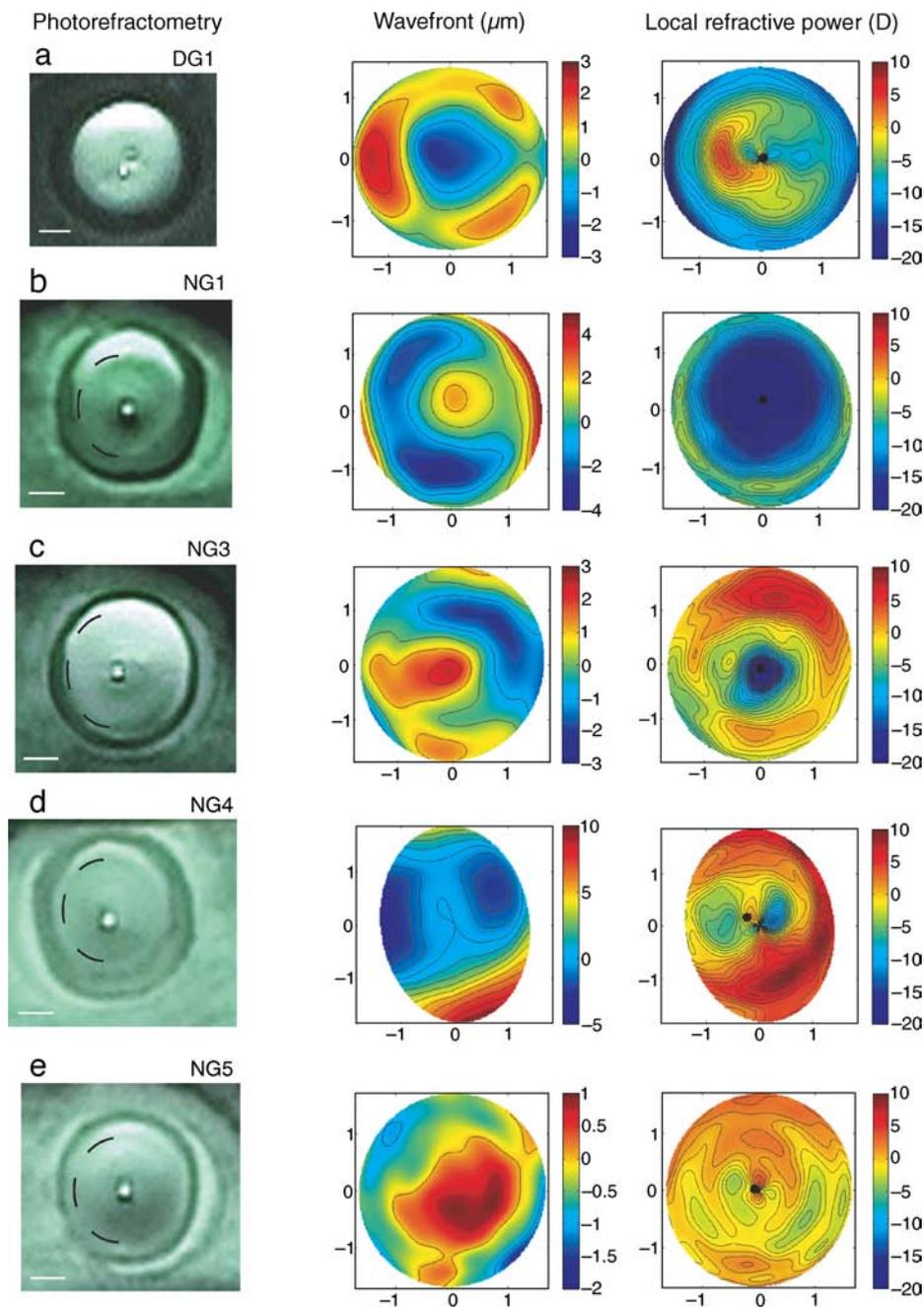
Figure 5. Results of the two optical methods used in this study. Photorefractometric pictures (left column) of (a) one day gecko and (b–e) four helmet geckos. Scale bar, 1 mm. In the helmet gecko pictures, the broken lines indicate the concentric zones with distinct refractive power transitions suggesting multifocality. DG1 (a) shows no such concentric zones, which suggests a monofocal optical system. The wavefront graphs (middle column) show the change in optical path lengths for light passing through different parts of the pupil (scale, micrometer). The maps of local refractive powers (right column) show how the refractive power changes over the pupil (scale, diopter). The black dots indicate the determined optical center. Two evident differences between the species are that in the (a) day geckos the refractive power decreases monotonically toward the outer parts of the pupil, while the refractive power in the (b–e) helmet geckos are increasing, and not in a monotonic manner. Blue color indicates low refractive power in the region and red color indicates high refractive power. Values at the axes indicate pupil sizes (in millimeters). See text for further explanation.
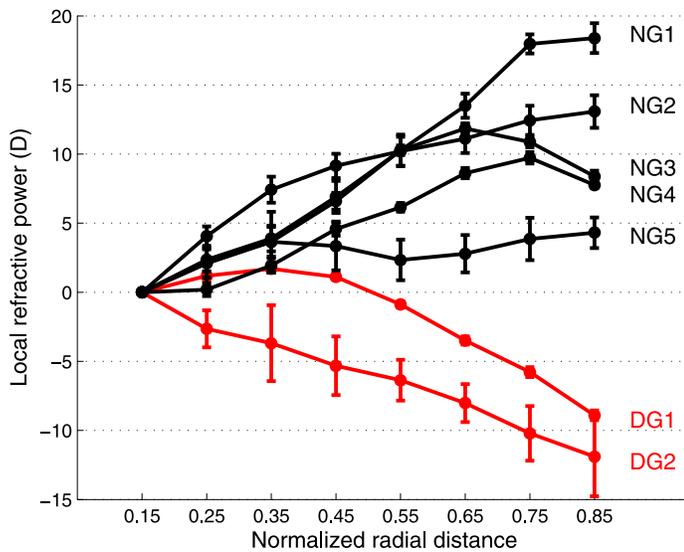
Figure 6. Radial profiles of the distribution of the refractive power over the pupil of diurnal (red) and nocturnal geckos (black). The change in refractive power is given relative to the center of the pupil (set to zero diopters). The dots and the error bars show the average values and the standard deviations of three measurements in the left eyes. See text for further explanation.

except for spherical aberration, which was positive, 0.11 ± 0.07 μm, for the nocturnal geckos and negative, -0.10 ± 0.06 μm, for the diurnal geckos (values for 2-mm pupil). This can also be seen in the local refractive power maps of Figure 5 and in the average profiles of Figure 6; the local refractive power in the diurnal gecko system decreases toward the edges of the pupil, whereas the nocturnal geckos have higher power in the outer parts of the pupil.

The wavefront results presented in Figures 5 and 6 confirm the observations made with photorefractometry (note that the size of the pupil is not the same in the left- and rightmost column of Figure 5 and the transitions in refractive power might therefore appear to be located differently). No distinct zones can be seen in the local refractive power of the diurnal geckos; both DG1 and DG2 lacked sharp optical changes and the refractive power decreased monotonically toward the edge of the pupil, thus confirming a monofocal optical system. This is in contrast to all measured nocturnal helmet geckos, which have a refractive power that increases from the center to the periphery of the pupil. We would in particular like to emphasize that this change is not monotonic as expected in a monofocal system. Instead, Figure 6 shows that the refractive power profile flattens toward the edge of the pupil. Additionally, ring-like zones with different powers can be seen both in the local refractive power maps of Figures 5b–5e and as "bumps" in the profiles of the nocturnal geckos of Figure 6, most clearly in NG3–5.

As can be seen in Figure 6, the results vary in between the individual geckos, which probably is a combination of

intraspecific variation and variation in viewing angle. In an attempt to estimate the multifocality, i.e., the difference in refractive power between different zones (which are not always exactly coinciding with the annular zones used in Figure 6), the six local refractive power maps of each helmet gecko were compared: The optical system of NG1 (Figure 5b) has an inner zone with one surrounding ring-shaped zone, and the difference in refractive power of the zones is approximately 15 diopters. This is similar to the eyes of NG3 and NG4 (Figures 5c and 5d). NG2 has a similar appearance with a somewhat smaller difference of 10 diopters. NG5 (Figure 5e) on the other hand has no clear inner zone but two concentric ring-shaped zones of similar powers are visible with a weak difference of approximately 5 diopters. This weaker multifocality for NG2 and NG5 is in agreement with the results from the photorefractometry and might be an effect of their young age. In general there seems to be a difference of approximately 15 diopters between the refractive zones in the eyes of adult helmet geckos.

Discussion

In this study, we found that the optical systems of helmet geckos make their eyes very light-sensitive, which probably is the reason why they can afford to have color vision at night. We studied the optical differences between two species of geckos, to qualitatively and quantitatively describe the monofocal eyes of day geckos and the multifocal eye of helmet geckos. In order to do so, we developed an adapted Hartmann–Shack wavefront sensor. Studies of animals with relatively large eyes, such as owls and cats, have included surgery and fixation of the head (Harmening et al., 2007; Huxlin et al., 2004). In this study, we demonstrate that it is possible to obtain high-resolution wavefront measurements of small, unharmed gecko eyes without completely controlling the gaze or the accommodation of the animal eyes.

The eyes of day geckos have monofocal optical systems. Since their optical systems are adapted to day activity including long focal lengths and small pupils, day geckos are subjected to little blur from chromatic aberration and would thus not benefit from multifocality. The eyes of all nocturnal helmet geckos examined showed multifocal optical systems with distinct concentric zones of different refractive powers even though the intraspecific variation was large. Large variation between individuals has been observed earlier in Hartmann–Shack studies on humans (Atchison, 2005; Thibos, Hong, Bradley, & Cheng, 2002). In our case, it might also be a result of caring conditions.

There are only minor insignificant variations present when comparing repeated measurements of the same eye or the differences between left and right eyes of the same
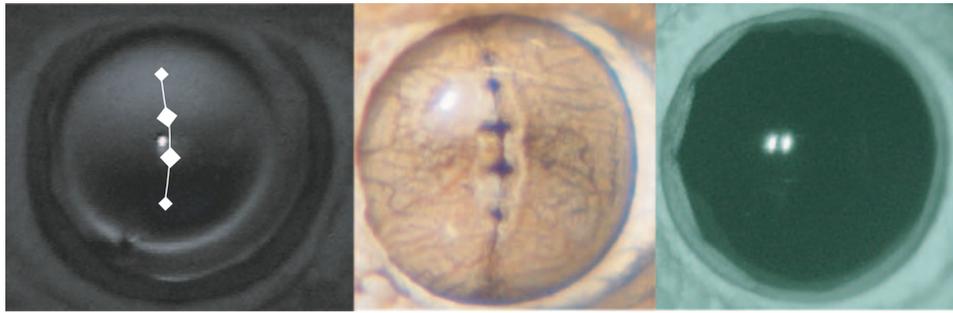
Figure 7. Photorefractometric image (left) of the eye of NG6 with the light-adapted pupil indicated by white lines and dots. The light-adapted (middle) and dark-adapted (right) pupils of the eyes of NG6 are shown for comparison.

gecko. In addition, the results of the Hartmann–Shack detector correlate well with the results from photorefractometry, which allows us to have good confidence in both optical methods used in this study.

The advantage of multifocal optical systems in night-active animals with semi- or fully opened pupils is that light of different ranges of wavelengths could be focused simultaneously on the retina. Our results show at least two zones of different refractive powers in nocturnal geckos with 15 diopter difference. This difference correlates well with the photopigments and spectral visual range of the geckos. A majority of the cones in nocturnal geckos are most sensitive to short (452–470 nm) and long wavelengths (520–533 nm), respectively, and only a small fraction is sensitive to very short wavelengths (363–366 nm; Crescitelli et al., 1977; Loew, Govardovskii, Röhlich, & Szél, 1996). With a posterior nodal distance of 3.5 mm the helmet geckos would need a difference of approximately 25 diopters to be able to focus their whole visual spectrum 300–600 nm, and only around 13 diopters for the range of their visual pigments' maximum sensitivity, assuming a reduced eye model and eye media with refractive index similar to that of water. Hence, the changes in refractive powers in the multifocal eyes of helmet geckos are within the expected magnitude for eyes with adaptations to focus different ranges of wavelengths on the retina.

Another possible advantage of multifocal optical systems could be that the concentric zones in a multifocal eye focus objects at different distances. The multifocal eye would thereby generate a sharp image for at least two different depths, which would otherwise be impossible if one considers the morphological dimensions and retinal organization. Ultimately, having more experimental data from the same species and also on closely related species with different activity patterns could generate a better understanding of the significance of multifocality for image formation on the retina.

We also investigated whether the pupil shape of the helmet geckos is related to their optical system. In NG6 the openings of the pupil at maximum constriction all fall within the inner refractive zone of the optical system (Figure 7). We can therefore not confirm the hypothesis that the concentric zones in the multifocal optical systems match in location with the two pairs of pupils in the helmet geckos (Kröger et al., 1999). The function of the multiple-pinhole pupil remains a mystery, partly because helmet geckos are not normally active at daytime when their pupils close to four openings. Murphy and Howland (1986) suggested the multiple-pinhole pupil to be a mean for the geckos to estimate distances in bright light. An object not focused properly on the retina appears quadrupled, while an object in focus appears single. The light-adapted pupil could also, in addition to the ability to effectively shut out light and protect the light-sensitive retina, function as camouflage, as suggested already by Cott (1940). A round pupil is more conspicuous and attracts possible predators more than the irregular shape of a multiple-pinhole pupil. During the day when the helmet geckos are basking the light-adapted pupil and irregular pattern of the iris, being color-matched to the body might help them stay hidden from birds and other predators.

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