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Diversity and Limits of Colour Vision in Terrestrial Vertebrates

Lina S. V. Roth

Doctoral Thesis
Lund, November 2008

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Academic thesis in fulfilment of the degree of Doctor of Philosophy at the Faculty of Science at Lund University. The thesis defence will take place in the Zoology Building, Helgonavägen 3, Lund, Sweden at 10.00 am, November 7, 2008. Faculty opponent: Natalie Hempel de Ibarra, University of Exeter, United Kingdom.

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Abstract

Most terrestrial vertebrates have colour vision, some perceive a less colourful world and others actually discriminate a wider colour spectrum than humans do. Still, we can all make use of the valuable colour information, which is more rigid than just brightness. However, at night when the light is dim, the lack of photons makes colour vision difficult. Nevertheless, some hawkmoths and bees can see colours at night. In my thesis I have studied whether there are any terrestrial vertebrates with the same ability and what adaptation for colour vision they have. My emphasise lies on the arrhythmic horse and a nocturnal gecko.

The horse is nowadays well-known to have dichromatic colour vision during the day. In behavioural experiments we found that horses perceive their colour space as a continuum of colours, which is different from how we perceive our trichromatic colour space (Paper 2). The horse is also in possession of one of the largest terrestrial eyes, and a large aperture and a short focal length enhances the signal-to-noise ratio by concentrating the photons on few photoreceptors. Still, the colour vision of horses fails at night. Thus the large eye of the horse does not appear to be adapted for nocturnal colour vision but rather for achromatic vision in dim light (Paper 3). Reptiles have also been proven to have colour vision during the day and we became especially interested in the nocturnal geckos. Due to their evolutionary history, the geckos have only cones in their retina, but they have adapted their cones and their optical system to allow for vision at low light intensities. We show that the eye of the nocturnal helmet gecko is almost 400 times more light-sensitive than our own eye (Paper 4). The adaptations of the cones for vision in dim light made us wonder whether geckos could use colour vision at night. In behavioural studies we found that helmet geckos can distinguish colours even at light intensities similar to dim moonlight (Paper 1). Still, for the nocturnal gecko it is unknown when the colours fade.

Key words: Camera-type eyes, Vertebrates, Colour vision, Dim light vision, Reptiles, Mammals, Geckos, Horses

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List of contribution

Paper 1	AK and LR contributed to the original idea and LR performed the experiments, analysed the results, and wrote the paper in consultation with AK.
Papers 2 & 3	All authors decided on the ideas and planned the experiments. LR and AB performed the behavioural experiments, LR performed the morphology preparations and the optical calculations, analysed the results and wrote both papers in consultation with the other authors.
Paper 4	The idea of the project involved RK, AK, LR and PU. LR performed the photorefractometry, the morphology preparations and the calculations of the optical sensitivity. LL and LR performed the Hartmann-Shack wavefront sensor experiments and LL analysed the data obtained with that method. LR wrote the paper except for the Hartmann-Schack part, which LL wrote. AK contributed with valuable knowledge and consultations in the writing process.

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AKNOWLEDGEMENTS

MÅNGFALD OCH BEGRÄNSNINGAR FÖR FÄRGSEENDET HOS LANDLEVANDE RYGGRADSDJUR

Färger innehåller mer tillförlitlig information än vad bara skillnader i ljusintensitet gör. Det är därför inte svårt att förstå varför de allra flesta landlevande ryggradsdjur har färgseende. Några upplever en färgfattigare värld än vi människor, men många ser fler färgnyanser. På kvällen däremot gör bristen på ljus det svårt att se färger. Trots detta finns det nattaktiva ryggradslösa djur som till exempel svärmare och bin som ser färger i mörker. I min avhandling undersöker jag om det finns ryggradsdjur med förmågan att se färg om natten, samt vilka förutsättningar de har för detta. Första delen sammanfattar jag ögat och synsinnet hos landlevande ryggradsdjur innan jag vidare undersöker specifikt däggdjurens och kräldjurens färgseende.

Ryggradsdjur har ögon av kameratyp, med en hornhinna och en lins som fokuserar ljuset på näthinnan. Näthinnan består vanligen av två typer av ljuskänsliga fotoreceptorer, stavar och tappar. Generellt används stavarna för ett färglöst mörkerseende och tapparna för färgseende vid högre ljusintensiteter.

Färgseende kräver minst två tapptyper, känsliga för olika våglängder av ljus. Deras signaler jämförs i näthinnan och en ”färgkod” skickas vidare till hjärnan och ger upphov till en färgupplevelse hos djuret. På kvällen motverkas emellertid färgseendet av den relativt förhöjda brusnivån som följer av bristen på ljus i fotoreceptorerna. Ett större öga med större pupill, tillsammans med en kortare fokallängd som koncentrerar ljuset på färre fotoreceptorer, förbättrar avsevärt ljusinsamlingen och därmed signal-brusförhållandet. Signalen kan också göras starkare genom att den summeras i tid och rum. Snabba rörelser och fina detaljer går då förlorade till förmån för en ljusare och mer tillförlitlig bild på näthinnan.

Hästar är aktiva både på dagen såväl som på natten. De har ett av de största ögonen bland landlevande djur. Detta gjorde oss nyfikna på deras förmåga till färgseende under olika ljusförhållanden. Hästen, liksom de flesta däggdjur, har två typer av tappar och är därför dikromater. Jämförelsen av de två tapparnas signaler ger upphov till en endimensionell färgvärld med korta våglängder som blått på ena sidan av skalan och långa våglängder såsom grönt och gult på andra sidan. Vi har genom beteendestudier på hästar visat att dikromater upplever sin färgvärld om dagen som en kontinuerlig färgskala och att de kan lära sig färger på ett relativt sätt (Artikel 2). När vi däremot sänkte ljusintensiteten i liknande beteendeexperiment visade det sig hästen förlorade sitt färgseende vid samma ljusintensitet som vi människor (Artikel 3). Trots hästens stora öga och pupill så är den optiska känsligheten i hästögat liknande den för människans öga när man bortser från signalsummering. När den färglösa signalen från stavarna tar över om natten är dock hästens syn troligen överlägsen vår. Men för hästar, människor och förmodligen andra däggdjur, bleknar de informationsrika färgerna tyvärr om natten.

Även kräldjur har färgseende. Vi blev intresserade av de nattaktiva geckoödlorna då dessa endast har tappar i näthinnan. Detta är ett resultat av att de härstammar från ödlor med ett, evolutionärt sett, långt dagaktivt förflutet, vilket lett till att ödlor helt saknar stavar för mörkerseende. När geckoödlorna blev nattaktiva anpassades tapparna och blev större och ljuskänsligare och även optiken förändrades för att bättre klara av de nya förutsättningarna (Artikel 4). När vi testade nattaktiva hjälmgeckoödlor i beteendeexperiment visade det sig att de vid svagt månljus fortfarande kan urskilja färger (Artikel 1). Vid den ljusintensiteten är vi människor själva färgblinda men geckoödlorna kan alltså fortsätta att använda sig av den värdefulla färginformationen om natten.

SUMMARY

Most terrestrial vertebrates have colour vision, some perceive a less colourful world and others actually discriminate a wider colour spectrum than humans do. Still, we can all make use of the valuable colour information, which is more rigid than just brightness. However, at night when the light is dim, the lack of photons makes colour vision difficult. Nevertheless, some hawkmoths and bees can see colours at night. In my thesis I have studied whether there are any terrestrial vertebrates with the same ability and what adaptation for colour vision they have. My emphasise lies on the arrhythmic horse and a nocturnal gecko.

In most vertebrates the retina includes two different classes of photoreceptor; the rods that operate in dim light intensities and the cones that allow for most animals to see colours during the day. Colour vision is achieved when signals from at least two different photoreceptor types with pigments of different spectral sensitivities are compared. For colour vision at night the great limiting factor is noise in the photoreceptors, which becomes relatively larger as the photons becomes scarce. A large eye with large aperture and a short focal length that concentrates the photons on few photoreceptors enhances the signal-to-noise ratio. In addition the signal could be summed in space and time and also spectrally even though the latter would cause colour vision to suffer.

Most non-primate mammals have two different cone pigments most sensitive to blue and yellow light respectively. The comparison of signals from two cone types gives raise to a one-dimensional chromatic space. In behavioural experiments on horses we found that horses perceive the chromatic space as a continuous scale of colours and that they can learn colours in a relative manner, preferring the colour most different from the negative training colour (Paper 2). When we gradually lowered the light intensity in a similar experiment they lost their ability to discriminate colours at the same intensity as humans. Hence, the large eye of the horse does not appear to be adapted for nocturnal colour vision but rather for achromatic vision in dim light (Paper 3).

Reptiles have also been proven to have colour vision and we became especially interested in the nocturnal geckos. Due to their evolutionary history, the geckos have only cones in their retina, but they have adapted their cones and their optical system to allow for vision at low light intensities. We show that the eye of the nocturnal helmet gecko is almost 400 times more light-sensitive than our own eye (Paper 4). The adaptations of the cones for vision in dim light made us wonder whether geckos could use colour vision at night. In behavioural studies we found that helmet geckos can distinguish colours even at light intensities similar to dim moonlight (Paper 1). Still, for the nocturnal gecko it is unknown when the colours fade.

THE VERTEBRATE EYE

The sun is emitting radiation of a wide spectrum of wavelengths including light that is used for vision by many animals. Light reflected by the surroundings provides very detailed and fast information about the environment, possible predators, food, and potential mates. It is therefore easy to understand why selection in evolution has had and still has such a strong impact on many animals' eyes and vision.

In the vertebrate camera-type eye, a cornea and a lens function as the light focusing parts. A pupil regulates the amount of light entering the eye and lastly, the light-sensitive retina captures photons and does the early processing of the visual signal. Depending on evolutionary constraints and ecological demands, the eyes of different vertebrates have adapted in a number of ways to optimally fulfil special needs such as colour vision and vision in dim light. Visual information important for survival is collected and processed while irrelevant information can be filtered out.

In this thesis I discuss the sensitivity and colour vision of terrestrial vertebrates. After a broad introduction I focus on the question whether there is any vertebrate that can overcome the constraints of dim light vision and make use of the valuable colour information even at night? I have chosen to work on a small nocturnal gecko that has huge light-sensitive eyes relatively to its body size, and the arrhythmic horse that has one of the largest terrestrial eyes in absolute size.

The Pupil

The aperture of the eye, the pupil, holds the important function of modulating the number of photons reaching the retina. If the number of photons is too small, the generated signal is too weak in relation to the overall noise in the photoreceptors. Accordingly, no reliable contrasts can be distinguished (Land, 1981; Land & Nilsson, 2002). As the light becomes dimmer the pupil dilates and allows for a larger amount of light to enter the eye. A large pupil is therefore of greatest importance to make an eye more light-sensitive at night. If the same eye in addition has a short posterior nodal distance (often called focal length, which is the calculated distance from posterior nodal point to retina in a well-focused eye) each photoreceptor receives a large number of photons and a strong signal can be generated even in dim light.

Night-active and crepuscular animals, which have a large pupil during the night, need to constrict the aperture to a great extent during the day in order to protect the very light-sensitive retina from being harmed. A strictly nocturnal animal however can manage to have a less constricting pupil if it is not exposed to bright light during the day.

Some extant mammals today are arrhythmic and active during both day and night. Thereby they face a tough challenge to meet the requirements of vision both in bright and dim light intensities. Many arrhythmic ungulates have met these challenges with a large and broadly oval horizontal pupil. In addition to the constriction of the pupil, finger-like protrusions of the pigmented retinal layers form the *corpora nigra*, which in bright light enlarge and shield the retina of arrhythmic ungulates from the strongest daylight. Similar shading structures are found in the Rock Hyrax (*Procavia*), which has a U-shaped pupil with the iris bulging out above the pupil (Fig. 1; Walls, 1942; Land & Nilsson, 2002).

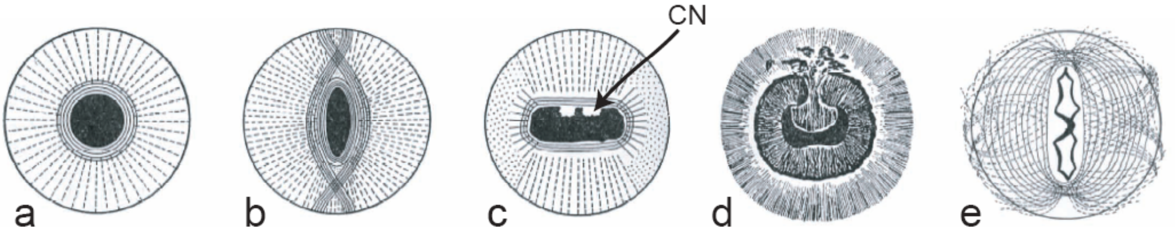

Figure 1. The iris musculature and the shape of the light-adapted pupil in human (a), a cat (b) and a horse (c) where also the light-shielding *corpora nigra* (CN) can be found in the latter. The pupil shape and the expansive iris in a hyrax (d) and the almost fully light adapted pupil and iris musculature of a nocturnal gecko (e), *Tarentola mauretanica* (From Walls 1942).

The pupil can obviously be of many appearances, ranging from a round shape like our own pupil and that of most birds, to oval and slit pupils, for instance those of most cats and some reptiles (Fig. 1). A slit pupil has the advantage to be more effective in constricting and cutting out light compared to a round pupil, especially if the slit is vertical since partly closed eyelids can help to cut out light furthermore (Walls, 1942). An even more efficient pupil, however, is the pupil of many nocturnal geckos. Their pupil is a so-called multiple-pinhole pupil and closes to two pairs of tiny apertures in a vertical line in the light-adapted state. A difference in pupil area of almost 300 times has been measured in the large Tokay gecko, *Gekko gekko*, between the light-adapted pupil and the fully opened dark-adapted pupil (Denton, 1956). In the nocturnal helmet geckos, *Tarentola chazaliae*, the difference is 100-150 times (Fig. 2; Paper 4), which can be compared to only 16 times in humans. The reason for the two-folds difference between the nocturnal gecko species is the absolute eye size, since the pupillary openings in the light adapted state hardly can be any smaller in any of the two geckos, limited finally by the optical quality of the image that becomes worse with smaller aperture, due to diffraction.

The function of the multiple-pinhole pupil however still puzzles the scientists. One explanation is the concealment of the pupil against the eye and the environment, which makes it difficult for predators to discover, compared to a round pupil, especially if the iris is camouflaged to match the body. A round black pupil has been shown to be very conspicuous and attract attention even among larger irregular objects (Cott, 1940). The light-adapted pupil of nocturnal geckos thus allows them to bask securely during the day. An additional suggestion deals with distance estimations in bright light. An object that is not in the plane of focus viewed with the tiny four pupil openings generates a quadruple image on the retina compared to only one image when the object is in focus (Murphy & Howland, 1986).

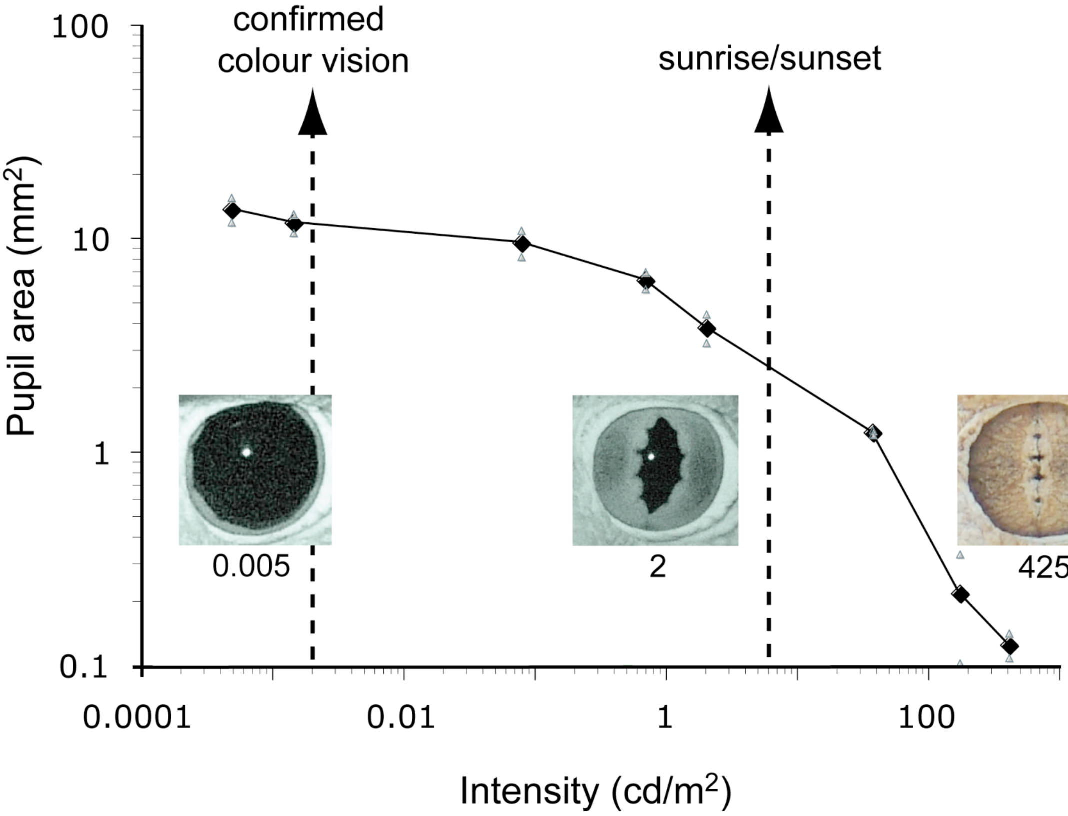

Figure 2. Pupil dynamics at different light intensities in the nocturnal helmet gecko, *Tarentola chazaliae*. The three pictures within the graph show the pupil size at the certain light intensities, which is written in cd m^{-2} below each picture. The pupillary area of helmet geckos differs by a factor of 100-150 between the light-adapted and fully dark-adapted pupil (Paper 4).

The Optical System

The camera eye of vertebrates has a lens and a cornea that focus the light onto the retina. In aquatic animals, the cornea has little influence on the refraction of light since the refractive indices of the cornea and water are very similar. In aquatic eyes the lens is therefore close to spherical and optically powerful. However, in air, the cornea, which has no optical function in water, becomes optically powerful. Therefore, in the terrestrial vertebrate eye the cornea serves as an important refracting lens. For diurnal animals the lens is flattened and the curved cornea does most of the refraction in the eye (Fig. 3). In humans, for example, the cornea answers for approximately two thirds of the focusing power, which leaves the lens with the task of fine accommodation (Table 1). Nocturnal animals usually depend more on a large spherical lens as the main focusing lens that, together with the cornea results in a short focal length of the eye (Table 1; Fig. 3; Walls, 1942; Land & Nilsson, 2002). For amphibian animals, such as the frog, it is of double importance to have a more powerful lens than cornea. Still, in water, they would be very long-sighted if this was not corrected for (Du Pont & De Groot, 1976).

Table 1. Eye refractive powers, and F-numbers in decreasing order

	Powers (dioptres)				
Species, (D, N, C)	Cornea	Lens	Whole eye	F-number (PND/A)	
Human (D) *Homo sapiens*	43.0	19.1 (32%)	59	2.1	(17.1/8)
Ostrich (D) *Struthio camelus*	25.4	27.5 (60%)	46	1.9	(21.8/11.5)
Tokay gecko (N) *Gekko gekko*	–	–	111	1.1	(6.5/6)
Rat (N) *Rattus norvegicus*	112.6	244.0 (81%)	301	1.0	(3.3/3.3^{B})
Frog (D/N/C) *Rana esculenta*	109.0	198.3 (81%)	245	1.0	(4.1/4)
Cat (N, C) *Felis catus*	38.9	53.0 (68%)	78	0.91	(12.5/13.8)
Opossum (N) *Didelphis marsupialis aurita*	72.1	157.1 (80%)	196	0.85	(5.1/6.0)
Horse (D/N/C) *Equus caballus*	19.5	15.0 (47%)	32	0.83	(25.0^{A}/30^{A})
Rabbit (C) *Oryctolagus cuniculus*	44.6	75.0 (74%)	101	0.82	(9.8/12^{B})
Helmet gecko (N) *Tarentola chazaliae*	–	–	294	0.74	(3.4/4.6)

The refractive powers of the lens and the cornea and the lens contribution for the whole eye. Minimal F-numbers are calculated from the posterior nodal distance (PND) divided by maximal pupil (A) diameter where a low F-number indicates a light-sensitive eye. The activity of the species is indicated by D (diurnal), N (nocturnal) or C (crepuscular). Sources of data: of human, (Helmholtz, 1924, cited from Hughes, 1977), ostrich (Martin et al., 2001), tokay gecko (Citron & Pinto, 1973), rat (Hughes, 1979), frog (Du Pont & De Groot, 1976), cat (Vakkur & Bishop, 1963), opossum (Oswaldo-Cruz et al., 1979), horse (Sivak & Allen, 1975), rabbit (Hughes, 1972), helmet gecko (Paper 4). ^{A}Paper 3; ^{B}Estimation from pictures in reference.

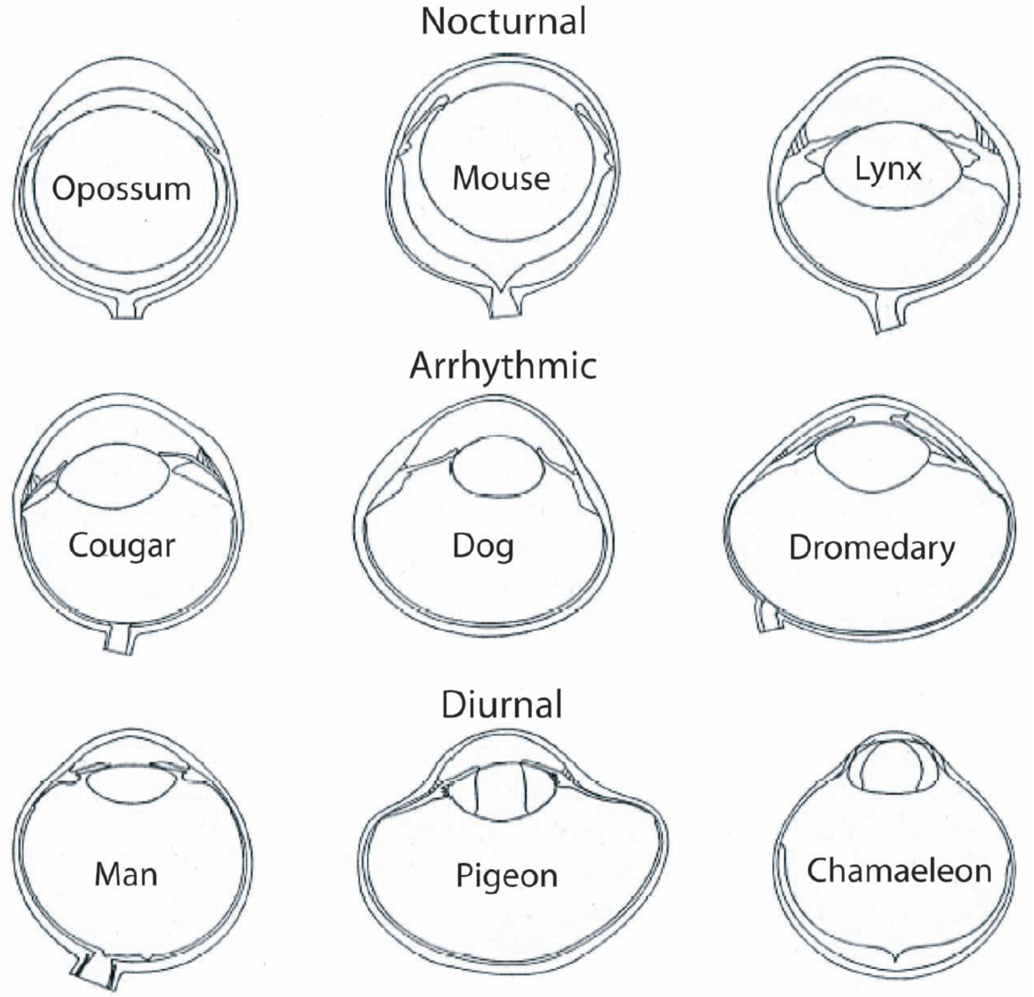

Figure 3. Different optical designs for the terrestrial life in the eyes of vertebrates. Diurnal animals have usually decreased the size and the curvatures of the lens, while nocturnal animals have a large round lens and a short posterior nodal distance (from Walls, 1942).

From the proportions of the optical system it is possible to obtain an indication of the eye's light gathering ability, i.e. sensitivity. A large pupil diameter (A) and a short posterior nodal distance (PND) concentrates much light on few photoreceptors in the retina, making the signal strong and the eye very light-sensitive (Table 1). An eye of equal size, but with a long PND, however, favours spatial resolution when light is abundant, since the angle between photoreceptors in the retina is small. That is, the light will be spread on many photoreceptors and small details in the image can be discerned (Land & Nilsson, 2002).

The ostrich and the horse have among the largest terrestrial eyes in absolute size, but still, the much smaller nocturnal geckos have eyes with relatively better light gathering ability than both of them. The nocturnal helmet gecko has a very light-sensitive optical system with a large aperture in the dark-adapted state and a short PND, yielding an F-number (PND/A) of only 0.74, slightly more sensitive than the eye of the domestic cat with an F-number of 0.91 (Vakkur & Bishop, 1963). Since the brightness of the retinal image is proportional to $(A/PND)^2$ (Hughes, 1977; Land & Nilsson, 2002) the helmet gecko eye produces an eight times brighter retinal image than the eye of humans when viewing the same scene (see also section on dim light vision).

Multifocal Optical Systems

Many vertebrates that depend on their vision in dim light have been found to have a multifocal optical system in their eye (Kröger et al., 1999). Hence, their lens and maybe even cornea have distinct concentric zones of different refractive index. Eyes with small F-numbers are more exposed to longitudinal chromatic aberration. As a result of chromatic aberration, light of short wavelengths, such as blue, are refracted stronger at a surface than light of long wavelengths, such as red, and this causes defocus on the retina if not corrected for. According to Kröger and colleagues (1999), a multifocal optical system corrects for some of the defocused light, but there is a question mark what happens to the light of wavelengths that enters the "wrong" concentric zone dedicated for another range of wavelengths.

A fast method to qualitatively determine multifocal optics is photorefractometry (Schaeffel at al., 1987), where distinct concentric zones with different refractive indices show as ring-like patterns when illuminated with infrared light (Fig. 4; Paper 4). To measure the wavefront that exits an illuminated eye, a Hartmann-Shack wavefront sensor can be used (Liang et al., 1994). In short, the eyes of the animal are illuminated with a far-red light beam and the wavefront reflected from the retina is focused onto a sensor and can then be analysed. Without knowing where the animal focuses, it is difficult to obtain a quantitative measure of the multifocality, but with our analyses of the Hartmann-Shack wavefront results a good estimation can be made (Paper 4).

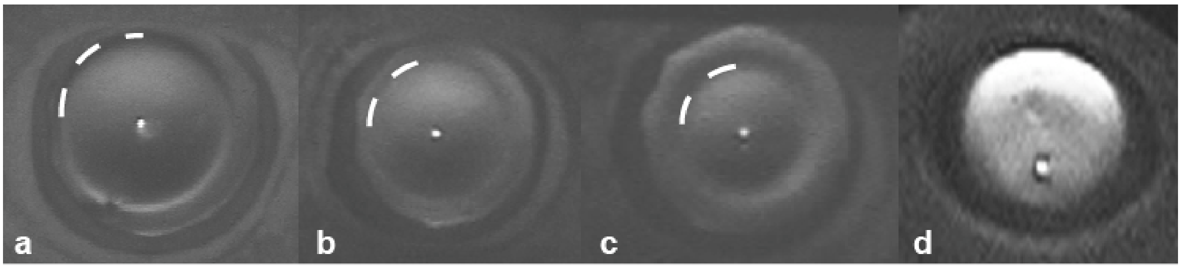

Figure 4. Nocturnal helmet geckos, *Tarentola chazaliae* (a-c), show more or less distinct rings (indicated by broken lines) in photorefractometric pictures suggesting multifocality. Day geckos, such as *Phelsuma grandis*, show no rings in photorefractometric pictures (d), which suggest that they are monofocal (Paper 4). The brightness difference between pictures is due to different intensities of the infrared illumination.

Our results from the Hartmann-Shack wavefront sensor suggest at least two concentric zones of different refractive power in the helmet gecko. In general the difference was 15-20 diopters between zones, which is within the expected magnitude needed for eyes of similar dimension to focus light of their whole visual spectrum (300-600 nm; Paper 4). Our results are thereby in agreement with those of Kröger and colleagues (1999), the multifocal eye of geckos could be an adaptation to correct for some of the defocus on the retina caused by chromatic aberration.

Filters within the Eye

Daylight contains a wide range of wavelengths, some of which are filtered out by the lens and the cornea. Generally these cut-off filters reduce sensitivity since only part of the spectrum is allowed to pass. Yellow pigments filter out short wavelenghts and make, for example, the bright blue sky appear dimmer for an animal during the day (Lythgoe, 1979). The lens of many diurnal vertebrates such as diurnal rodents, diurnal geckos, insectivores and ourselves is yellow to various degrees and protects the eye from harmful ultraviolet light. In addition, cutting out the ultraviolet and blue light reduces longitudinal chromatic aberration in the eye (Walls, 1942; Jacobs, 1981). Nocturnal animals usually lack yellow filters since they capture all available photons to enhance sensitivity at night (Walls, 1942; Lythgoe, 1979).

The Retina

In the inverted retina of vertebrates, the light-sensitive photoreceptors are oriented away from the light, lying in the outer portion of the retina in the back of the eye. The light therefore has to pass through multiple cell layers (ganglion cells, amacrine cells, bipolar- and horizontal cells), before it reaches the photoreceptors. There are two classes of photoreceptor in most vertebrate retinae, i.e. rods and cones, which are specialised for different light intensities (Rodieck, 1973). This system is called a dual or duplex retina. The rods are in general the most light-sensitive photoreceptors and respond to single photons (Barlow, 1956). They are used for vision at low light levels (scotopic vision, night vision) when photons are sparse.

As the light intensity increases the other class of photoreceptor, the cones, start to operate. As the light intensity increases further the rods slowly become saturated. The signals resulting from single photons are much smaller in the cones than in the rods, but in abundance of light and with a high density of cones high resolution is achieved during the day (photopic vision). In addition, if the retina contains different cone types with different spectral sensitivities their signals can be compared and thus make it possible to discriminate colours (see the colour vision section).

The density of photoreceptors and ganglion cells gives an indication of the spatial resolution of the eye. The highest resolution is achieved when each ganglion cell is receiving signals from only one or few photoreceptors. This type of connection is usually restricted to one or a couple of small areas in the retina, for example the fovea of humans, and in these areas, the visual image has the highest resolution and allows for discrimination of finest details (Rodieck, 1973). However, in most regions of the retina, ganglion cell density and thus spatial resolution is low. There might be one exception though, and that is in the diurnal and nocturnal gecko retina. Here the photoreceptor to ganglion cells ratio has been found to be close to one in the entire retina (Röll, 2001a). The effect on the signal summation in dim light is however not known.

Vertebrate photoreceptors consist of an inner and an outer segment, the latter of which contains the visual pigment responsible for absorbing the light. The outer segment of rods is cylindrical and built out of membranes organized in a large number of separated disks. The normally tapered outer segment of cones consists mainly of infolding of the membranes. The membranes are the sites of light transduction and are packed with light-absorbing photopigment that changes its structure when light is absorbed (Rodieck, 1973).

The length of the outer segment is of importance since the proportion of light absorbed increases with the length of the photoreceptor. However, very long photoreceptors also create more noise since they contain more photopigment that can spontaneously activate the transduction process. They also have a broader sensitivity spectrum than a short photoreceptor since the light that reaches the outer end of the receptor is heavily filtered by the visual pigment itself, a process called self-screening (Warrant & Nilsson, 1998). Long photoreceptors therefore have a negative effect on the resolution of wavelengths since the spectral sensitivity curves of large photoreceptors overlap more and the colour information declines.

The inner segment of the photoreceptor, besides being the power plant of the cell also guides the light into the outer segment and transports the electrical signal via the synapses to the bipolar cells that continue to process the visual signal. In birds, reptiles, marsupials and monotremes the inner segment can also contain an oil droplet that functions as a lens and often filters out light of short wavelengths before the light enters the light-absorbing outer segment of the photoreceptor (Walls, 1942). Coloured oil droplets function as cut-off filters that can actually make a difference for the animal's colour vision by sharpening the sensitivity curves of the photoreceptor and by displacing its maximum by several nanometres (Vorobyev, 2003). Just as a yellow lens, the oil droplets are likely to decrease chromatic aberration and glare since they absorb light in the short wavelength part of the spectrum.

The Reptilian Photoreceptors

The reptilian retina consists of double and single cones and even though double cones are present in most vertebrate groups their function has puzzled scientists for a long time (Walls, 1942; Underwood, 1970). A candidate for the most complex retina among reptiles, which might even account for all vertebrates, is the retina of the red-eared turtle, *Trachemys scripta elegans*, which contains one rod pigment and four types of cone pigments with sensitivities ranging from UV to far-red. The cone pigments are, in addition, expressed in different single or double cones with differently coloured oil droplets. Oil droplets of red, orange, yellow, pale green colours and colourless droplets have been found within the same retina resulting in seven different cone subtypes (Loew & Govardovskii, 2001).

During the evolution diurnal lizards have lost the typical vertebrate dual retina with both rods and cones. Instead they are left with different types of single and double cones (Walls, 1942; Underwood, 1951; Underwood, 1970). The photoreceptors of diurnal lizards contain oil droplets as well, even though they do not show the same diversity in colouration as in turtles or birds. Lizards are mostly restricted to green, yellow or colourless droplets (Loew et al., 2002; Bowmaker et al., 2005).

Adaptations in Nocturnal Geckos

At some point in the evolution, a group of lizards, the geckos, turned nocturnal, foraging in dim light conditions when temperature is much lower than during the day. The reasons might have been less competition for food during the night or to avoid overheating in hot arid regions during the day (Underwood, 1970). Foraging at night puts their visual system at very high demands since the gecko retina contains only different cone types and no rods. Consequently, the cone outer segments of nocturnal geckos have become larger, more rod-like and thereby more sensitive to light than those of diurnal lizards (Walls, 1942; Crescitelli & Karvaly, 1983; Röll, 2000).

The outer segments of cones of nocturnal geckos, such as *Eublepharis macularis* and *Gekko gecko*, have lengths of 47 μm and 39 μm respectively (Dunn, 1969), and even larger outer segments of 60 μm length, with a diameter of 10 μm have been found in the nocturnal gecko *Paroedura pictus* (Röll, 2000). These figures correspond well to my own measurements of the nocturnal helmet gecko, *Tarentola chazaliae*, where the outer segments are 30-40 μm long and have a width of 10 μm (Fig. 5a; Paper 4).

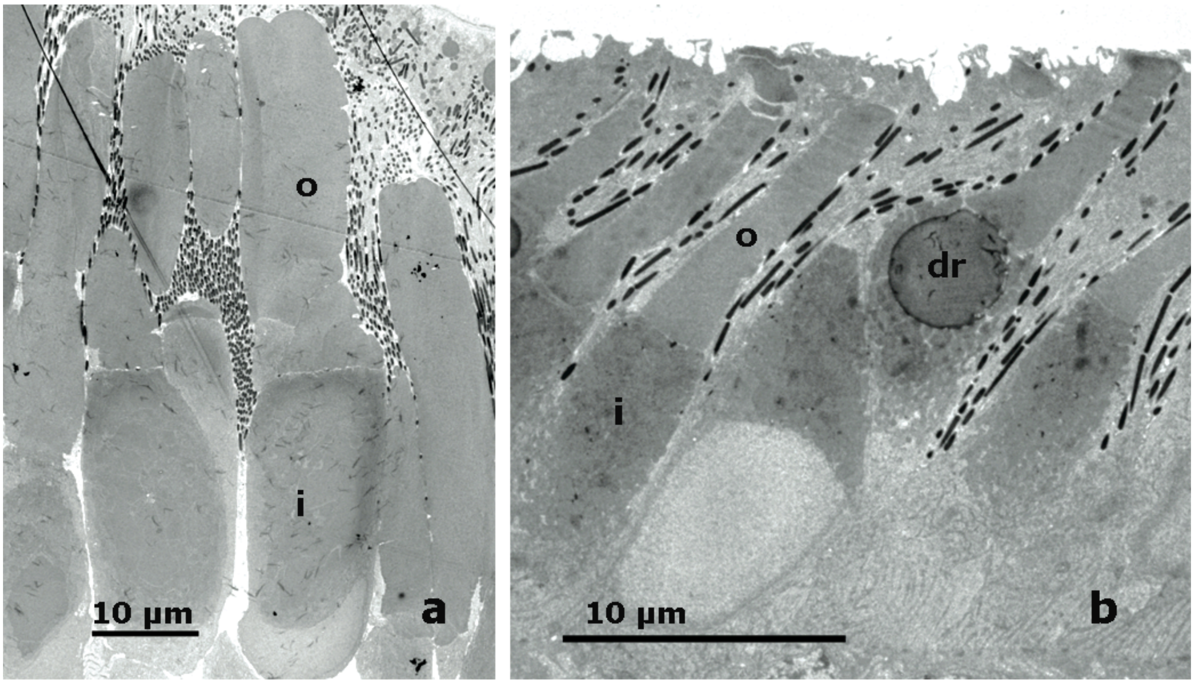

Figure 5. Electron micrographs of cones in the retina of a nocturnal and diurnal gecko with inner (i) and outer (o) segments and oil droplets (dr). In the nocturnal helmet gecko (a), *Tarentola chazaliae*, the outer segment of the cones measures 30-40 µm in length and 10 µm in width (Paper 4). In the day gecko (b), *Phelsuma abbotti checkei*, the same measurements are 10 µm and 2 µm respectively, demonstrating that the nocturnal gecko has both longer and wider and thereby more light-sensitive cones.

The diurnal geckos have again reverted from the nocturnal to a diurnal life style (Walls, 1942). Consequently they have also reverted their cones to be smaller because of the abundance of light where large photoreceptors are not needed. Röll has measured twelve diurnal gecko species to have cone outer segments of 6-12 µm length and 1-2 µm width (Röll, 2000). My own measurements on the day gecko, *Phelsuma abbotti checkei* show outer segments lengths of approximately 10 µm and widths of 2 µm (Fig. 5b).

In contrast to their diurnal ancestors the oil droplets in the photoreceptors of most nocturnal geckos have disappeared. In diurnal geckos the oil droplets are again abundant in some of the double cones outside the fovea, but all droplets are colourless. Instead the lens is yellow, filtering out the scattering light of short wavelengths (Walls, 1942; Röll, 2001b).

Underwood (1970) named the different cone types (Fig. 6a) and also described the regular mosaic pattern of the photoreceptors within the lizard retina. The pattern differs somewhat between diurnal lizards and geckos but generally consists of rows of large type B double cones, alternating with rows of type A single cones and type C double cones (Fig 6b; Underwood, 1970).

In the gecko retina the B double cone is most numerous and has symmetric outer segments, both of which are most sensitive to green light. Also the A single cones are sensitive to green light just like the thicker member of the C double cones. The majority of the thinner members in the C double cones are sensitive to blue light while 10-20% are most sensitive to ultraviolet light. A third type of C double cones has also been found to occupy the same positions in the retinal mosaic as other C double cones. In these cones both members seem equal in size and both are sensitive to green light (Loew et al., 1996).

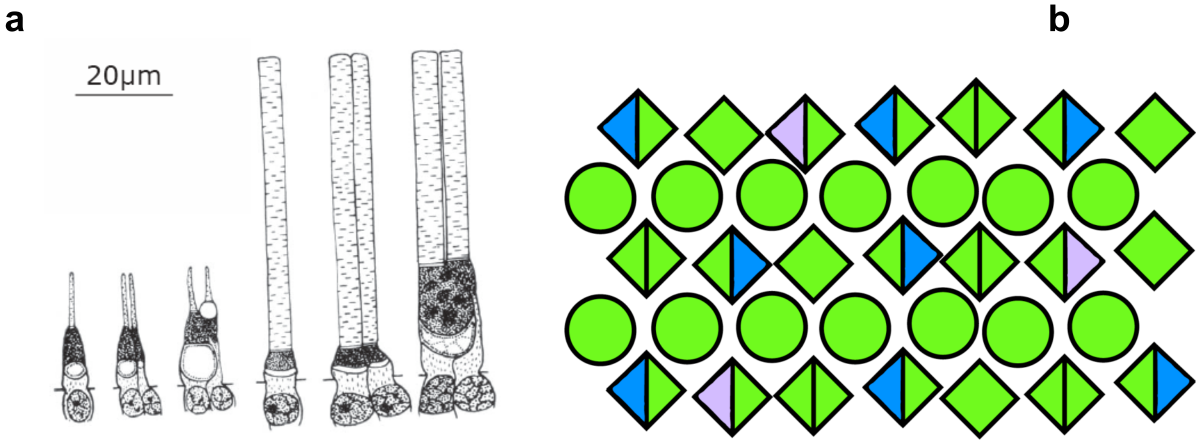

Figure 6. The three cone types (a) of a diurnal gecko with oil droplets (o) and short and slender outer segments and those of a nocturnal gecko with long and thick outer segments and inner segments generally without oil droplets (From Underwood, 1970). A regular mosaic pattern in a retina of a general nocturnal gecko (b). Rows of B double cones (rings) are altenated with A single cones (diamonds) and C double cones (divided diamonds). The colour within the symbols illustrates the colour to which the photoreceptors are most sensitive to, with violet signifying ultraviolet light (redrawn from Loew et al., 1996).

Mammalian Photoreceptors

Because of their nocturnal ancestry most extent non-primate mammals have a rod-dominated retina with two spectral types of cones. One possible reason for the retention of at least some cones in all mammalian retinae might lie in the pathways and circuitry of rods and cones. The classical mammalian rod pathway involves cone bipolar cells before the signal reaches ganglion cells (for review see Wässle, 2004).

Depending on the time of day when the animals are most active, the composition and ratio of rods and cones varies even though rods outnumber the cones in most mammalian retinae. Even so, both cones and rods have been found even in exclusively nocturnal or diurnal mammals (Peichl, 2005). The density of cones however, ranges from a few percent of all the photoreceptors, e.g. around 0,5% in the giant pouched rats (Peichl & Moutairou, 1998), to 86-99% in various diurnal mammals such as the tree shrew, prairie dog and ground squirrels (West & Dowling, 1975; Muller & Peichl, 1989). Hence, in the latter group the cones actually outnumber the rods while usually the opposite is the case.

Photopigments and their Evolution

Photopigments consist of a 7-helix transmembrane protein called opsin and a chromophore. The chromophore is 11-*cis*-retinal (retinal, vitamin A_1) in all mammals, but in fishes, amphibians and reptiles another chromophore, 11-*cis*-3.4-dehydroretinal (vitamin A_2), has been found (Lythgoe, 1979). The spectral sensitivity of a given pigment is determined by the amino acid sequence of the opsin and by the chromophore. 11-*cis*-3.4-dehydroretinal (together with the opsin called porphyropsin) shifts the spectral sensitivity curve towards longer wavelengths compared to 11-*cis*-retinal.

When the photopigment absorbs a photon, retinal changes its structure and associates with a G-protein, transducin. The biochemical cascade that follows results in closure of cGMP-gated channels in the membrane resulting in a hyperpolarisation of the cell (for review and comparison with invertebrates see Hardie & Raghu, 2001).

Studies in the lamprey, a jawless (agnathan) fish at the base of the vertebrate linage, show that already the ancestral vertebrates had five opsin genes, four of which were cone opsins (Bowmaker, 1998; Collin et al., 2003). Most vertebrate classes, such as birds, reptiles and fishes have preserved the ancient four cone opsins and thus have the potential for tetrachromatic colour vision (Kelber et al., 2003).

Table 2. Absorption maxima of photoreceptors of diurnal lizards

Species	Absorption maxima (nm)				Reference
	LWS	MWS	SWS	UVS	
Anolis carolinensis	625	503	462	365*	(Provencio et al., 1992) *(Loew et al., 2002)
16 other Anolis species	564	495	455	365	(Loew et al., 2002)
Chamaeleo dilepsis	555-615	477-507	440-447	383	(Bowmaker et al., 2005)
Ctenophorus ornatus	571	493	440		(Barbour et al., 2002)
Furcifer pardalis	555-610	491	444	375	(Bowmaker et al., 2005)

Sensitivity peaks of four photopigments of diurnal lizards have been found at 555-625 nm, 477-507 nm, 440-462 nm and 365-383 nm respectively.

Table 3. The absorption maxima of photoreceptors of geckos

Species	Geographic range	N/ D/ Ar	Absorption maxima (nm)	Reference
Gekko gekko	Pakistan to China and Indonesia, USA	N	521, 467, -	(Crescitelli et al., 1977)
Gekko gekko	Pakistan to China and Indonesia, USA	N	521, 467, 364	(Loew, 1994)
Goonatodes albogularis	Gr + Lesser Antilles, Central/South America	D	542, 475, 362	(Ellingson et al., 1995)
Gymnodactylus caspius	Middle Asia and Crimea	Ar	534, 452, -	(Govardovskii et al., 1984)
Gymnodactylus caspius	Middle Asia and Crimea	Ar	532, 467, NR	(Govardovskii et al., 2000)
Gymnodactylus fedchenkovi	Middle Asia and Crimea	Ar	535, 451, -	(Govardovskii et al., 1984)
Gymnodactylus kotchyi	Middle Asia and Crimea	Ar	537, 460, -	(Govardovskii et al., 1984)
Gymnodactylus russovi	Middle Asia and Crimea	Ar	534, 452, -	(Govardovskii et al., 1984)
Hemidactylus frenatus	Cosmopolitan	N	520, 466, -	(Crescitelli et al., 1977)
Hemidactylus frenatus	Cosmopolitan	N	521, 463, NR	(Govardovskii et al., 2000)
Hemidactylus garnotii	India to Oceania	N	521, 464, 363	(Loew et al., 1996)
Hemidactylus turcicus	Cosmopolitan, Europe	N	526, 467, 366	(Loew et al., 1996)
Platyurus platyurus			527, 465, -	(Crescitelli et al., 1977)
Pthychosoon linotum	Myanmar, Thailand, Malaysia	N	523, 470, -	(Crescitelli et al., 1977)
Teratoscincus scincus	Arab Peninsula to W China	N	533, 452, 365	(Loew et al., 1996)
Teratoscincus scincus	Arab Peninsula to W China	N	536, 466, -	(Govardovskii et al., 1984)

Sensitivity peaks of three photopigments of geckos have been found at 521-542 nm, 451-475 nm and 362-366 nm respectively. The geographic range for all species except *Gymnodactylus spp*. is taken from from Henkel and Schmidt (2003) and the activity of the species are indicated by N (nocturnal), D (diurnal), or Ar (arrhythmic). NR = intentionally not recorded during the study.

Reptilian Photopigments

Because of the slenderness of diurnal lizard cones, it was earlier rather difficult to study their spectral sensitivity using microspectrophotometric techniques. There were, however, early indications from electroretinogram measurements that there was more than one photopigment present in the retina of lizards (Crescitelli, 1972). Many diurnal lizards have now been established to have four different cone pigments and are thus probably tetrachromats (Table 2).

In addition, a couple of lizard species have retinae with a mixure of rhodopsin and porphyropsin, that is, both Vitamin A_1- and A_2-based visual pigments, of which the latter shift the sensitivity peak towards longer wavelengths. This mixture was found in four recently studied species of chameleons (Bowmaker et al., 2005) and also in the lizard *Anolis carolinensis* (Provencio et al., 1992). Other Anolis lizards and the Ornate dragon lizard, *Ctenophorus ornatus*, have only Vitamin A_1-based visual pigments (Barbour et al., 2002; Loew et al., 2002).

For a long time geckos were thought to have only two cone pigments, one short-wavelength-sensitive (SWS) and one middle-wavelenght-sensitive (MWS) pigment. However, since Loew's findings of a pigment with an absorption maximum in the ultraviolet spectrum (UVS) in the Tokay gecko, *Gekko gekko* (Loew, 1994), more studies support the presence of a UV-sensitive photopigment in geckos (Table 3; Ellingson et al., 1995; Loew et al., 1996). Three different photopigments theoretically allow for trichromatic colour vision in geckos.

Mammalian Photopigments

More than 225 million years ago mammals diverged from other vertebrates. Most information about mammals comes from their teeth, which fortunately turn out to be very informative about the animals' lifestyle. There is evidence indicating that the early placental mammals were small insectivores, and probably nocturnal and solitary in behaviour. Their olfactory lobes were large, indicating the importance of their sense of smell (Walls, 1942; Pough et al., 2002). During the mammals' nocturnal phase of evolution when they depended on other senses rather than vision they are believed to have lost two of the ancestral four cone opsins (Jacobs & Rowe, 2004). Thus, they lost the possibility of tetrachromatic colour vision. Hence, most extent mammals have one short-wavelength-sensitive cone type (SWS cone) and one long-wavelength-sensitive cone type (LWS cone) operating during the day and thus the preconditions of dichromatic vision.

Later and quite recently, about 30 million years ago, the primates evolved a third photopigment sensitive to middle-to-long wavelengths (MWS cone). The reason for the evolutionary selection of this mutation is thought to be the primates' higher degree of diurnal activity with a need of finding and distinguishing food such as ripe fruit (Osorio & Vorobyev, 1996). It has been suggested that the trichromacy only could evolve and be functional in an eye where one-to-one connections between cones and ganglion cells were established. A mutation, which created MWS and LWS cones would otherwise be lost because of the complex network that pools signals from many cones (for review see Wässle, 2004). However, a recent groundbreaking study on transgenic mice explains how a third cone type and trichromatic vision can evolve and be of immediate use in animals. Jacobs and colleagues (2007) show trichromacy in gene-manipulated mice expressing a third LWS cone type. The mammalian retina thus seems very adjustable to changes in the photoreceptor organisation since small alterations can be of immediate value to the animal.

Interestingly enough, one crepuscular frugivorious species of megabat, the *Haplonycteris fisheri*, has recently been found to also possess duplicated gene opsins for the MWS and LWS pigments. In two other bats, the crepuscular megabat, *Pteropus dasymallus formosus*, and the nocturnal microbat, *Myotis verlifer*, only two opsins most sensitive to ultraviolet light and light of long wavelengths were found (Wang et al., 2004). Furthermore, there are behavioural and microspectrophotometric results from the arrhythmic fat-tailed dunnart (*Sminthopsis crassicaudata*) and additional confirming microspectrophotometry data from the crepuscular honey possum, *Tarsipes rostratus* that suggest trichromacy in some Australian marsupials (Arrese et al., 2002; Arrese et al., 2006). Like in the bats the trichromacy does not unite all marsupials since studies on the crepuscular tammar wallaby confirm dichromatic colour vision (Hemmi, 1999a; Hemmi, 1999b). Given the new findings of a third opsin is animals that are not strictly diurnal – bats and marsupials – it is intriguing to think about the selective pressure underlying this evolution since the third opsin, at least in the fat-tailed dunnart, has been suggested to be fully functional and contribute to trichromatic colour vision.

There are also mammals which have lost cone types, usually the SWS cone type, and are left with monochromatic vision. This is true for the nocturnal owl monkey, *Aotus*, even though a non-functional pigment gene has been found (Jacobs et al., 1993). The loss of the SWS cone type is also confirmed in e.g. marine mammals and African giant rats peich (Peichl & Moutairou, 1998; Peichl, 2001).

COLOUR VISION

Definitions and Benefits

As Newton discovered already in the 17th century, light rays are not coloured (Newton, 1671). A colour is only created when the light is perceived by an observer with colour vision. To have colour vision an animal must possess at least two photoreceptor types with photopigments of different spectral sensitivities. The photoreceptors need to operate over approximately the same light intensities, look approximately in the same direction and have a distribution in the retina that enables the signals to be compared by subsequent cell layers to generate colour information. Hence, the different receptors need to be fairly close to each other in the retina. However, several photopigments are only an indication of the animal's colour vision. To be absolutely positive that an animal can use the colour information, behavioural studies need to be done. According to a commonly used definition, colour vision is the ability to distinguish different objects of the same shape, size, texture and brightness that only differ in the spectral composition of the reflected light (Jacobs, 1981; Kelber et al., 2003).

Colour makes it possible to discern objects from each other in a patchy environment. An edge where the brightness changes, might be an indication of a boundary between two different objects, or it could just be created by shadows and your eyes might be fooled. A hue boundary on the other hand represents surfaces that differ in reflectance and thus probably different objects. Colour information is therefore a more reliable cue than brightness. However, colour vision itself is meaningful only as it relates to the behaviour and survival of the animal. It provides animals with important information about e.g. food, potential partners or enemies and landmarks (Kelber et al., 2003).

For humans the most familiar form of colour vision is trichromatic colour vision since most humans have three cone types with different pigments to compare signals from: the short-wavelength-sensitive (SWS) cone, the middle-wavelength-sensitive (MWS) cone and the long-wavelength-sensitive (LWS) cone. In the retina a signal comparison is performed between the SWS cone and both the MWS and LWS cone types, which is called the blue-yellow opponent system. A second comparison is made between the MWS and LWS cone types, forming the red-green-opponency (Wässle, 2004). The three cone pigments give raise to a two-dimensional chromatic space in which we can perceive two qualities of colour; the hue, which is the attribute of the tint of a colour such as blue and green, and the saturation of a colour (Wyszecki & Stiles, 1982). Saturation is related to the spectral purity of a colour; to what degree a chromatic stimulus differs from an achromatic stimulus such as white and grey regardless of their brightness. An unsaturated colour contains much white or grey whereas e.g. blue colour with very small degree of grey/white is highly saturated. However, even though there are other animals with trichromatic colour vision we do not know whether they perceive the very same chromatic qualities as humans.

Tests for Colour Vision

The presence of several photopigments in the retina of an animal are a good indication of its ability to discriminate colours. However, not all photoreceptors are necessarily used for colour vision; for instance, our rods do not contribute to a tetrachromatic system. Therefore, to confirm that an animal perceive colours a behavioural study is needed.

In our studies on geckos and horses we have used a method resembling that developed by Karl von Frisch when he trained bees to colours in the beginning of the 20th century (Frisch, 1914). His idea was to present a coloured stimulus among several shades of grey to make brightness an unreliable cue for the animal. To make the experiment easier for non-flying animals we did instead present the animals with two choices at a time, a so-called dual choice behavioural experiment (Fig. 7). Still, we made brightness an unreliable cue since we presented several brighter and darker versions of all colours used.

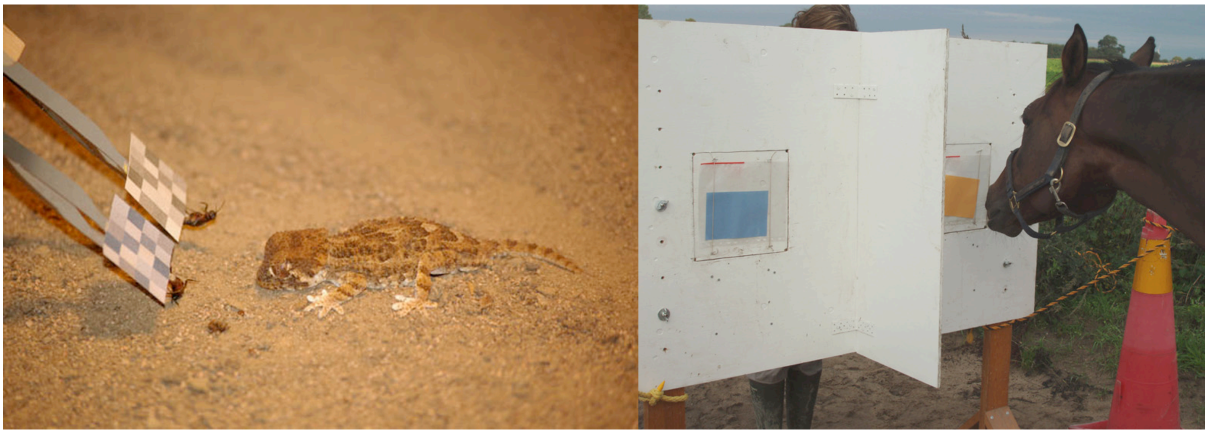

Figure 7. We tested behaviourally our colour vision hypotheses in geckos (left) and horses (right) with dual choice experiments. For the horses pieces of carrot were the reward when choosing the positive stimuli and for the gecko the reward was a well-tasting cricket. (Paper 1, 2 & 3)

It is possible to calculate how bright a certain colour appears to an animal (Eqn. 1). The reflectance of the colours is measured with a spectroradiometer to obtain their reflectance $S(\lambda)$. In addition, the light spectrum $I(\lambda)$ and the spectral sensitivity $R_i(\lambda)$ of the animal's photopigments need to be known to calculate the relative number of quanta absorbed by the photoreceptor i (Q_i). It is then possible to match colours in brightness for the specific animal tested. Additional darker and brighter versions should, however, also be used to account for possible inaccuracies in the calculations.

$$Q_i = \int S(\lambda)I(\lambda)R_i(\lambda)d\lambda \qquad \text{Eqn. 1}$$

During our experiments, the animal received a reward when it chose the positive stimulus and experienced absence of a reward when it chose the negative stimulus. If the animal chose the negative stimulus we changed stimuli and presented the animal with a new stimulus combination. To simplify training in the very beginning of the learning period, the animal was repeatedly presented with the same stimulus combination until it chose the positive stimulus and received a reward.

In the experiments with the geckos, a cricket had to be presented even when the animal chose the negative stimulus. Therefore, we combined the negative stimulus with a cricket dipped in saturated solution of salt water, which was not appreciated or eaten by the geckos. The positive stimulus was associated to a tasty cricket dipped in water. This method was used by Wagner (1932) and is a valid method since the geckos could not discriminate between both crickets before making a choice.

Colour Vision in Reptiles

Even though it was early known that many reptiles have four cone photopigments and thus the potential for tetrachromatic vision, few behavioural studies have been made. The reason is probably that reptiles, just like amphibians, are difficult to train and handle in behavioural experiments. Just as most animals, reptiles have a wide range of sensory systems for locating food, communication and predator avoidance. In lizards a relationship between foraging mode and the use of chemical senses and vision has been confirmed (Cooper, 1994). Carnivores that forage actively rely on chemical cues and use tongue-flicking behaviour, which increases in rate, in response to chemical cues from prey animals. Ambush predators do not use tongue flicking while foraging but rely primarily on vision for detecting prey.

With his stimulus associated with salty or non-salty crickets, Wagner concluded as early as 1932 that diurnal lizards have colour vision. Swiezawska who worked in the same lab did not use Wagners negative training but did instead only reward her lizards when they chose a specific colour (Swiezawaska, 1949). A somewhat stronger negative training was used by Benes (1969) on whiptail lizards, *Cnemidophorus tigris*. She presented the stimuli sequentially instead of simultaneously so that the animals could not compare the stimuli. When the lizard chose the mealworm associated with the negative stimulus a small electric shock was given the lizard. Independently of method used, all studies show colour vision in lizards.

Nickel (1960) performed behavioural experiments on alligators, which demanded an angelic patience of her. In the beginning of her study the animals tried to use brightness cues instead of colour cues and they often became frustrated with the experiments. Finally however, after small modifications of the set-up Nickel showed that alligators also discriminate colours.

For a tetrachromatic retina with differently coloured oil droplets, Arnold and Neumeyer (1987) behaviourally showed three wavelength ranges of best discrimination ability in the turtle *Pseudemys scripta elegans*. They also found ranges of reduced wavelength discrimination ability in the turtle, due to the effect of the oil droplets. Thus, the oil droplets filter out light of short wavelengths which both sharpen and shift the effective spectral sensitivities of the cones.

Colour Vision in Dichromatic Mammals

Walls (1942) suggested that the cones of non-primate mammals had no colour vision capacity. Accordingly, in the early 20th century, the behavioural studies on mammals were quite ambiguous. This applies especially to the experiments performed on predatory animals such as dogs and cats, which Rosengren (1969) summarizes in her paper together with her own results that confirm colour vision in dogs. She trained cocker spaniels to food dishes equipped with lids painted in different colours. Each colour and the negative stimulus, grey, were presented in different brightness versions to eliminate achromatic cues for the dogs. The ambiguity from the earlier studies might originate from the difficulty in training for example cats, and the importance of smell in dogs. It could also be caused by the choice of stimuli colours, which I soon will explain.

Grzimek (1952) performed behavioural experiments in horses confirming colour vision. Subsequent studies are in agreement with Grzimek's results, even though there are small inconsistencies between studies, dealing with which colours the horses could discriminate (Pick et al., 1994; Macuda & Timney, 1999; Smith & Goldman, 1999).

Even though colour vision among mammals is recognised to be widespread today (Jacobs, 1993; Kelber et al., 2003), it is still not well known how dichromatic mammals perceive their colour space. A dichromat has two different cone types to compare signals from and thus a one-dimensional chromatic space. In this colour space, between colours that lead to a full response of the SWS cones only and the colours that lead to full response of the LWS cones, there is a location called neutral point (Fig. 8). The neutral point corresponds to the wavelength of monochromatic light that leads to similar responses of both cone types as white and grey do. Hence, the perception of white and grey shades is indistinguishable from the monochromatic light at the neutral point for a dichromat. This phenomenon could explain at least some of the inconsistencies in the early colour vision studies on dogs and horses since some green and blue colours are located very close to the neutral point in the dichromatic colour space and therefore difficult to discriminate from grey.

One question that arises with the neutral point is whether it splits up the one-dimensional colour space in two parts, each with one colour, the saturation of which increases towards the end points (Fig. 8b), or alternatively the dichromats might perceive the colour at the neutral point as any other colour they perceive and the entire colour range as a continuum (Fig. 8c).

Hemmi (1999a) suggested after a behavioural experiment on tammar wallabies, *Macropus eugenii*, that dichromats perceive their chromatic space as a continuous scale of colours. His wallabies showed tendencies of relative colour learning and we became curious and wanted to explore this further. Our own behavioural experiments on horses (Paper 2) are in agreement with Hemmi's hypothesis. We used horses as model species since the colour vision ability of horses has been confirmed many times (Grzimek, 1952; Pick et al., 1994; Macuda & Timney, 1999; Smith & Goldman, 1999). From our results it is clear that horses treat the colours at the neutral point as any other colour they can perceive, which demonstrates that they perceive a continuous colour space.

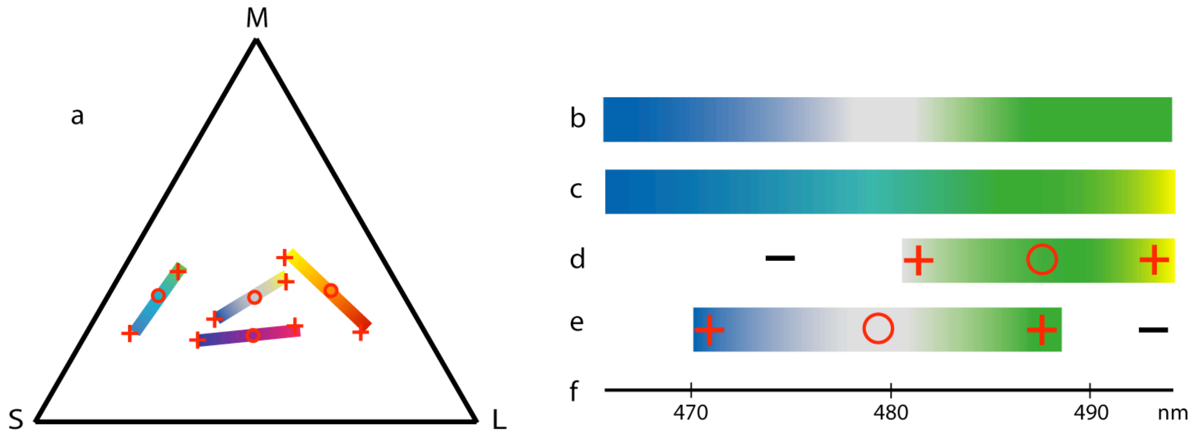

Figure 8. Illustration of a trichromatic colour space (redrawn from Jones et al., 2001) and suggested dichromatic colour spaces (Paper 2). Chicks were turned into trichromats by excluding UV light from the experimental set-up (a). A red cross indicates a positive stimulus in a dual choice experiment and a red ring indicates the test colour. The chicks generalized between all combinations of positive colours shown in the triangle except between blue and yellow, which are located on opposite sides of the grey point (Jones, Osorio & Baddeley, 2001). In dichromats, the neutral point has been suggested to divide the colour space into two colour categories (b; Jacobs & Deegan, 1994). Hemmi (1999a) suggested that a continuous scale of colours should be perceived (c). Two dual choice experiments (d and e) were performed on horses where a red cross indicates a positive stimulus and a black bar the negative stimulus. Red rings correspond to colours only presented in tests. All horses generalized between the positive colours. Note that in (e), the test colour coincides with the neutral point of horses (480 nm). All colours in the figure are vizualized as they appear to humans and the corresponding wavelengths for the horse are also calculated (f).

Our results show that the dichromatic colour space of horses differs qualitatively from the trichromatic colour space, which has been studied in similar experiments on chicks. Chicks are tetrachromatic like most other birds, but by excluding all UV light in the experiment the chicks were left with trichromatic colour vision (Jones et al., 2001). When the chicks were trained to two similar colours such as red and yellow they preferred the intermediate colour, orange, in tests. However, when trained to blue and yellow they refused to choose the intermediate wavelength, grey, suggesting that they treat the grey point as achromatic just as trichromatic humans do (Fig. 8a).

In our experiments we found that the horses could learn to generalize over their neutral point located at 480 nm (Geisbauer et al., 2004) just as well as they could generalize between colours located on the same side of the neutral point (Fig. 8d-f; Paper 2). Thus they treated colours at the neutral point as any other colour they can perceive.

For human dichromats, the perceived (more accurately, the reported) colour depends on the luminance. At moderate luminance, colours at the neutral point in the spectrum are reported as green, and with increasing luminance changes to be grey and white (personal communication Thomas Wachtler; Wachtler, Dohrmann & Hertel, 2004). Wachtler's results together with Hemmi's study support that the conclusions drawn from our results may be generally true for dichromats – the neutral point does not split up the dichromatic colour space, as earlier suggested by Jacobs and Deegan (1994) and as the grey point does for the trichromatic chicks and for us. Instead dichromats perceive a continuous scale of colours.

In addition, in our experiment the horses learnt colours in a relative manner, preferring in tests, the colour most different from the negative stimulus, even when novel colours were presented. Relative colour learning is a capability only found with one-dimensional chromatic spaces and has been indicated earlier in Hemmi's experiments on tammar wallabies. In his experiment, one wallaby that was supposed to choose white over different colours, avoided white when presented with colours of longer wavelengths than 490 nm (their neutral point; Hemmi, 1999a). This happened after the animal had been trained and rewarded to distinguish 640 nm from colours of shorter wavelengths. The wallaby had obviously learned the relative wavelength difference. Our result on horses and Hemmi's study on wallabies, suggest that relative colour learning may be generally true for dichromatic mammals.

DIM LIGHT VISION

Less Photons and more Noise

To see well in dim light puts the visual system at high demands. As the light intensity declines the photons become more and more scarce. The most severe constraint in dim light vision is noise: both the random arrival of photons, which becomes a relatively larger problem at lower light intensities and the thermal noise in the photoreceptor that creates a signal just as a photon does (Barlow, 1956). As the photons become scarce the signal-to-noise-ratio becomes smaller because of the low signal intensity. Making photoreceptors large with much photopigment increases the probability of light being absorbed and thus makes them more sensitive to light.

However, at the same time thermal noise becomes a larger problem in large photoreceptors since more pigment causes more spontaneous thermal activation (Warrant, 1999). Thermal noise in dark-adapted rods has been found to correlate with the absolute behavioural threshold (Aho et al., 1988). Aho and colleagues tested the absolute behavioural threshold for toads, man, and also for frogs at different temperatures and found that a lower body temperature lowered the rate of thermal noise and thus absolute threshold. Ectothermic animals such as nocturnal geckos active at low temperatures would thus benefit from a lower level of thermal noise in the photoreceptors.

However, even though the signal properties of cones and rods are similar, the cone pigment has been shown to isomerize much more often spontaneously than rod pigment (Kefalov et al., 2003). This difference is suggested to be due to the looser binding of the cone chromophore (Ala-Laurila et al., 2004). The looser chomophore pocket in the cone opsin assures rapid regeneration in bright light, to the price of the thermally less stable pigment generating high levels of noise.

Besides having larger photoreceptors than diurnal geckos, nocturnal species also have photopigment peaks shifted towards shorter wavelengths relative to diurnal reptiles (Table 2 and 3). The most probable reason for this pigment shift towards shorter wavelengths is thermal noise. It was early suggested that a displacement of the maximum sensitivity of a pigment towards shorter wavelengths could make it more stable towards thermal noise (Barlow, 1957). Donner and colleagues (1990) investigated the bullfrog retina where the rods of the dorsal part contain mostly porphyropsin ($\lambda_{max} \approx 523$ nm) while those of the ventral part contain rhodopsin ($\lambda_{max} \approx 502$ nm). They found eight times more dark noise in the bullfrog porphyropsin rods than in rods with rhodopsin. When they compared the bullfrog results with earlier results from porphyropsins of sturgeons ($\lambda_{max} \approx 538$ and 549 nm) the thermal noise was again found to be higher in pigments of longer wavelength sensitivity maximum. These results suggest that there should be an advantage for nocturnal animals to shift their long-wavelength-sensitive photopigments towards shorter wavelengths obtaining less thermal noise and a stronger signal-to-noise ratio.

This shift in pigment sensitivity could also be an adaptation to the shift towards the shorter wavelengths of the light during twilight (Munz & McFarland, 1973; Johnsen et al., 2006). However, since the spectrum of moonlight and starlight are spectrally neutral or shifted towards longer wavelengths, compared to sunlight, the advantage of a shorter wavelength peak in the middle-wavelength-sensitive pigment would be limited to the short dawn and dusk periods.

Optical and Anatomical Adaptations

As mentioned before, an optical adaptation to dim light intensities is to dilate the pupil, allowing more light to enter the eye. Obviously since the aperture cannot be larger than the eye diameter, large eyes are common among animals that depend on vision in dim light. However, large eyes are costly and the size of the skull is usually the limiting element. The problem is most obvious for small nocturnal animals, which need relatively larger eyes and thus do not follow common allometric functions. One example is the nocturnal owl monkey (*Aotus trivirgatus*), which is a member of the new world monkeys, with most of its skull consisting of eye sockets. Similarly, the small nocturnal geckos have eyes which are very large relative to their head and body size (Fig. 9; Werner, 1969; Werner & Seifan, 2006).

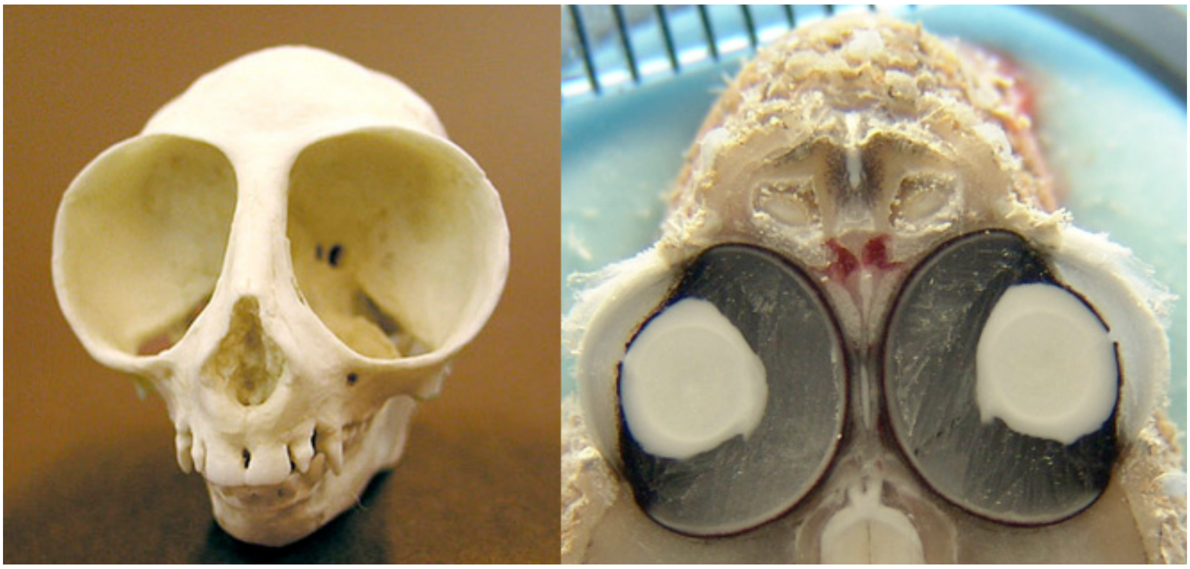

Figure 9. In the nocturnal owl monkey, *Aotus trivirgatus* (left), and the nocturnal helmet gecko, *Tarentola chazaliae* (right), the eyes take up most of their skulls in order to have large pupils at night allowing more photons to enter the eye (owl monkey picture with kind permission from Bone Clones, USA; gecko picture from Paper 4).

One fast comparison of the sensitivity of different eyes is to use F-numbers (focal length/pupil diameter; Table 1). The arrhythmic horse, for instance, has one of the largest eyes among extent land mammals (Walls, 1942) with an F-number indicating that it is much more light sensitive than e.g. humans. For a more extensive comparison, including both eye and photoreceptor dimensions, the optical sensitivity of single receptors for white light by Warrant and Nilsson can be used (1998; Eqn. 2; Fig. 10). The optical sensitivity equation gives a value of the photons absorbed by a photoreceptor when looking at an extended source of white light, where k is the absorption coefficient of the receptor, A is the pupil diameter, d and l is photoreceptor outer segment diameter and length and f is the focal length (Land, 1981). The value obtained from the equation can then be compared between animals.

$$S_w = (\pi/4)^2 A^2 (d/f)^2 (kl/(2.3+kl)) \qquad \text{Eqn. 2}$$

According to equation 2, the horse has almost a tenfold more light-sensitive eye than humans when the rods operate at night, suggesting that horse achromatic night vision is superior to ours (Table 4). However, calculating on the small cone outer segments in the horse (4 μm) at colour vision threshold (0.02 cd/m^2, Paper 3), instead of the longer rod outer segments, the sensitivity of horse cone vision (0.1 μm^{2}sr) is similar to human cone vision (0.08 μm^{2}sr) at the threshold. Another interesting comparison, between the very large eye of the horse and the very small eye of the gecko, shows that the gecko eye still is about 300 times more light-sensitive (Table 4).

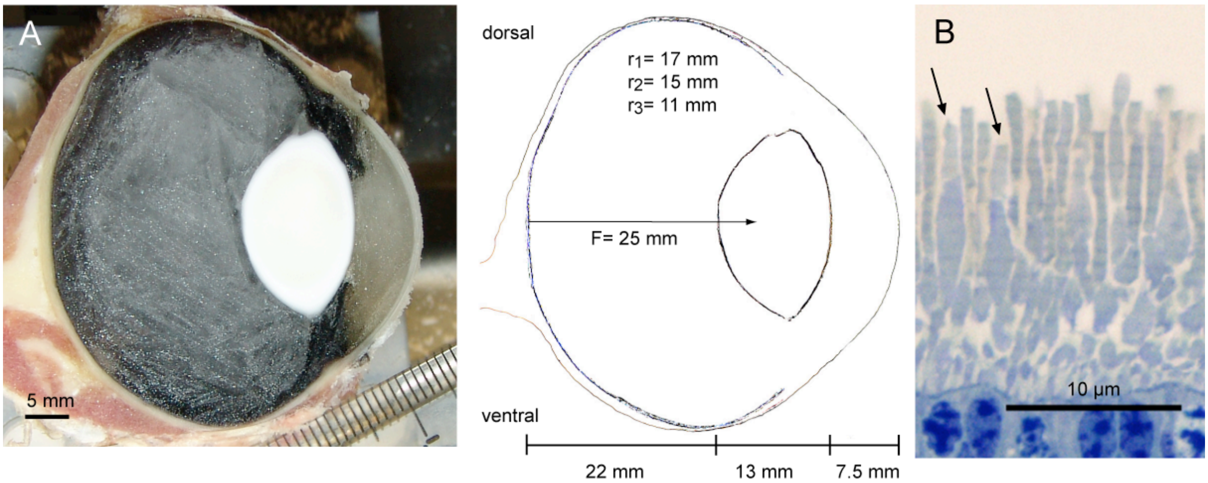

Figure 10. The horse is in possession of one of the largest eyes (A), in absolute size, among terrestrial vertebrates. Their photoreceptors (B) are however small and slender where the outer segments of the bulkier cones (arrows) have a diameter of 1 μm and a length of 4 μm (Paper 3).

An additional optical feature that increases absorption of the rare photons in dim light is the tapetum lucidum. A tapetum is composed of reflecting cell layers functioning as a mirror in the back of the eye and it is the tapetum that makes the animals' eyes glow from the headlights of our car as we (hopefully) drive past them at night. The tapetum gives the non-absorbed photons a second chance to be captured by the photoreceptors and thereby enhances the visual sensitivity. Tapeta are found in many vertebrates that are active during dim light conditions even though the composition and location of the reflecting cells differs. The eye-shine varies between groups of animals and even within a species, depending on age, since the tapetum takes a couple of months to mature (Ollivier et al., 2004). However in bright light a tapetum decreases the visual acuity and is therefore absent for example in most primates, squirrels or birds, which usually are diurnal.

Table 4. The optical sensitivity of single photoreceptor for white light

	Human[A] Max. threshold	**Human**[A] Colour threshold	**Horse**[B] Max. threshold	**Horse**[B] Colour threshold	**Helmet gecko**[D] Max. threshold	
Absorption coefficient, k	0.028	0.035	0.035	0.035	0.035	
Pupil diameter, A (μm)	8000	7000	30000	30000	4600	(4000)
Focal length, f (μm)	16700	16700	25000	25000	3.4	
Photoreceptor diameter, d (μm)	1.5	1.5	1	1	10[E]	
Length of outer segment, l (μm)	30	30	20[C]	8[C]	35	
Optical sensitivity for white light, S_w	0.09	0.08	0.13	0.1	38	(30)

The maximum optical sensitivity of single photoreceptor and the optical sensitivity at colour vision threshold for white light (Eqn. 2; Warrant & Nilsson, 1998) in the diurnal human and the arrhythmic horse, *Equus caballus*. In the nocturnal helmet gecko, *Tarentola chazaliae*, sensitivity at maximum threshold and within brackets values at intensity with confirmed colour vision are shown. [A] Land (1981), Warrant and Nilsson (1998) and Wyszecki and Stiles (1982). An average of the human photoreceptor diameter in parafovea and perifovea is used. [B] Warrant and Nilsson (1998) and Paper 3. [C] Twice measured value because of the reflecting tapetum in the back of the retina. [D] Warrant and Nilsson (1998) and Paper 4. [E] Summed average diameter of the double cones.

Neural Adaptations

In addition to optical mechanisms and adaptations of the receptors, neural adaptations can also enhance sensitivity. One neural way to improve the signal-to-noise ratio in dim light is to sum the signals from several neighbouring photoreceptors. The ganglion cells will then receive a much more reliable signal even though the price of this summation is a loss of spatial resolution, which can be thought of as fewer but larger pixels in the image (Pirenne & Denton, 1952).

Another neural strategy to improve vision in dim light is to sum the signals in time, comparable to a longer shutter time in a camera. A brighter image is obtained but motion will be blurred as the integration time increases (Lythgoe, 1979).

Animals that need to see well in dim light are found to benefit from sacrificing both spatial and temporal resolution to various degrees depending on their lifestyle and visual needs in dim light. Humans have been known for a long time to pool neighbouring rod signals to generate a stronger visual signal in dim light (Pirenne, 1948). The sensitivity values in Table 4 on maximum threshold in humans and the horse are therefore underestimated since signal summation is ignored. Analytical models also suggest, that sit-and-wait predators such as toads, preying on small slowly moving arthropods, just like the helmet geckos do, should benefit mostly from sacrificing temporal resolution (Warrant, 1999). This seems also to be the reality since very long integration times of around 1.6 sec have been measured for toad photoreceptors in behavioural experiments under dark conditions (Aho et al., 1993).

The nocturnal geckos have adapted their cones to be more sensitive (wide and long outer segments) and less noisy (shorter maximum wavelength sensitivity peak) but since the cone:ganglion cells ratio is close to one, there might not be much spatial summation in the retina (Röll, 2001a). If there is, photoreceptor signals may be summed using a “running average” which could reduce resolution to a slightly smaller degree. In addition this should allow them to change the degree of summation with intensity. Apart from possible spatial summation, the temporal summation together with the optically very light-sensitive eye will brighten the retinal image and enhance the visual signal a great deal in dim light. But is this enough to utilize colour information at night?

COLOUR VISION IN DIM LIGHT

For a long time it was thought that animals, just like humans, sacrifice colour information at night to benefit absolute sensitivity. However, this preconception has turned out to be wrong. After a behavioural study by Kelber and colleagues (2002) on three nocturnal hawkmoth species; *Deilephila stellatarum*, *Hyles liniata* and *Hyles gallii*, it was obvious that these animals use the valuable colour information even at night. At intensities corresponding to dim starlight (0.0001 cd m^{-2}), when it became difficult for the handler to discriminate the animals, the hawkmoths still chose the positive colour stimulus. Among night-active invertebrates nocturnal colour vision turned out to be more common than earlier thought and recently also the Indian carpenter bee, *Xylocopa tranquebarica* has been shown behaviourally to see colours at night (Somanathan et al., 2008).

However, invertebrates do not have a dual retina and their phototransduction is faster and less noisy than in vertebrates (for review see Hardie & Raghu, 2001). Therefore nocturnal colour vision in invertebrates is less surprising. My main question in this thesis is whether there are any vertebrates that just like these nocturnal invertebrates make use of the colour information at night.

Nocturnal Colour Vision in Geckos

The nocturnal gecko eye is very light-sensitive compared to other vertebrate eyes (Table 4). Geckos also have three different photopigments in cones that are adapted to dim light intensities. The adaptations of gecko eyes for colour and dim light vision made us hypothesize that nocturnal geckos, such as the nocturnal helmet gecko, *T. chazaliae,* could discriminate colours at night.

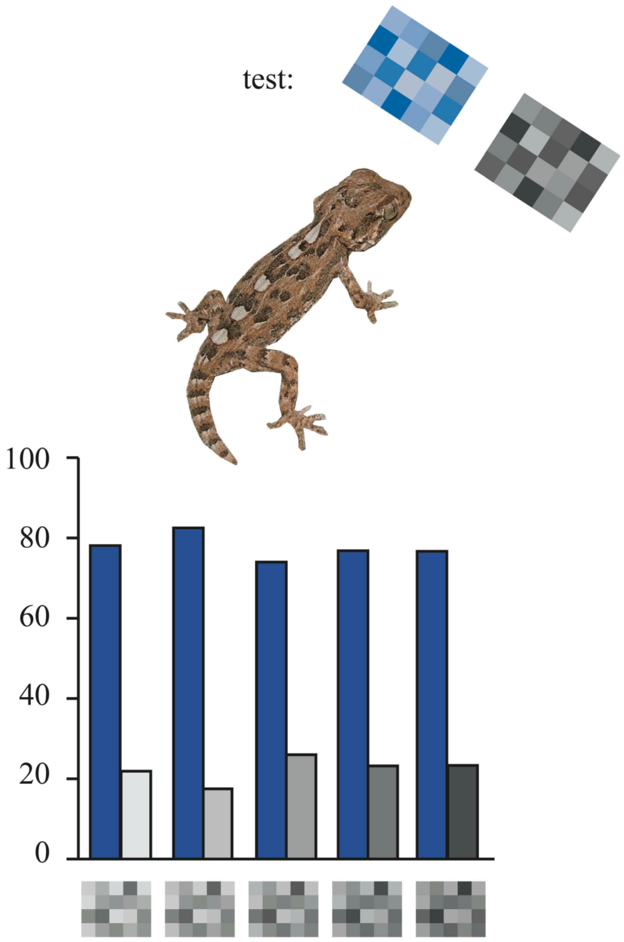

Figure 11. The choice frequency of two helmet geckos, *Tarentola chazaliae*, of which both could discriminate the blue pattern from all grey versions. On top is a helmet gecko with a positive blue stimulus and a negative grey (Paper 1; Kelber & Roth, 2006).

We used a method similar to that Wagner (1932) used in his experiment. We trained two animals to a positive blue chequered stimulus and a negative grey stimulus in light intensities of 0.002 cd m^{-2}, similar to dim moonlight. Grey stimuli were also produced in two darker versions and in two brighter versions to avoid any achromatic cues to be used by the geckos. Both geckos were able to discriminate the blue stimulus from all shades of grey. Even though human colour vision fails in these low light intensities our results show that the nocturnal geckos perceive colours in dim moonlight (Fig. 11; Paper 1; Kelber & Roth, 2006).

Colour vision in dim light is strongly limited by noise and when photons are scarce animals usually sacrifice colour vision to increase the visual sensitivity. However, in dim moonlight intensities, the optical properties and the large cones of the nocturnal gecko, makes the eye almost 400 times more light sensitive than the human eye at the lowest light intensities when humans see colour (Tabel 4; Paper 4). This difference alone could explain that the colour vision threshold of geckos is at least ten times dimmer than in humans. Note that we did not determine the threshold for gecko colour vision. Experiments at dimmer intensities are not easy to perform since the experimenter then has difficulties to see and since this type of experiment cannot be controlled automatically and is very time-consuming.

In addition to the optical adaptations of the gecko eye, strategies that normally enhance sensitivity could be used to generate more reliable colour vision signals at night, i.e. summing signals in time, sacrificing the temporal resolution, or in space by summing colour signals from neighbouring cells with the same photopigment.

Since adaptations that make nocturnal colour vision possible are quite common there might be other vertebrates that can use colour information at night? Toads and frogs have two types of rod with different photopigments. Hence, there is a possibility to compare signals and produce colour signals with the rods. Toads use colour cues for mate choice (Gnyubkin et al., 1975). They are mostly active at night and it has been suggested that they can make colour choices in dim light (personal communication Vadim V. Maximov). There is, however, nothing yet known published about amphibian rod colour vision and controlled behavioural experiments need to be performed before any conclusions can be drawn. It is possible that nocturnal geckos, even though lacking rods in a true meaning, may have followed a similar strategy sacrificing temporal resolution, just as the toads, to maintain colour vision at night. It would suggest that the vertebrate optics together with temporal summation makes colour vision in dim light possible and very useful. It would also suggest that it is beneficial to keep colour vision even at low light levels since a colour signal is always more robust to changes in the illumination than single photoreceptor signals (Kelber & Roth, 2006; Kelber et al., 2003).

Dichromatic Colour Vision in Moonlight

As mentioned before, mammals have lost two cone opsins during the nocturnal phase in the beginning of the mammalian evolution. This might be of advantage for animals active in a wide range of light intensities. When cone signals are compared to generate a colour signal photoreceptor noise adds up while the signal does not. Therefore, since dichromatic colour vision includes only one opponent system with two cone types to compare signals (and add noise from) it is more efficient and less noisy than a trichromatic colour vision system in dim light intensities. Thus, in low light conditions dichromacy allows for better stimulus discrimination than trichromatic or even tetrachromatic colour vision (Vorobyev, 1997).

Very little is known about the absolute threshold of colour vision in any mammal, except humans. Besides behaviourally confirming colour vision in dogs, Rosengren (1969) also tested the colour vision of one dog in twilight when human colour vision started to fail. The dog still managed to find the correct colour but as twilight deepened the number of incorrect choices increased and the dog was no longer able to discriminate colours (Rosengren, 1969). In our study on horses we behaviourally tested their colour vision threshold and compared it to that of humans (Paper 3). Surprisingly, horse colour vision failed at a similar light intensity as that of humans in our dual choice experiment. Both humans and horses in our study discriminated colours down to intensities corresponding to bright moonlight (Fig. 12).

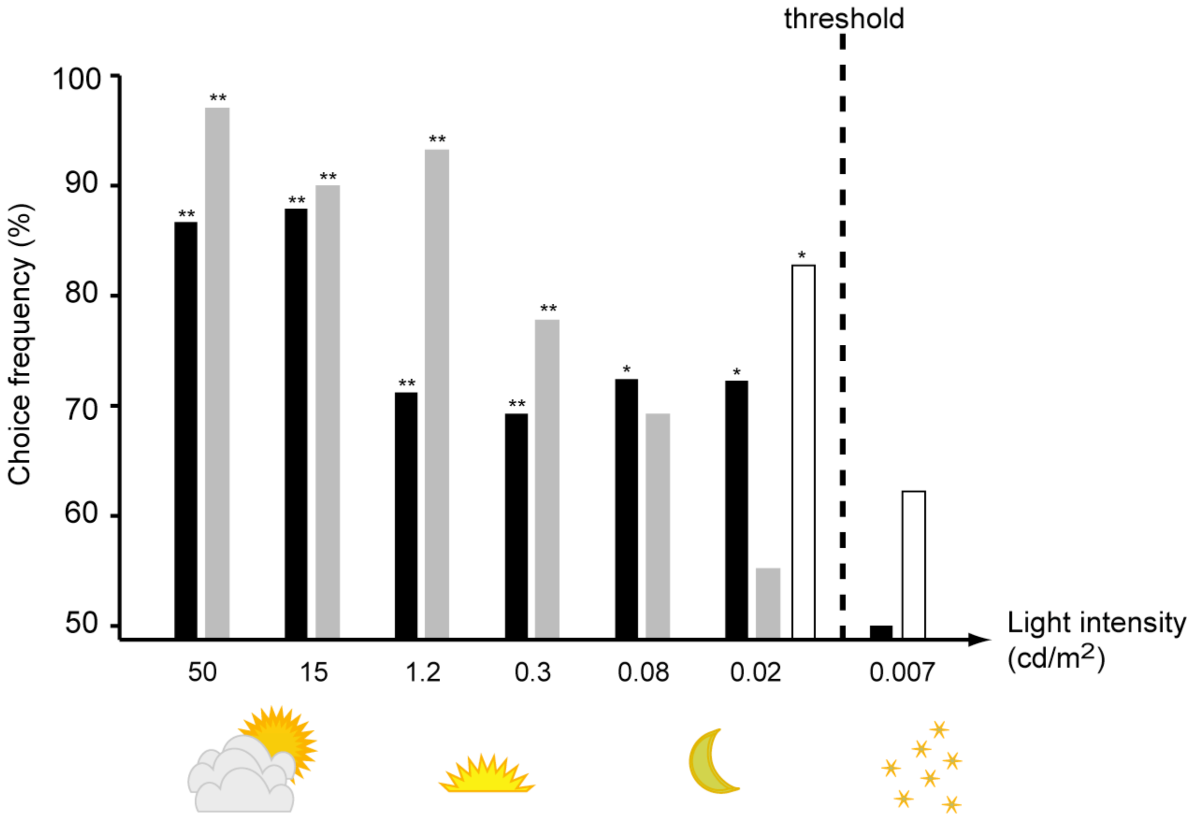

Figure 12. The choice frequencies at different light intensities for two horses (black and grey bars) and six human subjects (white bars show mean). The horse and humans have similar colour vision threshold and discriminate colours at a light intensity corresponding to bright moonlight (0.02 cd m^{-2}; From Paper 3). Binominal tests are for individual results; *P<0.05, **P<0.01).

The reason why horses cannot make use of the colour information at night might be found in the optical sensitivity of their eye (Eqn. 2). Calculating the optical sensitivity of cone vision, the horse eye is similar to the human eye (Table 4; Paper 3). In addition, the horse has, such as most non-primate mammals, a retina with a high rod-to-cone ratio without a rod-free area such as the inner part of the human fovea. Instead the ganglion cells are dispersed compared to the human retina. As spatial summation in dim light increases, the rod signals become stronger and the weakened colour signals fade. Hence, horse colour vision fails when the bright moon darkens but most certainly horses use their optically highly light-sensitive eyes for achromatic visual capabilities at night that puts human vision in the shade.

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