

Supplementary Material for:

The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals

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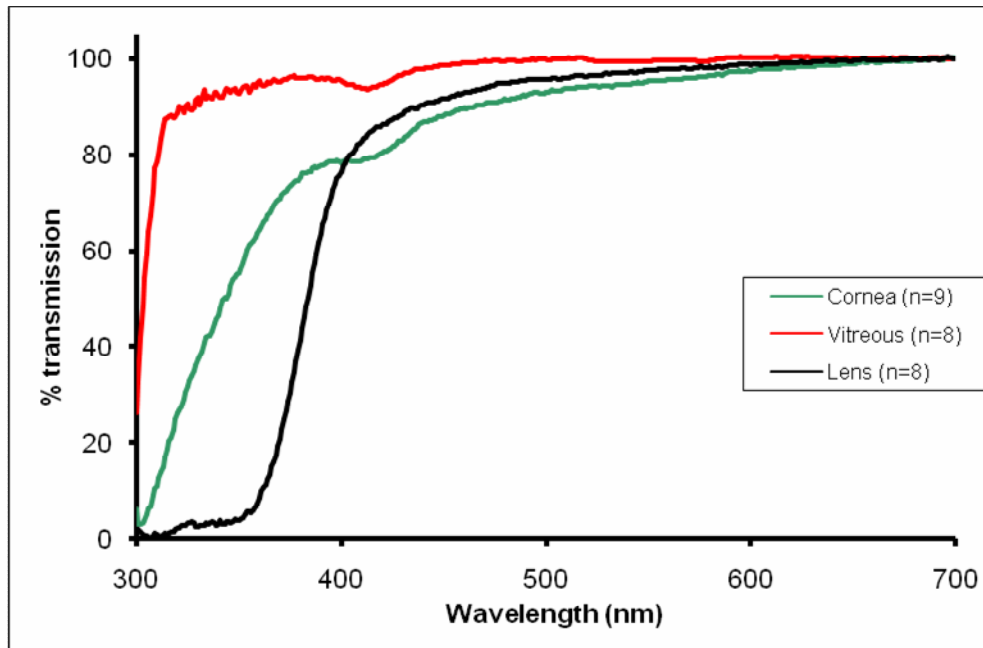
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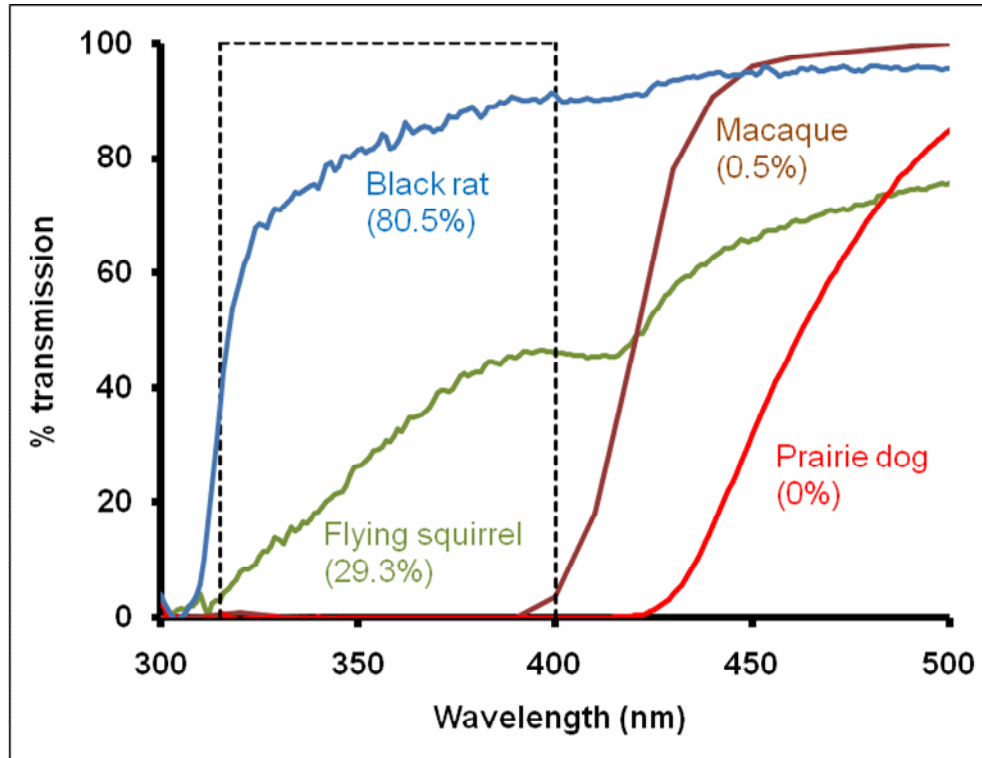
S1. Comparison of lens transmission to that of the aqueous humour and cornea.

Although lenses were examined in all 38 species, due usually to the small size of the eye, corneas were only scanned from 30 and the vitreous in only 2. In all animals tested the lens always removed more short wavelength radiation than either the cornea or the vitreous.



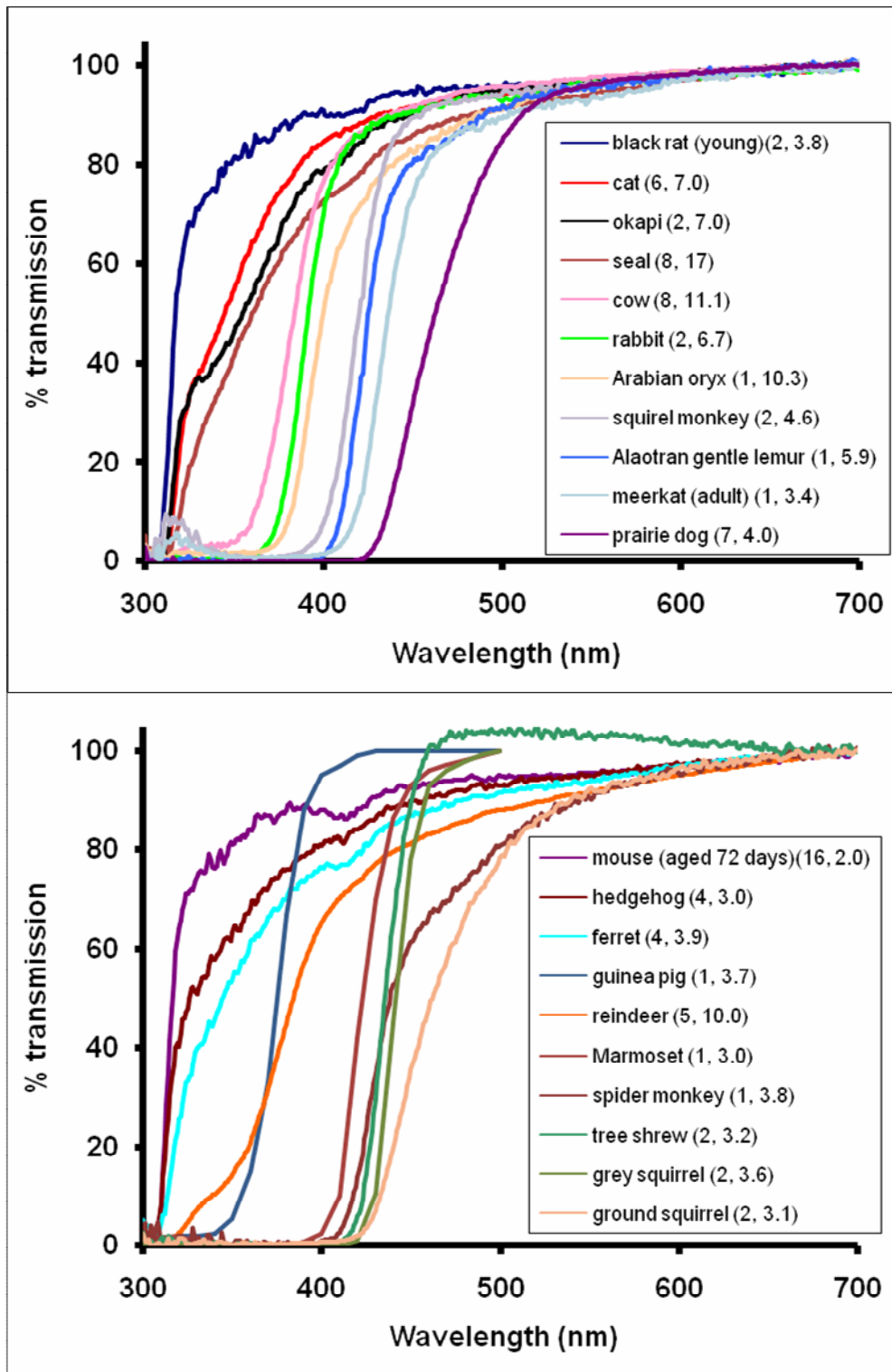
Supplementary figure 1 Spectral transmission of bovine ocular media. The small irregularities in the transmission of the cornea and vitreous around 400nm are caused by contamination with blood.

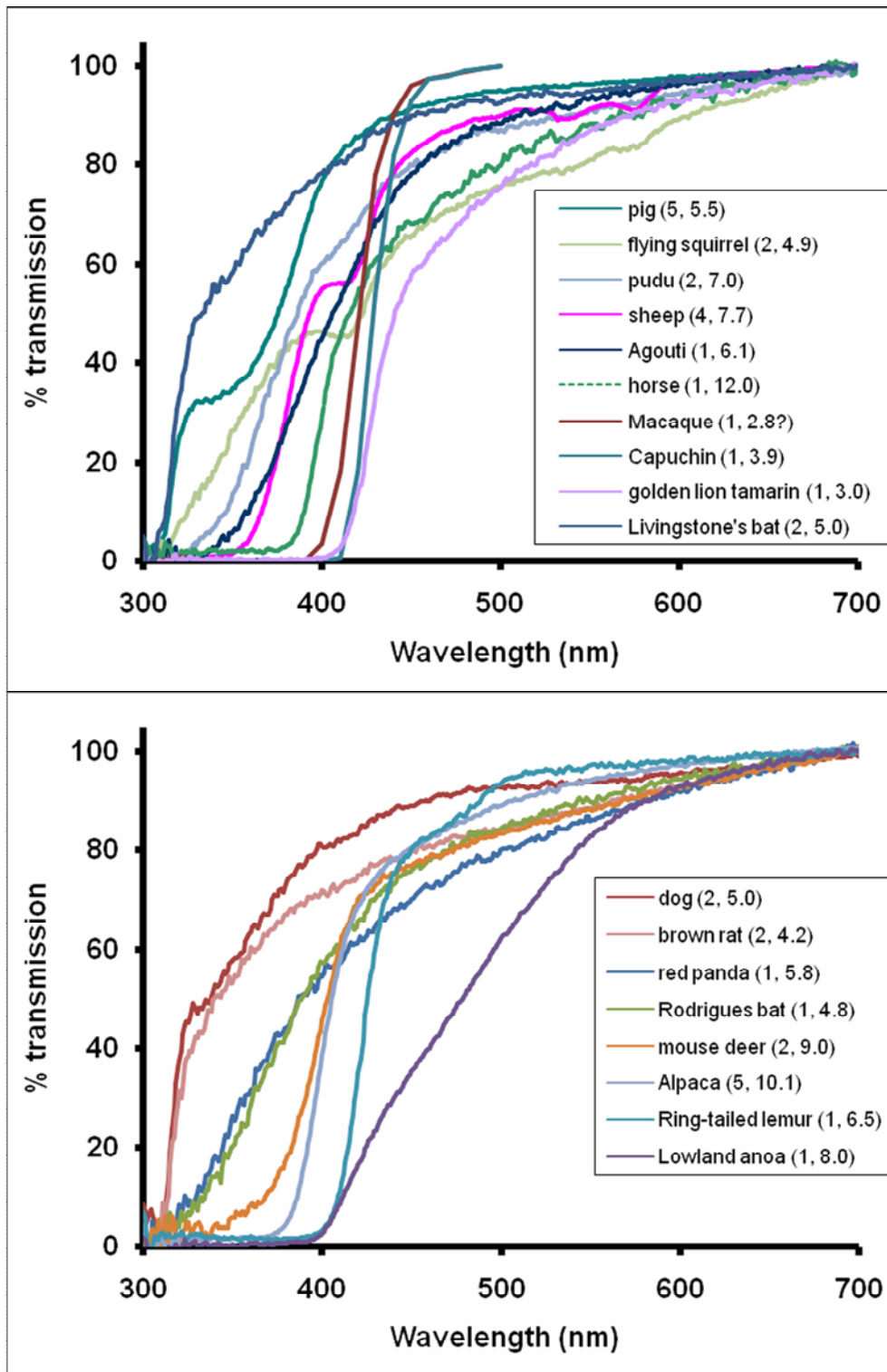
S2. Calculation of the proportion of UVA transmitted by the lens



Supplementary figure S2 The proportion of UVA transmitted by the lenses of four species of mammal. This represents the area under the curve for each species within the rectangle shown expressed as a percentage of the area of the entire rectangle. Equivalent values for all species are shown in table 1.

S3. Spectral transmission of lenses from all species examined





Supplementary figures 3a-d Spectral transmission of all mammalian lenses used in this study.

In most cases average scans of all available lenses are shown. However, in 4 species lenses of a variety of sizes were scanned whose spectral transmission differed (black rat, mouse, Livingstone's bat and meerkat). For these species the transmission of a specific lens size is shown. The legends indicate the number of lenses scanned that make up each curve and the average pathlength of the lens.

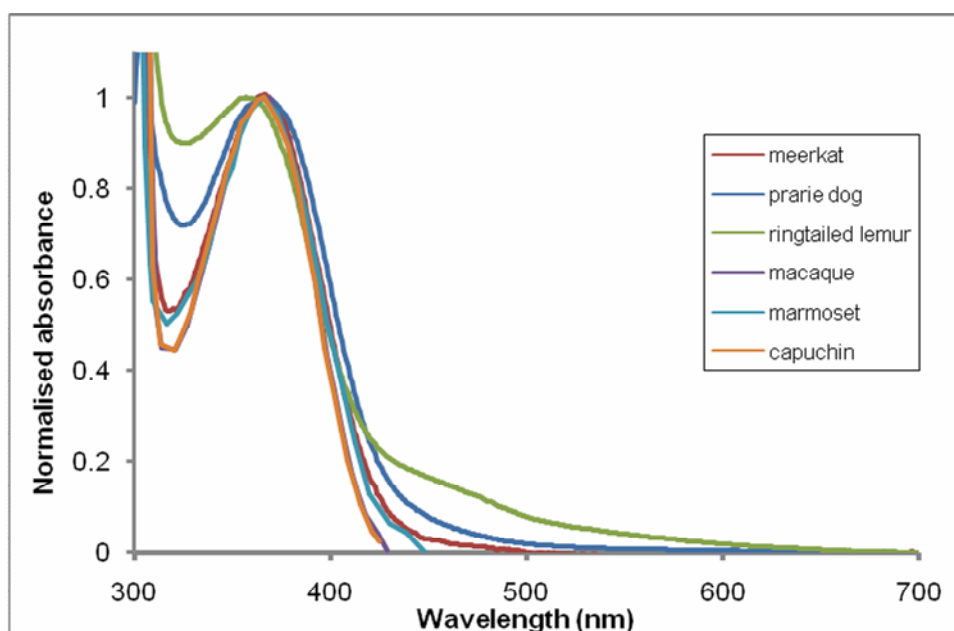
The data for 5 lenses (shown in figs S3b&c) came from a previous unpublished study in which lens transmission was zeroed at 500nm, while for all other lenses transmission at 700nm was set at zero. Zeroing at 500nm will steepen the curve somewhat and shift the wavelength of 50% transmission slightly towards shorter wavelengths.

S4. Extraction and identification of lens pigments

The lenses of grey squirrels and various primates (human, baboon, rhesus monkey, squirrel monkey & bush baby) contain pigments with absorption maxima around 360-370nm [1,2].

The nature of the pigment responsible for the visibly yellow colouration of lenses in six of the species studied here was investigated by homogenising lenses in distilled water and centrifuging (4°C, 100,000g) the homogenate for 10-30 minutes and scanning the supernatant. In some species the supernatant was further purified by ultrafiltration under nitrogen using Amicon YM2 membranes with pore size 100da fitted to a 50ml Amicon cell, before scanning.

We extracted pigments with absorption spectra similar to those described by Cooper & Robson [1,2] in the lenses of four more primates (macaque, marmoset, capuchin, ringtailed lemur), prairie dogs and meerkats (Fig S4).



Supplementary figure 4 Normalised absorbance spectra of aqueous extracts of the yellow lenses from 6 species of mammals

The pigments of primate and grey squirrel lenses have been identified as kynurenine-related tryptophan derivatives [3-11]. Similar pigments have been characterised in ground squirrels [12] and even some fish [13,14].

To confirm this identification in the macaque and marmoset their lyophilised lens pigment extracts were prepared for isocratic reverse-phase high performance liquid chromatography (HPLC) by redissolving ca. 5mg in 1ml 20% methanol and filtering through a C-18 Cep Pak cartridge. A Whatman 10 ODS-3 semi-preparative column (250x9.4mm internal diameter) protected by a Bondapak C-18 pre-column was fitted to the system. A mobile phase of 20% methanol and 0.1% acetic acid (filtered and degassed prior to use) was used as detailed by Dunlap et al. [15], with a flow rate of 2ml/min. Filtrate absorbance was monitored on a Knauer variable wavelength detector at 340nm and recorded on a Shimadzu C-R6A Chromatopac recorder. Up to 0.5ml of sample was injected into the system and any peaks were

collected, pooled and lyophilised. Dried purified samples were redissolved in 1ml 20% methanol and scanned on a Shimadzu UV-250 spectrophotometer. Pigments were identified by injecting an equal volume of a known standard with the sample and monitoring any increase in absorbance at the retention time of the standard.

The lens pigments of both the marmoset and macaque co-chromatographed with 3-hydroxykynurenine glucoside, the major pigment in human lenses, suggesting that perhaps all mammals with yellow lenses contain 3-hydroxykynurenine or a modified derivative of this molecule.

S5 Correlation between lens transmission and retinal morphology

Species	% UVAttransmitted	% cones	Peak cone density (mm ²)	Peak rod density (mm ²)
Mouse (<i>Musmusculus</i>)	81.4 ^a	2.8 ¹⁶	15,170 ¹⁶	505,208 ¹⁶
Black rat (<i>Rattusrattus</i>)	80.5 ^b	1.0-2.0 ¹⁷	-	-
Hedgehog (<i>Erinaceuseuropaeus</i>)	65.5	1.5-2.7 ¹⁸	6,500 ¹⁸	350,000 ¹⁸
Dog (<i>Canis lupus familiaris</i>)	61.3	-	23,080 ¹⁹	501,000 ¹⁹
Livingstone's fruit bat (<i>Pteropuslivingstonii</i>)	60.8 ^c	0.4-0.6 ²⁰	11,000 ²⁰	800,000 ²⁰
Cat (<i>Felis catus</i>)	58.9	-	26,500 ²¹	460,000 ²¹
Ferret (<i>Mustelaputoriusfuro</i>)	56.1	-	26,183 ²²	349,428 ²²
Brown rat (<i>Rattusnorvegicus</i>)	55.8	0.9 ²³	-	-
Okapi (<i>Okapiajohnstoni</i>)	53.4	-	-	-
Pig (<i>Sus scrofa</i>)	43.6	11.1 ²⁴	39,000 ²⁴	151,000 ²⁴
Guinea pig (<i>Caviaporcellus</i>)	34.6	8.0-17 ²⁵	30,000 ²⁵	290,000 ²⁵
Red panda (<i>Ailurusfulgens</i>)	30.2	-	-	-
Flying squirrel (<i>Glaucomysvolans</i>)	29.3	-	-	-
Rodrigues flying fox (<i>Pteropusrodricensis</i>)	28.1	0.4-0.6 ²⁰	11,000 ²⁰	800,000 ²⁰
Reindeer (<i>Rangifertarandus</i>)	26.5	-	-	-
Pudú (<i>Pudupuda.</i>)	25.0	-	20,600 ²⁶	-
Cattle (<i>Bospremigenius</i>)	22.1	-	21,000 ²⁷	250,000 ²⁷
Sheep (<i>Ovisaries</i>)	15.2	8.3 ²⁸	28,000 ²⁸	270,000 ²⁸
Agouti (<i>Dasyproctapunctata</i>)	15.0	10-19 ²⁹	14,000 ²⁹	64,000 ²⁹
Rabbit (<i>Oryctolagusuniculus</i>)	12.7	3.5-4.5 ³⁰	16,000 ³⁰	308,000 ³⁰
Java mouse deer (<i>Tragulusjavanicus</i>)	12.4	-	19,900 ²⁶	-
Arabian oryx (<i>Oryx leucoryx</i>)	8.5	-	32,700 ²⁶	-
Alpaca (<i>Vicugna pacos</i>)	6.0	-	-	-
Horse (<i>Equusferus caballus</i>)	4.6	<10 ³¹	20,000 ³²	170,000 ³³
Squirrel monkey (<i>Saimirisciureus sciureus</i>)	2.8	9.3 ³⁴	138,021 ³⁴	37,939 ³⁴
Ring-tailed lémur (<i>Lemurcatta</i>)	2.0	-	-	-
Meerkat (<i>Suricatasuricatta</i>)	1.7 ^d	-	-	-
Marmoset (<i>Callithrixjacchus</i>)	0.9	24.3 ³⁴	132,813 ³⁴	79,102 ³⁴
Lowland anoa (<i>Bubalusdepressicornis</i>)	0.6	-	-	-
Ground squirrel (<i>Urocitellusrichardsonii</i>)	0.6	85.5 ³⁵	49,550 ³⁵	13,000 ³⁵
Macaque (<i>Macacafascicularis</i>)	0.5	6.1 ³⁶	210,000 ³⁶	184,000 ³⁷
Spider monkey (<i>Atelespaniscus</i>)	0.4	-	-	-
Golden lion tamarin (<i>Leontopithecusrosalia</i>)	0.4	-	-	-
Alaotrangentlelemur (<i>Hapalemuralaotrensis</i>)	0.3	-	-	-

Treeshrew (<i>Tupaia glis</i>)	0.3	<i>94-96</i> ³⁸	<i>36,000</i> ³⁸	<i>3,500</i> ³⁸
Grey squirrel (<i>Sciurus carolinensis</i>)	0.0	<i>64.0</i> ³⁹	-	-
Prairie dog (<i>Cynomys ludovicianus</i>)	0.0	<i>89.3</i> ³⁹	-	-
Capuchin (<i>Cebus apella</i>)	0.0	<i>7.9</i> ³⁴	<i>164,062</i> ³⁴	<i>174,967</i> ³⁴

Supplementary table S5 Retinal parameters of species ranked in order of the amount of UVA that penetrates the lens.

Where possible retinal data from the same species used in this study are shown. If these were unavailable data were taken from species within the same genus (these values are shown in italics). In several instances more than 1 author produced retinal data for the same species. In such cases the most recent or comprehensive was used. There were some differences between various studies on the same species but not significant enough to affect the overall conclusions. The shaded values indicate arbitrary thresholds of more than 20% cones or a peak density of over 100,000 cones/mm², which are indicative of a retina specialised for high spatial acuity. Where the transmission of the lens varied with age, the % UVA on the retina was calculated using specific ages/lens sizes as follows; a - aged 69-72 days with lens pathlength 2.2mm, b – pathlength 3.8mm, c – pathlength 5.0mm, d – pathlength 3.4mm.

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