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Ecology drives spectral transmission in the ocular lenses of frogs and salamanders

Kate N. Thomas[1], David J. Gower[1], Jeffrey W. Streicher[1], Rayna C. Bell[2,3], Matthew K. Fujita[4], Ryan K. Schott[3,5], H. Christoph Liedtke[6], Célio F. B. Haddad[7], C. Guilherme Becker[8], Christian L. Cox[9], Renato A. Martins[10], Ron H. Douglas[11]

[1]Department of Life Sciences, The Natural History Museum, London SW7 5BD, UK

[2]Department of Herpetology, California Academy of Sciences, San Francisco, CA 94118, USA

[3]Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0162 USA

[4]Department of Biology, Amphibian and Reptile Diversity Research Center, The University of Texas at Arlington, Arlington, TX 76019, USA

[5]Department of Biology, York University, Toronto M3J 1P3, Canada

[6]Ecology, Evolution and Development Group, Department of Wetland Ecology, Estación Biológica de Doñana (CSIC), 41092 Sevilla, Spain

[7]Departamento de Biodiversidade and Centro de Aquicultura (CAUNESP), I.B., Universidade Estadual Paulista, C.P. 199, 13506-900 Rio Claro, SP, Brazil

[8]Biology Department, The Pennsylvania State University, University Park, PA 16802, USA

[9]Department of Biological Sciences and Institute for the Environment, Florida International University, FL 33199, USA

[10]Programa de Pós-graduação em Conservação da Fauna, Universidade Federal de São Carlos, São Carlos 13565-905 Brazil

[11]Division of Optometry & Visual Science, School of Health Sciences, City, University London, Northampton Square, London EC1V 0HB, UK

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Conflicts of Interest

None of the authors have any conflicts of interest.

Author Contributions

RHD, DJG, JWS and KNT designed the study with input from RCB and MKF. JWS, DJG, RCB, MKF, CFBH, CGB, RAM, CLC, KNT, and HCL collected samples. KNT, JWS, DJG, RCB, MKF, RKS, and CFBH assigned ecological traits to species. RHD and KNT collected spectral transmission measurements from lenses, analysed the data, and drafted the manuscript. HCL ran evolutionary model comparisons. All authors participated in revising the draft manuscript and gave final approval for publication.

Data availability statement

All data presented here and details for individual specimens (including museum catalogue numbers) are available from the Natural History Museum Data Portal: https://doi.org/10.5519/a7ficgr8 (Thomas et al., 2021). Genetic data used to help identify 19 species are accessioned on GenBank (MW830045-MW830063). R code to reproduce analyses is available at https://github.com/knthomas/amphibian-lens-transmission.

Abstract

1. The spectral characteristics of vertebrate ocular lenses affect the image of the world that is projected onto the retina, and thus help shape diverse visual capabilities. Here, we tested whether amphibian lens transmission is driven by adaptation to diurnal activity (bright light) and/or scansorial habits (complex visual environments).
2. Spectral transmission through the lenses of 79 species of frogs and 6 species of salamanders was measured, and data for 29 additional frog species compiled from published literature. Phylogenetic comparative methods were used to test ecological explanations of variation in lens transmission and to test for selection across traits.
3. Lenses of diurnal (day-active) and scansorial (climbing) frogs transmitted significantly less shortwave light than those of non-diurnal or non-scansorial amphibians, and evolutionary modelling suggested that these differences have resulted from differential selection.
4. The presence of shortwave-transparent lenses was common among the sampled amphibians, which implies that many are sensitive to shortwave light to some degree even in the absence of visual pigments maximally sensitive in the UV. This suggests that shortwave light, including the UV, could play an important role in amphibian behaviour and ecology.
5. Shortwave-absorbing lens pigments likely provide higher visual acuity to diurnally active frogs of multiple ecologies and to nocturnally active scansorial frogs. This new mechanistic understanding of amphibian visual systems suggests that shortwave-filtering lenses are adaptive not only in daylight conditions but also in scotopic conditions where high acuity is advantageous.

Keywords: Activity period, Anura, Caudata, diurnal, scansorial, sensitivity, UV

Introduction

Animal vision is extraordinarily diverse, and animals obtain different information from their external world depending on the optics, structure, and physiology of their eyes. Eyes may differ in, for example, the colours they can detect (spectral sensitivity), the level of detail they can resolve (spatial acuity), and the amount of light they can capture (sensitivity), and these differences affect how animals interact with each other and their environment (Land & Nilsson, 2012). When light passes through an eye, it may encounter ocular filters that change the spectrum of light that ultimately reaches the photoreceptors, and these are diverse in form and function across animals (reviewed in Douglas & Marshall, 1999). In vertebrates, as well as a few cephalopods (e.g. Thomas et al., 2017) and spiders (Hu et al., 2012, 2014), the lens or cornea can act as long-pass filters with important implications for sensitivity, acuity, and colour vision. Here, we examine the spectral characteristics of amphibian lenses and test whether aspects of ecology that affect visual environment and behaviour drive differences across species.

In a camera-type eye, the main function of the lens is to focus light from the external world onto the retina, yet spectral properties of lenses are also important in shaping vision. Light from the environment must pass through the ocular media (cornea, lens, humours) to activate the visual pigments within the retina for visual perception to occur. The longest wavelengths of light a vertebrate can see are determined by the spectral sensitivity of its visual pigments, because transmission by the ocular media is uniformly high at longer wavelengths (Douglas & Marshall, 1999). At short wavelengths (ca. 300–450nm), however, the limits of vision in vertebrates are determined primarily by the lens (Douglas & Marshall, 1999).

Light below approximately 300 nm will not reach the retina in any vertebrate because it is absorbed by the nucleic acids and structural protein components of the cornea, lens, and aqueous and vitreous humours (Douglas & Marshall, 1999). Between about 300–450 nm, however, ocular media transmission varies among species. In some vertebrates, all the ocular media are as transparent as biologically possible and transmit short wavelengths well. In others, the lens (and in some fishes, the cornea) contains pigments that absorb short wavelengths, preventing them from reaching the retina (Douglas & Marshall, 1999 for review). Species with shortwave-transparent ocular media presumably benefit from increased absolute sensitivity and potential perception of short wavelengths, including ultraviolet (UVA = 315–400nm). Species with pigmented ocular media, on the other hand, sacrifice sensitivity to protect the retina from damaging short wavelengths that can induce retinal toxicity (Glickman, 2011; Van Norren & Vos, 2016) and potentially improve spatial acuity by removing that part of the spectrum most prone to chromatic aberration and Rayleigh scattering (Douglas & Jeffery, 2014). Thus, the balance of costs and benefits of shortwave-filtering lenses should vary across species that are active in distinct light environments or have different visual needs, potentially leading to different evolutionary trajectories for spectral lens transmission.

Shortwave-absorbing ocular filters are found in the lenses of diverse species across all major vertebrate groups examined, including fishes (Douglas & McGuigan, 1989; Thorpe et al., 1993; Siebeck & Marshall, 2000, 2001, 2007), humans (Kessel et al., 2010) and other mammals (Douglas & Jeffery, 2014), reptiles (Simões et al., 2016; Gower et al., 2019), birds (Lind et al., 2014; Olsson et al., 2021) and frogs (Yovanovich et al., 2019, 2020). However, we currently have little understanding of the forces driving the evolution of lens pigmentation in any group, and only recently have studies started to use phylogenetic comparative methods to investigate the evolution of this trait (e.g. Yovanovich et al. 2020, Olsson et al. 2021). To our knowledge, no study has found evidence for correlated evolution of lens transmission and any aspect of ecology. Considering the important implications of spectral lens transmission for acuity, sensitivity, and colour vision, studying the distribution and evolution of this trait not only provides insights into how ecology drives sensory evolution, but also into the potential visual capabilities of diverse animals.

Previous surveys have highlighted two factors likely to be important in the evolution of shortwave lens transmission in vertebrates. Many vertebrates with shortwave-transparent lenses are nocturnal, presumably to maximize absolute sensitivity in scotopic environments, while most vertebrates with pigmented lenses that absorb short wavelength light are diurnal. For instance, low shortwave transmission has been observed in the lenses of diurnally active species of birds (Lind et al., 2014), mammals (Douglas & Jeffrey, 2014), snakes (Simões et al., 2016), and fishes (Douglas & McGuigan, 1989; Thorpe et al., 1993; Seibeck & Marshall, 2001; Seibeck & Marshall, 2007). Exceptions are mesopelagic fishes (and squids), which live in the equivalent of permanent nocturnality, yet have lenses containing shortwave-absorbing filters that are presumed to increase the visibility of bioluminescent signals and camouflage (Douglas & Thorpe, 1992; Muntz, 1976; Thomas et al., 2017). Lens pigmentation has also been noted in vertebrates with morphologies, physiologies, and behaviours associated with high acuity, such as mammals with high cone densities in the retina (Douglas & Jeffrey, 2014), raptors with large tubular eyes and high electrophysiological spatial resolution (Lind et al., 2014; Gaffney & Hodos, 2003), and snakes that are highly visual hunters (Simões et al., 2016). Thus, explanations for the scattered presence of vertebrate lenses containing shortwave-absorbing pigments rely mostly on loose or anecdotal associations with ecological, anatomical, and physiological variables. Here, using extensive taxonomic sampling paired with phylogenetic comparative methods, we aimed to test whether activity period and the need for high acuity are forces driving the adaptive evolution of lens pigmentation in a vertebrate group.

Frogs (Amphibia: Anura; includes toads) and salamanders (Amphibia: Caudata; includes newts) exhibit a range of ecologies and natural histories that make them good candidates for testing hypotheses about the adaptive evolution of lens transmission. Although predominantly nocturnal, amphibians have repeatedly evolved diurnal activity patterns across diverse families (Anderson & Wiens, 2017) and therefore may benefit from the protection of shortwave-absorbing pigments in their lenses. Further, many frogs inhabit ground- and water-associated habitats, but scansorial species specialized for climbing and jumping in foliage may require high spatial acuity for navigating visually complex environments. Therefore, scansorial species may benefit from the increased acuity that lens pigmentation can provide. Little previous work on amphibian lens transmission has been carried out. Recent surveys of spectral lens transmission among 36 of the approximately 7,370 living species of frogs reported similar variation to that observed among other vertebrates, but perhaps surprisingly found no correlations with potential ecological drivers of lens pigmentation, including activity pattern (Yovanovich et al., 2019, 2020).

The aims of the current study were to measure spectral lens transmission in an expanded sample of ecologically and phylogenetically diverse amphibians in order to test several explanatory hypotheses, including 1) that in amphibians, lens transmission is not driven by ecology and species variation is primarily explained by phylogeny (Yovanovich et al., 2020), 2) that diurnal activity drives low shortwave lens transmission as an adaptation to bright environments, and 3) that scansorial behaviour drives low shortwave lens transmission as an adaptation to complex visual environments where high acuity is advantageous. We measured ocular lens transmission in 79 species of frogs and six species of salamanders distributed within 29 families, and, together with published data for 29 anuran species, used phylogenetic comparative methods to test for ecological and evolutionary drivers of spectral transmission in amphibian lenses.

Materials and Methods

Animals

The spectral transmission (300–700 nm) of 171 lenses from 123 post-metamorphic individuals belonging to 85 species of frogs (n = 79) and salamanders (n = 6) was examined (Table 1). Both lenses from two tadpoles (*Dendropsophus minutus* and *Alytes muletensis*) were also measured. Most animals were collected in the field, although 40 specimens representing 27 species were obtained from commercial dealers or captive colonies as noted in Table 1 (see also archived data), with ethical approval and collection permits from relevant organizations for each site (see Acknowledgments for granting bodies and project numbers). Animals were sacrificed by immersion in 5g/L Tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate to a pH of 7.0–7.5 for approximately 5–10 minutes. Lenses were removed from enucleated eyes and frozen dry, then transported to City, University of London (UK) on dry ice for scanning. In most cases, species were identified based on morphology, but mitochondrial 16S rRNA molecular barcoding was used to assist identifications for 19 specimens. Briefly, DNA was extracted from adult muscle or liver tissue and tadpole tail snips, then universal 16S AR/BR primers were used to amplify and sequence a ~580 bp fragment following Jameson et al. (2021). Sequences were aligned and cleaned using Geneious Prime v.2019.2.3 and species identities confirmed using an initial NCBI BLAST search of GenBank followed by reference sequence verification using primary taxonomic literature. Spectral lens transmission data from fresh lenses of 29 additional species of frogs were obtained from published literature (Yovanovich et al. 2019, 2020).

Transmission measurement

Lenses were thawed, briefly rinsed in phosphate buffered saline (PBS) and placed in an aluminium holder that fit into a cuvette mounted in front of an integrating sphere within a Shimadzu 2101 UVPC spectrophotometer. The lenses were positioned within holes in the aluminium blocks, ranging in diameter from 1.0–9.0 mm in 0.5 mm increments, so that they were in line with the measurement beam of the spectrophotometer (Supplementary Material S1). Several lenses were small (equatorial diameter <2mm; Table 1), so the scans were sometimes noisy, in which case data from multiple scans per lens were averaged.

To further reduce noise, the average scan for each lens was smoothed by an 11-point running average and transmission at 700 nm taken as 100%. The only exceptions were lenses from four species (*Ambystoma mexicanum*, *Cynops pyrrhogaster*, *Lithobates pipiens*, *Salamandra salamandra*), which were scanned from 300–500 nm as part of an earlier study

(Thorpe, 1991) and were normalised at 500 nm. Some lenses were slightly smaller than the hole in the holder in which they were placed, especially those that were < 1 mm in diameter. This resulted in some light bypassing the lens, so transmission did not reach zero at 300 nm as it should, since wavelengths below this do not penetrate the structural components of the lens (Douglas & Marshall, 1999). Thus, all scans were further adjusted so that transmission at 305 nm was 0%. Lens transmission is expected to be high and approximately spectrally flat at longer wavelengths (Douglas & Marshall, 1999), so scans from lenses whose transmission at 450 nm was below 70% were assumed to be compromised and excluded (see Supplementary Material S2).

Supplementary experiments indicated freezing and thawing had no effect on the spectral transmission of *Rana temporaria* lenses (Supplementary Material S3). This view is supported by the observation that there was little difference between the transmission spectra of fresh lenses measured in a previous study (Yovanovich et al., 2020) and those measured here in previously frozen lenses of the same species (*n* = 7, Supplementary Material S4).

Right and/or left lenses were scanned. When both lenses from the same animal were scanned, there was no systematic difference between them and the adjusted curves from each lens were averaged to find mean lens transmission for the specimen. Post-metamorphic individuals sampled within a species were then averaged to represent the spectral transmission of the lenses for that species. The only exception to this was *Hylodes phyllodes*, where a larger and a smaller individual were kept separate because they showed substantially different transmission spectra (Fig. 1b).

The degree of shortwave transmission by a lens is expressed in this study by both the wavelength of 50% normalized transmission (t50) and the total amount of UVA light transmitted by the lens (%UVA). Although t50 is the traditional measure of lens transmission, we believe the %UVA gives a more accurate representation of a lens's overall transmission at short wavelengths (Supplementary Material S5).

Measurement of lens size

The amount of shortwave radiation transmitted by a lens depends not only on the concentration and identity of any shortwave-absorbing pigment within it but also on its axial length (thickness/pathlength), with larger lenses transmitting less light (Douglas & Marshall, 1999). Thus, it is important to determine the dimensions of each lens as closely as possible. Although aquatic tadpoles have lenses that are close to spherical, the lenses of terrestrial adult amphibians are to some degree flattened, with the equatorial diameter exceeding the axial length (Sivak et al., 1985). Because thawed amphibian lenses are somewhat fragile and prone to lose their shape when manipulated, pathlength could not be measured directly, but we were able to approximate equatorial lens diameter by the size of the hole in the mounting holder in which the lens fit most closely without deformation (see above; Fig. S1). Although the degree of asphericity of the adult amphibian lenses varies with species, the equatorial diameter is never more than 22% greater than the axial dimension and is usually significantly less than this (e.g. Sivak et al., 1985; Volonteri et al., 2017). Thus, any error involved in estimating equatorial rather than axial diameter will be relatively modest.

Species traits

Adult ecology was categorized into binary states for activity pattern and habitat using peer-reviewed literature, online natural-history resources, field guides, and field observations: 1) diurnal or non-diurnal, and 2) scansorial or non-scansorial (Table S1). Categorizations were simplified versions of those used by Thomas et al. (2020). Species were classed as diurnal if adults are primarily active in daylight above ground; crepuscular, cathemeral, and nocturnal species were classified as non-diurnal. Species were categorized as scansorial if adults are primarily found climbing among plants. Ideally, we would have scored species for visual acuity directly, but such data are unavailable for the vast majority of amphibians (see Discussion). Scansoriality was a readily scored trait that is likely reliant on visual acuity for navigating complex environments and for precision jumping.

Phylogeny

Comparative analyses used the phylogenetic hypothesis of Jetz and Pyron (2018), which combined a molecular phylogeny of 56% of extant amphibians with taxonomic inference of remaining species to produce a full amphibian species tree. Tip labels of the phylogeny were matched to species in the dataset with AmphiNom v.1.0.1 in R (Liedtke, 2019), which uses the Amphibian Species of the World online database (Frost, 2021) to match taxonomic synonyms across datasets. The phylogeny was pruned to the species in the dataset and polytomies were resolved randomly with the multi2di function in ape v.5.4.1 (Paradis et al., 2004; Paradis & Schliep, 2019). Data were plotted alongside the phylogeny using ggtree v.2.4.1 in R (Yu et al., 2016; R Core Team, 2019).

Testing ecological explanations of variation in lens transmission

To test whether activity pattern or habitat are correlated with spectral lens transmission while accounting for evolutionary relationships, the comparative approach of Freckleton et al. (2002) was used. Phylogenetic linear models with maximum-likelihood (ML) estimation of phylogenetic signal (λ) were implemented with caper v.1.0.1 in R to test for differences in lens transmission (t50 and %UVA, separately) between binary ecological states (Orme et al., 2018). These models did not include lens size, because previously published transmission data did not include diameters of the lenses scanned (Yovanovich et al., 2019; 2020). Species means for adult lens transmission measured in this study (*n* = 85) and from the literature (*n* = 29) were both included in these models; however, because %UVA was defined as total transmission from 315–400 nm, %UVA for 10 species from the literature where transmission was not measured to 315 nm could not be calculated. This led to differences in species sampling for models using t50 (*n* = 114) and those using %UVA (*n* = 104). Analyses exploring the relationship between lens size, spectral transmission, and ecology can be found in the Supplementary Materials (S6).

Testing models of evolution across ecological groups

To test whether species with different ecological phenotypes are under different selective regimes for lens transmission, different models of evolution were fit to the lens transmission data and compared. These models included only the post-metamorphic species data collected in the present study (*n* = 85) so that lens size (approximating pathlength) could be accounted for as a covariate, and included identical species sampling for t50 and %UVA. First, ancestral states for each node of the phylogeny were estimated separately for activity pattern (diurnal or non-diurnal) and habitat (scansorial or non-scansorial). Node states were determined based on marginal likelihoods calculated from 99 stochastic character maps constructed using make.simmap in phytools v.0.7.70 (Revell, 2012), with Q set to "empirical" and an equal rates transition model. These reconstructions (Supplementary Materials S7) were then used to fit four models of the evolution of lens transmission (separately across activity patterns and habitats) with log lens size as a covariate in mvMORPH v.1.1.4 (Clavel et al., 2015): 1) a single-parameter Brownian motion (BM) model (BM1), 2) a single-parameter Ornstein-Uhlenbeck (OU) model (OU1), 3) a multiple-parameter BM model (BMM), and 4) a multiple-parameter OU model (OUM). The multiple-parameter models fit different parameter regimes to branches or clades reconstructed to have different activity patterns or habitat preferences. The best-fit model was determined by ranking Akaike information criterion (AIC) scores for each model, with a ΔAIC ≥ 2 between models considered a significant difference in model fits.

Results

Variation in spectral lens transmission across species

Post-metamorphic spectral lens transmission among the 85 amphibian species measured here varied substantially. The spectral transmission curves for the lenses of representative species highlighting the diversity of shortwave transmission are shown in Fig. 1, and those of all species measured are given in Supplementary Materials S8 (Figs. S8a–i). Table 1 gives further details of all species studied.

The lenses of 52% of the species examined showed no evidence of substantial pigmentation, displaying a smooth decline in transmission below ca. 350 nm and transmitting on average 56% of incident UVA (range 92%–37%). A few species' lenses (13%) contained high pigment concentrations resulting in a sharp decline in transmission around 390–420 nm, with little transmission of UVA (mean 12%, range 21%–2%). Between these two extremes, the remaining lenses (35%) contained lesser amounts of shortwave-absorbing pigments, resulting in an irregular decline in transmission in the UVA (mean 30%, range 55%–17%).

The most highly pigmented lenses were measured from a diurnal frog, *Hylodes phyllodes*, with 2 mm diameter lenses. A smaller individual of the same species with 1 mm diameter lenses still showed evidence of pigmentation but transmitted substantially more UVA light (Fig. 1b, Table 1). We sampled more tadpole diversity than presented here, but due to the small size of tadpole lenses (often < 0.5 mm) the scans were noisy, and most did not meet our data quality standards (above). The two species of tadpoles successfully measured, *Alytes muletensis* and *Dendropsophus minutus*, both had shortwave-transparent lenses with no evidence of pigmentation. We also measured the transmission of adult *A. muletensis* lenses, which were also unpigmented but removed slightly more short-wavelength radiation than the tadpole lens, consistent with the increased pathlength of the larger lens (Fig. 1b). Although a range of adult lens sizes were scanned in several other species, we observed no changes in lens transmission relating to size within species or across species (Fig. S6).

Ecological explanations of shortwave lens transmission

Both activity pattern and habitat had significant effects on shortwave transmission by amphibian lenses (Figs. 2 & 3). Activity patterns differed significantly in t50 (PGLS: $F_{(1, 112)} = 6.72$, $R^2_{adj.} = 0.05$, $\lambda = 0.78$, $p = 0.01$) but not %UVA (PGLS: $F_{(1, 102)} = 2.00$, $R^2_{adj.} = 0.01$, $\lambda = 0.79$, $p = 0.16$), with the lenses of diurnal species ($n = 14$, $\mu = 366$, $SE = 15.1$) exhibiting higher t50s than non-diurnal species ($n = 100$, $\mu = 347$, $SE = 7.5$; $t = 2.59$, $p = 0.01$). Diurnal species ($n = 10$, $\mu = 46$, $SE = 12.6$) also had a lower mean %UVA than nondiurnal species ($n = 94$, $\mu = 55$, $SE = 6.4$), though this difference was not statistically significant.

Habitats differed significantly in t50 (PGLS: $F_{(1, 112)} = 9.04$, $R^2_{adj.} = 0.07$, $\lambda = 0.82$, $p = 0.003$) and %UVA (PGLS: $F_{(1, 102)} = 10.9$, $R^2_{adj.} = 0.09$, $\lambda = 0.73$, $p = 0.001$), with scansorial species exhibiting higher t50s ($n = 42$, $\mu = 367$, $SE = 6.8$; $t = 3.01$, $p = 0.003$) and lower %UVAs ($n = 37$, $\mu = 39$, $SE = 5.23$; $t = -3.30$, $p = 0.001$) than non-scansorial species (t50: $n = 72$, $\mu = 346$, $SE = 13.8$; %UVA: $n = 67$, $\mu = 56$, $SE = 9.7$). Thus, the lenses from both scansorial and diurnal species prevented more UV light from reaching the retina compared to non-diurnal and non-scansorial animals.

Patterns of evolution across ecological groups

Comparisons of neutral and non-neutral models of evolution suggested that, when modelled together with lens size, species active at different times of day are under different evolutionary regimes for the spectral transmission of their lenses. For t50, models that allowed different evolutionary parameters for diurnal vs. non-diurnal species (BMM and OUM) performed significantly better than those estimating a single parameter across all species (BM1 and OU1; Table 2). While the OUM model had the lowest AIC, it was not significantly better than the BMM (ΔAIC = 1.4). The BMM model suggested that the rate of neutral change (drift) is higher among diurnal species ($\sigma = 29.3$) than for nondiurnal species ($\sigma = 3.7$), while the OUM model estimated a higher evolutionary optimum (θ) for t50 in diurnal species ($\theta = 401$ nm) than for nondiurnal species ($\theta = 351$ nm). For %UVA among the same species, the OU model allowing for different parameter estimates for diurnal and non-diurnal species (OUM) performed significantly better than the three other models (ΔAIC > 9). This model estimated a lower optimum for UVA transmission in diurnal species ($\theta = 22.1\%$) than in non-diurnal species ($\theta = 50.5\%$).

Similar modelling of evolution across habitats consistently suggested that scansorial and non-scansorial species are under different selective regimes for spectral lens transmission. For t50, the OUM model was the best fit (ΔAIC > 12), with a higher estimated optimum t50 for scansorial species ($\theta = 396$ nm) than for non-scansorial species ($\theta = 352$ nm). Likewise, for %UVA the OUM model was best supported, with a lower optimum for UVA transmission in scansorial species ($\theta = 22.3\%$) than in non-scansorial species ($\theta = 51.2\%$).

Discussion

Among vertebrates, the spectral properties of ocular lenses determine how much shortwave light is transmitted to the retina, with implications for visual sensitivity and spatial acuity (Douglas and Marshall, 1999). Yet, no study to date has found evidence for correlated evolution of lens transmission and any aspect of ecology. This study generated new data for spectral lens transmission across ecologically and phylogenetically diverse amphibians and found evidence for ecologically driven patterns of lens transmission. Both diurnal and scansorial ecologies were associated with decreased shortwave lens transmission across sampled species. Further, evidence suggests that species active at different times of day or utilizing different habitats are under different selective regimes for the spectral transmission of their lenses. The evolutionary context and functional implications of these findings for vertebrate vision are discussed below.

Shortwave filtering in the lenses of diurnal species

Amphibians active primarily during the day transmitted less shortwave light through their lenses than non-diurnal (cathemeral, crepuscular, or nocturnal) species. This difference was significant when shortwave transmission was summarized by the wavelength at which transmission falls to 50% (t50), but not when quantified by the proportion of UVA light that passed through the lens (%UVA). This was likely due to reduced species sampling, because 4/14 diurnal species in the comparison of t50 had to be dropped from the comparison of %UVA due to lack of available data (see Methods); indeed, with the same four species dropped from analysis of t50 we had less evidence for the difference ($p = 0.06$). A previous study on frogs did not find any correlation between lens transmission (quantified by t50) and activity period with eight diurnal species in a sample of 37 species (Yovanovich et al., 2020). High variation in lens transmission among species and small sample sizes driven by the relative rarity of diurnality among amphibians (Anderson & Wiens, 2017), as well as the difficulty of scoring complex behavioural traits into discrete bins, may make correlations with activity pattern difficult to detect in small samples.

Diurnal amphibians will benefit from lenses that reduce shortwave transmission because this will both reduce light-induced retinal toxicity (Glickman, 2011; Van Norren & Vos, 2016) and improve image quality by removing those wavelengths most prone to chromatic aberration and scattering (Douglas & Marshall, 1999), while involving minimal costs from the reduced photon capture in their well-lit environments. In contrast, non-diurnal species will benefit from unpigmented ocular media, as the fundamental requirement of the visual system is to capture enough photons to activate the photoreceptors. Thus, amphibians living in photon-limited environments, such as experienced by nocturnal species, will benefit from ocular media that are as transparent as possible.

As in other vertebrates, low shortwave transmission was not observed in all diurnal amphibians sampled. For example, among diurnal dart frogs (family Dendrobatidae), *Dendrobates leucomelas* had shortwave-transparent lenses, while congener *D. auratus* had highly pigmented, shortwave-filtering lenses. Similarly, *Atelopus varius* was the only diurnal toad (family Bufonidae) in our dataset but it had more shortwave-transmissive lenses than the seven other, non-diurnal toads. If lens pigmentation is serving a protective function in species occupying bright habitats, then one might predict that the amount of UV radiation present in the environment could exert selection on the spectral characteristics of lenses. A previous study on 37 frogs found no associations between elevation or latitude (two proxies for UV light) and lens transmission (Yovanovich et al., 2020). Further study is needed to disentangle the relative importance of shortwave-induced retinal toxicity and sensitivity in the evolution of shortwave-filtering lenses among frogs.

Shortwave filtering in the lenses of scansorial species

The lenses of scansorial amphibians, which are primarily found climbing in trees and other plants, transmit significantly less shortwave light than those of species associated with ground or water habitats. Scansorial behaviour has evolved multiple times in frogs and is repeatedly associated with low shortwave lens transmission. Evidence for shortwave-absorbing lens pigmentation was seen across major radiations of tree frogs, such as hyperoliids (e.g. *Hyperolius riggenbachi*), rhacophorids (e.g. *Polypedates leucomystax*), centrolenids (e.g. *Cochranela granulosa*), and hylids (e.g. *Boana faber*, Figs. 2 & S8). Among 41 scansorial frogs measured to date, only four have lenses that transmit more than half of incident UVA. While indirect, this evidence is consistent with the hypothesis that increased acuity is adaptive for climbing species.

Because chromatic aberration and Rayleigh scattering are both greatest at short wavelengths (Douglas & Marshall, 1999; Cronin & Bok, 2016), the removal of this part of the spectrum by the lens may serve to increase spatial acuity in scansorial amphibian species. Climbing species tend to inhabit more complex 3-dimensional worlds compared to, for example, ground-dwelling animals, so high spatial acuity likely benefits several aspects of their visually guided behaviours. A tree frog, for example, might need to negotiate narrow twigs or make potentially precarious leaps to avoid a predator, capture prey, or simply move from one location to another to encounter a mate. Such movements would be aided by the ability to see fine detail.

High degrees of lens pigmentation have previously been observed alongside increased spatial acuity in non-amphibian vertebrates. For example, lens pigmentation is associated with high cone densities (a prerequisite for high spatial acuity) in mammals (Douglas & Jeffrey, 2014), with high-acuity aerial hunting in birds (Lind et al., 2014), and with visual predation in snakes (Simões et al., 2016). If lenses that filter out short wavelengths do indeed serve to improve the spatial resolution of the retinal image for scansorial amphibians, one might expect such species to have high acuities.

Spatial acuity is most reliably determined by behavioural means, which has been investigated in few amphibians (Birukow, 1937; Manteuffel & Himstedt, 1978; Aho, 1996). However, because the ability of vertebrate eyes to resolve spatial detail depends largely on the focal length of the eye and the density of retinal neurons (Land & Nilsson, 2012), these can be used to calculate a theoretical spatial resolving power for a species, which often closely matches that determined by behavioural means (Pushchin, 2019). Large eyes and an increased density of cones and retinal ganglion cells potentially result in higher spatial resolution. Unfortunately, such anatomically based estimates of spatial resolution have only been calculated for four anuran species (Pushchin, 2021 for review) and ganglion cell density alone has been determined in an additional eight (Pushchin, 2019 for review). From these limited behavioural and morphological data, maximum resolving power was highest in a scansorial hylid, *Boana* (*Hyla*) *raniceps* (Bousfield & Pessoa, 1980).

Thus, it is congruent that the lenses of scansorial species tend to absorb more shortwave radiation than those of non-scansorial amphibians (Fig. 3) and the highest theoretical spatial acuity of any amphibian was also calculated for a tree frog. Furthermore, scansorial frogs tend to have large relative eye size compared to other frogs (Thomas et al., 2020), potentially elevating spatial resolving power. All this is consistent with shortwave-filtering lenses being an adaptation for increased spatial resolution in scansorial amphibians. However, not all scansorial species have lenses that filter out shortwave light (e.g. *Phyllomedusa vaillantii*, Figs. 2 & S8g, Table 1), nor do they always have higher maximum ganglion cell densities than non-scansorial amphibians (Pushchin, 2019, 2021). Such variability in the relationship between lens transmission and acuity among scansorial species suggests no single explanation will account for the distribution of shortwave-filtering lenses in frogs.

One explanation for this might be that most amphibians, including the scansorial species in our dataset, are nocturnal, and lenses that block short wavelengths come with the cost of reduced absolute sensitivity for those species that are active in low light. Thus, some scansorial species may benefit more from the increased acuity afforded by a shortwave-absorbing lens, while for others the enhanced sensitivity resulting from a clearer lens will be at a premium. Morphological and behavioural study of acuity in tree frogs would be an interesting realm for future study and would allow for a direct examination of whether scansorial frogs with pigmented lenses have higher spatial acuity.

Evidence for selection

Comparisons of different evolutionary models suggested that the evolution of shortwave lens transmission differs across habitats and activity periods in amphibians. Scansorial frogs appeared to be under selection for increased lens pigmentation, whereas non-scansorial species were under selection for more transparent lenses. Diurnal and non-diurnal species also showed consistent differences in evolutionary dynamics for lens transmission, but it was less clear whether this resulted from an increased rate of neutral change (drift) or selection for reduced shortwave transmission in diurnal species. To our knowledge this is a first attempt to examine the evolutionary processes driving lens pigmentation, but the use of complex Ornstein-Uhlenbeck models compared via likelihood can be prone to error in small datasets and should be interpreted with caution (Cooper et al., 2016). As spectral transmission is measured in more species, it will be appropriate to undertake larger macroecological studies disentangling the ecological and functional drivers of patterns within and across diverse groups. Future work might examine whether selection for acuity, sensitivity, and retinal protection shape patterns of shortwave-absorbing lens pigmentation across amphibians and other vertebrate groups.

Lenticular pigments

The variation in shortwave lens transmission we observed across amphibians is almost certainly due to the presence of shortwave-absorbing pigments in the lenses of many species, and their absence in others. The identities of the lenticular pigments responsible for shortwave-filtering in vertebrates are known in detail only for mammals and fishes. In most mammals (Douglas & Marshall, 1999 for review; Haines et al., 2006; Douglas & Jeffery, 2014) and some fishes (Truscott et al., 1992; Thorpe et al., 1992) they have been identified as kynurenine-related tryptophan derivatives. By contrast, most shortwave-filtering in fish lenses is performed by a series of mycosporine-like amino acids (Dunlap et al., 1989; Thorpe et al., 1993). For other groups of vertebrates, the identity of the lens pigments is largely unknown, although an unidentified pigment with an absorption maximum around 345–347 nm has been extracted from the lenses of the frog *Lithobates pipiens* (Kennedy & Milkman, 1956; Thorpe, 1991). Although pigments were not extracted from the lenses in this study, their absorption maxima can be approximately determined when present in low concentrations because they result in an irregular decline in lens transmission at short wavelengths. Although most such pigments, in agreement with the pigment found in *L. pipiens,* absorb maximally around 350 nm (e.g. *Aplastodiscus leucopygius* & *Scinax rizibilis*, Figs. 1a & S8), it seems likely that, as in fishes, pigments absorbing most strongly at longer wavelengths may also be involved in some species (e.g. *Hylodes phyllodes*, Figs. 1b & S8). Future study of the identity, spectral characteristics, and distribution of lens pigments would shed light on how this trait has evolved across amphibians and among diverse vertebrates.

Implications for sensitivity to short wavelengths

Visual sensitivity at short wavelengths, including in the UV, is widespread among both vertebrates and invertebrates and can serve to increase overall sensitivity and extend the spectral range of vision or contribute to more specific visual tasks such as navigation, mate choice, foraging, predator and prey detection, or communication (Cronin & Bok, 2016). There is limited behavioural (Dietz, 1972; Przyrembel et al., 1995; Han et al., 2007; Secondi et al., 2012; Kats et al., 2012) and electrophysiological (Govardovskii & Zueva, 1974; La Touche & Kimeldorf, 1979; Perry & McNaughton, 1991; Deutschlander & Phillips, 1995; Martin et al., 2016) evidence thus far that some amphibians are also sensitive in this part of the spectrum. Representatives of all classes of vertebrate (Bowmaker, 2008), including amphibians (Ma et al., 2001; Korenyak & Govardovskii, 2013; Mège et al., 2016; Martin et al., 2016; Secondi et al., 2017), have visual pigments with maximum sensitivity (λ_{max}) in the UV, although among amphibians, only salamanders have been demonstrated to have cones with a UV λ_{max}, whereas the shortest wavelength cones identified in frogs all have maximum sensitivity in the violet (Donner & Yovanovich, 2020).

Not having a visual pigment with a λ_{max} in the UV, however, does not preclude UV-sensitivity. The range of wavelengths absorbed by visual pigments is broad, and all visual pigments have a secondary absorption maximum in the UV (the cis-peak or β-band). Therefore, as long as a species has ocular media that are transparent to short wavelengths, they will be sensitive to light in this part of the spectrum even in the absence of a visual pigment with a λ_{max} in the UV (Douglas & Jeffery, 2014). With the exception of a few fish corneas, within vertebrates the lens always absorbs more short wavelengths than the cornea or aqueous and vitreous humours (Douglas & Marshall, 1999). Thus, because most amphibian lenses transmit substantial amounts of UVA radiation, these wavelengths will impinge on the retinae of many species, resulting in sensitivity to the UVA portion of the spectrum. This suggests that UVA light may have important roles and consequences for visual ecology and evolution across amphibians. We hope the transmission spectra provided in this manuscript will be of value to providing a more thorough understanding of colour vision in amphibians once more visual pigment data become available.

Diversity and ontogeny

This study produced some of the first measurements of lens transmission in salamanders, but only six species were sampled and all showed substantial shortwave lens transmission. Salamanders are rarely diurnal as adults, but we did sample a diurnal terrestrial eft of *Notopthalmus viridescens* and saw no evidence of lens pigmentation or difference from adult transmission spectra. Further study of salamanders, including scansorial species (e.g. several plethodontids: Waldron & Humphries, 2005; Capshaw et al. 2019), is needed to make any generalizations about lens transmission in this group. Lens transmission data are unavailable for the third major lineage of extant amphibians, the mostly fossorial caecilians (Order Gymnophiona), though they have reduced visual systems and we expect their lenses to lack filtering pigments. Many amphibians have distinct life stages, and anurans are known to exhibit changes in visual systems at metamorphosis (e.g. Sivak et al., 1985; Shrimpton et al., 2021; Schott et al., 2021). Although we found that the tadpoles of two frog species had shortwave-transparent lenses, the adult stage of *Alytes muletensis* also had shortwave-transparent lenses (Fig. 1b). It is unknown how lens pigmentation may change across life stages in most vertebrates (except fishes: Thorpe & Douglas, 1993), and this could have important implications for colour vision, acuity, and sensitivity through ontogeny. Continued study of the visual systems (lens transmission, acuity, sensitivity, spectral sensitivity), behaviours, and natural histories of diverse vertebrates will make larger-scale and more powerful macroevolutionary studies of their visual evolution possible.

References

Anderson, S. J., & Wiens, J. J. (2017). Out of the dark: 350 million years of conservatism and evolution in diel activity patterns in vertebrates. *Evolution,* **71**(8), 1944-1959. https://doi.org/10.1111/evo.13284

Aho, A.C. (1996). The visual acuity of the frog (*Rana pipiens*). *Journal of Comparative Physiology A*, **180**(1), 19-24. https://doi.org/10.1007/s003590050023

Birukow, G. (1937). Untersuchungen über den optischen Drehnystagmus und über die Sehschärfe des Grasfrosches (*Rana temporaria*). *Zeitschrift für vergleichende Physiologie*, **25**(1), 92-142.

Bousfield, J. D., & Pessoa, V. F. (1980). Changes in ganglion cell density during post-metamorphic development in a neotropical tree frog *Hyla raniceps*. *Vision Research*, **20**, 501–510. https://doi.org/10.1016/0042-6989(80)90125-X

Bowmaker, J.K. (2008). Evolution of vertebrate visual pigments. *Vision Research*, **48**(20), 2022-2041. https://doi.org/10.1016/j.visres.2008.03.025

Capshaw, G., Soares, D., Carr, C. E. (2019). Bony labyrinth morphometry reveals hidden diversity in lungless salamanders (Family Plethodontidae): Structural correlates of ecology, development, and vision in the inner ear. *Evolution*, **73**(10), 2135-2150. https://doi.org/10.1111/evo.13837

Clavel, J., Escarguel, G., & Merceron, G. (2015). mvMORPH: an R package for fitting multivariate evolutionary models to morphometric data. *Methods in Ecology and Evolution*, **6**, 1311-1319. https://doi.org/10.1111/2041-210X.12420

Cooper, N., Thomas, G. H., Venditti, C., Meade, A., Freckleton, R. P. (2016) A cautionary note on the use of Ornstein Uhlenbeck models in macroevolutionary studies. *Biological Journal of the Linnean Society*, **118**, 64-77. https://doi.org/10.1111/bij.12701

Cronin, T. W., & Bok, M. J. (2016) Photoreception and vision in the ultraviolet. *Journal of Experimental Biology,* **219**, 2790-2801. https://doi.org/10.1242/jeb.128769

Deutschlander, M. E. & Phillips, J. B. (1995). Characterization of an ultraviolet photoreception mechanism in the retina of an amphibian, the axolotl (*Ambystoma mexicanum*). *Neuroscience Letters,* **197**, 93–96. https://doi.org/10.1016/03043940(95)11905-C

Dietz, M. (1972). Toads are able to recognize UV light. *Die Naturwissenschaften*, **59**(7), 316-316. https://doi.org/10.1007/BF00593366

Donner, K. & Yovanovich, C. A. M. (2020). A frog's eye view: Foundational revelations and future promises. *Seminars in Cell and Developmental Biology*, **106**, 72-85. https://doi.org/10.1016/j.semcdb.2020.05.011

Douglas, R.H., & McGuigan, C.M. (1989). The spectral transmission of freshwater teleost ocular media—an interspecific comparison and a guide to potential ultraviolet sensitivity. *Vision Research*, **29**(7), 871-879. https://doi.org/10.1016/0042-6989(89)90098-9

Douglas, R.H., & Thorpe, A. (1992). Short-wave absorbing pigments in the ocular lenses of deep-sea teleosts. *Journal of the Marine Biological Association of the United Kingdom*, **72**(1), 93-112. https://doi.org/10.1017/S0025315400048815

Douglas, R.H., & Jeffery, G. (2014). The spectral transmission of ocular media suggests ultraviolet sensitivity is widespread among mammals. *Proceedings of the Royal Society B: Biological Sciences*, **281**(1780), 20132995. https://doi.org/10.1098/rspb.2013.2995

Douglas, R.H., & Marshall, N.J. (1999). A review of vertebrate and invertebrate ocular filters. In *Adaptive mechanisms in the ecology of vision* (eds Archer S. N., Djamgoz, M. B. A., Loew E. R., Partridge J. C., & Vallerga S.). Springer, Dordrecht, 95-162.

Dunlap, W. C., Williams, D. M., Chalker, B. E., & Banaszak, A. T. (1989). Biochemical photoadaptation in vision: U.V.-absorbing pigments in fish eye tissues. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, **93**(3), 601–607. https://doi.org/10.1016/0305-0491(89)90383-0

Freckleton, R. P., Harvey, P. H., Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist*, **160**(6), 712-726. https://doi.org/10.1086/343873

Frost, D. R. (2021). Amphibian Species of the World: an Online Reference. Version 6.1 (*10 January 2021*). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001

Gaffney, M. F., & Hodos, W. (2003). The visual acuity and refractive state of the American kestrel (*Falco sparverius*). *Vision Research* **43**(19), 2053–2059. https://doi.org/10.1016/S0042-6989(03)00304-3

Glickman, R.D. (2011). Ultraviolet phototoxicity to the retina. *Eye & Contact Lens: Science and Clinical Practice*, **37**(4), 196-205. https://doi.org/10.1097/ICL.0b013e31821e45a9

Gower, D.J., Sampaio, F.L., Peichl, L., Wagner, H.J., Loew, E.R., Mclamb, W., Douglas, R.H., Orlov, N., Grace, M.S., Hart, N.S. Hunt, D.M., Partridge, J.C. and Simões, B.F. 2019. Evolution of the eyes of vipers with and without infrared-sensing pit organs.

Biological Journal of the Linnean Society, **126**(4), 796-823. https://doi.org/10.1093/biolinnean/blz003

Govardovskii, V.I, & Zueva, L.V. (1974). Spectral sensitivity of the frog eye in the ultraviolet and visible region. *Vision Research*, **14**(12), 1317-1321. https://doi.org/10.1016/0042-6989(74)90003-0.

Han, B.A., Kats, L.B., Pommerening, R.C., Ferrer, R.P., Murry-Ewers, M., & Blaustein, A.R. (2007). Behavioral avoidance of ultraviolet-B radiation by two species of neotropical poison-dart frogs. *Biotropica*, **39**(3), 433-435. https://doi.org/10.1111/j.1744-7429.2007.00268.x

Hu, Z., Liu, F., Xu, X., Chen, Z., Chen, J. & Li, D. (2012). Spectral transmission of the principal-eye corneas of jumping spiders: implications for ultraviolet vision. *Journal of Experimental Biology*, **215**(16), 2853-2859. https://doi.org/10.1242/jeb.065904

Hu, Z., Xu, X., Chen, Z., Li, H., Wang, X., Wu, L., Liu, F., Chen, J. & Li, D. (2014). The spectral transmission of non-salticid spider corneas. *Journal of Experimental Biology*, **217**(15), 2698-2703. https://doi.org/10.1242/jeb.099069

Jameson, T. J. M., Streicher, J.W., Manuelli, L., Head, J. J., & Smith, E.N. (2021) Miniaturization in direct-developing frogs from Mexico with the description of six new species. *Herepetological Monographs*. In Press.

Jetz, W., & Pyron, R.A. (2018). The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology and Evolution*, **2**(5), 850-858. https://doi.org/10.1038/s41559-018-0515-5

Kennedy, D., & Milkman, R. D. (1956). Selective light absorption by the lenses of lower vertebrates, and its influence on spectral sensitivity. *The Biological Bulletin*, **111**(3), 375-386. https://doi.org/10.2307/1539144

Kessel, L., Lundeman, J. H., Herbst, K., Andersen, T. V. & Larsen, M. (2010). Age-related changes in the transmission properties of the human lens and their relevance to circadian entrainment. *Journal of Cataract & Refractive Surgery*, **36**(2), 308-312. https://doi.org/10.1016/j.jcrs.2009.08.035

Korenyak, D. A., & Govardovskii, V. I. (2013). Photoreceptors and visual pigments in three species of newts. *Journal of Evolutionary Biochemistry and Physiology*, **49**, 399–407. https://doi.org/10.1134/S0022093013040038

Land, M. F. & Nilsson, D.-E. (2012) *Animal Eyes*. Oxford University Press, Oxford, 1-271.

La Touche, Y. D. & Kimeldorf, D. J. (1979). Spectral sensitivity of the newt *Taricha granulosa*, to visible and u.v. radiation. *Comparative Biochemistry and Physiology Part A: Physiology*, **63**(2), 313–317. https://doi.org/10.1016/0300-9629(79)90165-8

Liedtke, H. C. (2019). AmphiNom: an amphibian systematics tool. *Systematics and Biodiversity* **17**(1), 1-6. https://doi.org/10.1080/14772000.2018.1518935

Lind, O., Mitkus, M., Olsson, P., Kelber, A. (2014) Ultraviolet vision in birds: the importance of transparent eye media. *Proceedings of the Royal Society B: Biological Sciences,* **281**, 20132209. https://doi.org/10.1098/rspb.2013.2209

Ma, J.X., Kono, M., Xu, L., Das, J., Ryan, J. C., Hazard, E. S., Oprian, D. D., & Crouch, R. K. (2001). Salamander UV cone pigment: Sequence, expression, and spectral properties. *Visual Neuroscience*, **18**(3), 393–399. https://doi.org/10.1017/S0952523801183057

Manteuffel, G. & Himstedt, W. (1978) The aerial and aquatic visual acuity of the optomotor response in the crested newt (*Triturus cristatus*). *Journal of Comparative Physiology A*, **128**(4), 359-365.

Martin, M., Théry, M., Rodgers, G., Goven, D., Sourice, S., Mège, P., & Secondi, J. (2016). UV wavelengths experienced during development affect larval newt visual sensitivity and predation efficiency. *Biology* Letters, **12**(2), 20150954. https://doi.org/10.1098/rsbl.2015.0954

Mège, P., Ödeen, A., Théry, M., Picard, D., & Secondi, J. (2016). Partial opsin sequences suggest UV-sensitive vision is widespread in Caudata. *Evolutionary Biology*, **43**(1), 109-118. https://doi.org/10.1007/s11692-015-9353-4

Muntz, W. R. A. (1976). On yellow lenses in mesopelagic animals. *Journal of the Marine Biological Association of the United Kingdom*, **56**(4), 963-976. https://doi.org/10.1017/S0025315400021019

Olsson, P., Lind, O., Mitkus, M., Delhey, K. & Kelber, A. (2021) Lens and cornea limit UV vision of birds – a phylogenetic perspective. *Journal of Experimental Biology*, **224**(20), jeb243129. https://doi.org/10.1242/jeb.243129

Orme, O., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., & Pearse, W. (2018). caper: Comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. https://CRAN.R-project.org/package=caper

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Paradis, E., & Schliep, K. (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics,* **35**(3), 526–528.

https://doi.org/10.1093/bioinformatics/bty633

Perry, R. J., & McNaughton, P. A. (1991). Response properties of cones from the retina of the tiger salamander. *The Journal of Physiology*, **433**(1), 561–587. https://doi.org/10.1113/jphysiol.1991.sp018444

Pushchin, I. (2019). Retinal ganglion cell topography and spatial resolution estimation in the Japanese tree frog *Hyla japonica* (Günther, 1959). *Journal of Anatomy*, **235**, 1114–1124. https://doi.org/10.1111/joa.13075

Pushchin, I. (2021). Retinal ganglion cell distribution and spatial resolution in the Asiatic toad *Bufo gargarizans* (Günther, 1859). *Vision Research*, in press. https://doi.org/10.1016/j.visres.2021.10.001

Przyrembel, C., Keller, B., & Neumeyer, C., (1995). Trichromatic color vision in the salamander (*Salamandra salamandra*). *Journal of Comparative Physiology A*, **176**(4), 575–586. https://doi.org/10.1007/BF00196422

R Core Team. (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Revell, L. J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Secondi, J., Lepetz, V., & Théry, M., (2012). Male attractiveness is influenced by UV wavelengths in a newt species but not in its close relative. *PLoS One*, ***7***(1), p.e30391. https://doi.org/10.1371/journal.pone.0030391

Secondi, J., Martin, M., Goven, D., Mège, P., Source, S. and Théry, M. (2017). Habitat-related variation in the plasticity of a UV-sensitive photoreceptor over a small spatial scale in the palmate newt. *Journal of Evolutionary Biology*, **30**(6), 1229–1235. https://doi.org/10.1111/jeb.13076

Siebeck, U. E., & Marshall, N. J. (2000). Transmission of ocular media in labrid fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **355**, 1257-1261. https://doi.org/10.1098/rstb.2000.0679

Siebeck, U.E. & Marshall, N.J., (2001). Ocular media transmission of coral reef fish—can coral reef fish see ultraviolet light? *Vision Research*, **41**(2), 133–149. https://doi.org/10.1016/S0042-6989(00)00240-6

Siebeck U. E., & Marshall, N. J. (2007). Potential ultraviolet vision in pre-settlement larvae and settled reef fish – A comparison across 23 families. *Vision Research* **47**(17), 2337–2352. https://doi.org/10.1016/j.visres.2007.05.014

Simões, B. F., Sampaio, F. L., Douglas, R. H., Kodandaramaiah, U., Casewell, N. R., Harrison, R. A., Hart, N. S., Partridge, J. C., Hunt, D. M., & Gower, D. J. (2016). Visual pigments, ocular filters and the evolution of snake vision. *Molecular Biology and Evolution*, **33**(10), 2483–2495. https://doi.org/10.1093/molbev/msw148

Sivak, J. G., Levy, B., Weber, A. P. & Glover, R. F. (1985). Environmental influence on shape of the crystalline lens: the amphibian example. *Experimental Biology*, **44**(1), 29-40.

Schott, R. K., Bell, R. C., Loew, E. R., Thomas, K. N., Gower, D. J., Streicher, J. W. & Fujita, M. K. (2021). Genomic and spectral visual adaptation in southern leopard frogs during the ontogenetic transition from aquatic to terrestrial light environments. *bioRxiv*, 2021.02.19.432049. https://doi.org/10.1101/2021.02.19.432049

Thomas, K. N., Gower, D. J., Bell, R. C., Fujita, M. K., Schott, R. K., & Streicher, J. W. (2020). Eye size and investment in frogs and toads correlate with adult habitat, activity pattern, and breeding ecology. *Proceedings of the Royal Society B: Biological Sciences*. **287**, 20201393. https://doi.org/10.1098/rspb.2020.1393

Thomas, K.N., Gower D.J., Streicher, J.W., Bell, R.C., Fujita, M.W., Schott, R.K., Haddad, C.F.B., Cox, C.L., Martins, R.A., Liedtke, H.C., Becker, C.G., & Douglas, R.H. (2021). Data for: Ecology drives spectral transmission in the ocular lenses of frogs and salamanders. *The Natural History Data Portal.* https://doi.org/10.5519/a7ficgr8

Thomas, K.N., Robison, B.H. & Johnsen, S. (2017). Two eyes for two purposes: in situ evidence for asymmetric vision in the cockeyed squids *Histioteuthis heteropsis* and *Stigmatoteuthis dofleini*. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **372**(1717), 20160069. https://doi.org/10.1098/rstb.2016.0069

Thorpe, A. (1991). Spectral transmission and short-wave absorbing pigments in the lenses of fish and other animals. PhD thesis, City University, London.

Thorpe, A. & Douglas, R. H. (1993) Spectral transmission and short-wave absorbing pigments in the fish lens—II. Effects of age. *Vision Research* **33**(3), 301-307. https://doi.org/10.1016/0042-6989(93)90086-C

Thorpe, A., Douglas, R. H., & Truscott, R. J. W. (1993). Spectral transmission and short-wave absorbing pigments in the fish lens—I. Phylogenetic distribution and identity. *Vision Research*, **33**(3), 289–300. https://doi.org/10.1016/0042-6989(93)90085-B

Thorpe, A., Truscott, R. J., & Douglas, R. H. (1992). Kynurenine identified as the short-wave absorbing lens pigment in the deep-sea fish *Stylephorus chordatus*. *Experimental Eye Research*, **55**, 53-57. https://doi.org/10.1016/0014-4835(92)90091-6

Truscott, R. J. W., Carver, J. A., Thorpe, A., & Douglas, R. H. (1992). Identification of 3-hydroxykynurenine as the lens pigment in the gourami *Trichogaster trichopterus*.

Experimental Eye Research, **54**(6), 1015–1017. https://doi.org/10.1016/0014-4835(92)90167-Q

Van Norren, D., & Vos, J. J. (2016). Light damage to the retina: an historical approach. *Eye*, **30**(2), 169-172. https://doi.org/10.1038/eye.2015.218

Volonteri, C., Barrasso, D. A., Cotichelli, L., Basso, N. G. & Hermida, G. N. (2017). Eye ontogeny in *Pleurodema bufoninum*: a comparison with *Pleurodema somuncurense* (Anura, Leptodactylidae). *Journal of Morphology*, **278**(7), 896-906. https://doi.org/10.1002/jmor.20682

Waldron, J. L. & Humphries, W. J. (2005) Arboreal habitat use by the green salamander, *Aneides aeneus*, in South Carolina. *Journal of Herpetology*, **39**(3), 486-492. https://doi.org/10.1670/228-04A.1

Yovanovich, C. A. M., Grant, T., & Kelber, A. (2019). Differences in ocular media transmittance in classical frog and toad model species and its impact on visual sensitivity. *Journal of Experimental Biology*, **222**(12), jeb204271. https://doi.org/10.1242/jeb.204271

Yovanovich, C. A. M., Pierotti, M. E. R., Kelber, A., Jorgewich-Cohen, G., Ibáñez, R., & Grant, T. (2020). Lens transmittance shapes ultraviolet sensitivity in the eyes of frogs from diverse ecological and phylogenetic backgrounds. *Proceedings of the Royal Society B: Biological Sciences*, **287**(1918), 20192253. https://doi.org/10.1098/rspb.2019.2253

Yu, G., Smith, D. K., Zhu, H., Guan, Y., Lam, T. T. Y. (2016). ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, **8**(1), 28-36. https://doi.org/10.1111/2041-210X.12628

Tables and Figures

Table 1. Summary transmission data for species in the current study. Shown are: taxonomic information according to Frost (2021; Or. = Order, A = Anura, C = Caudata), number of animals and lenses scanned for each species, the approximate equatorial diameters (diam) of the lenses, and the species means for transmission at 450 nm (t450), the wavelength of 50% transmission (t50), and the amount of UVA transmitted (%UVA). All data are derived from scans normalised at 700 nm except for *Ambystoma mexicanum, Cynops pyrrhogaster, Lithobates pipiens,* and *Salamandra salamandra*, where scans began and were normalised at 500 nm. *Hylodes phyllodes* is listed twice, because lenses from differently sized individuals produced different results at short wavelengths. Scans are from post-metamorphic individuals unless specified (tad = tadpole). Species are ordered by %UVA transmitted by the lens. An asterisk (*) indicates data collected from specimens originating from commercial dealers or captive colonies; all other data are from wild-caught animals.

Or.	**Family**	**Species**	**lens *n***	**animal *n***	**diam (mm)**	**450t**	**t50**	**%UVA**
A	Hylodidae	*Hylodes phyllodes*	1	1	2.0	84.6	423	2.0
A	Hyperoliidae	*Hyperolius riggenbachi**	2	2	2.0	84.9	407	5.7
A	Hylidae	*Boana faber*	1	1	5.5	82.3	399	6.1
A	Hylidae	*Osteopilus septentrionalis**	2	1	3.0	85.0	397	10.3
A	Mantellidae	*Boophis erythrodactylus*	2	1	3.0	80.6	396	12.9
A	Hylidae	*Acris blanchardi*	5	4	1.5	89.6	394	13.3
A	Hylidae	*Osteocephalus taurinus*	6	4	3.5-5.0	78.2	395	14.4
A	Ranidae	*Rana temporaria*	5	4	2.0-4.0	97.5	392	15.2
A	Ranidae	*Lithobates pipiens**	16	8	2.7-3.9	99.9	392	15.4
A	Rhacophoridae	*Polypedates leucomystax**	1	1	4.0	91.3	391	16.6
A	Hylidae	*Dendropsophus minusculus*	2	1	1.5-2.0	69.9	402	17.7
A	Hylidae	*Boana calcarata*	1	1	3.0	77.0	398	18.5
A	Hylidae	*Scinax cruentomma*	1	1	2.5	72.2	397	20.6

A	Hylidae	*Boana diabolica*	2	1	2.5-3.0	70.0	396	20.9
A	Hylidae	*Bokermannohyla circumdata*	1	1	4.0	89.9	389	20.9
A	Mantellidae	*Mantella betsileo**	2	1	1.5	78.7	389	21.2
A	Hylidae	*Boana cinerascens*	2	1	2.0	80.1	389	21.8
A	Hylidae	*Scinax rizibilis*	1	1	2.5	93.3	388	22.8
A	Dendrobatidae	*Epipedobates tricolor**	1	1	1.0	79.8	413	23.1
A	Hyperoliidae	*Heterixalus alboguttatus**	1	1	1.5	88.0	397	23.5
A	Leptodactylidae	*Leptodactylus rhodomystax*	2	1	2.5	71.4	397	24.1
A	Hylidae	*Boana pardalis*	1	1	4.5	89.4	387	25.2
A	Leptodactylidae	*Leptodactylus knudseni*	2	1	6.0-7.0	78.6	380	25.7
A	Craugastoridae	*Pristimantis zeuctotylus*	2	1	2.5	74.5	388	25.9
A	Hylidae	*Scinax hayii*	1	1	3.5	88.2	387	26.6
A	Hyperoliidae	*Hyperolius fusciventris**	2	1	1.5-2.0	86.8	394	29.2
A	Hylidae	*Aplastodiscus leucopygius*	1	1	2.5	89.5	385	30.5
A	Megophridae	*Megophrys nasuta**	1	1	1.5	92.3	374	30.5
A	Ranidae	*Lithobates sphenocephalus*	2	1	3.5	87.7	381	33.1
A	Hylidae	*Dendropsophus leucophyllatus*	5	4	1.5-3.0	75.4	384	33.7
C	Plethodontidae	*Batrachoseps attenuatus*	3	2	1.0	73.3	360	34.4
A	Alytidae	*Alytes cisternasii**	2	1	1.5	76.0	380	37.1
A	Leptodactylidae	*Leptodactylus fuscus*	1	1	3.0	88.4	370	37.2
A	Hylidae	*Boana punctata*	1	1	1.5	80.7	379	37.8
A	Mantellidae	*Mantella viridis**	1	1	1.5	77.0	368	37.9
A	Hylidae	*Scinax boesemani*	1	1	1.5	85.2	376	41.5
A	Leptodactylidae	*Leptodactylus podicipinus*	1	1	2.5	79.1	365	42.2
A	Hylidae	*Dryophytes cinereus*	1	1	2.5	76.0	370	42.5
A	Bufonidae	*Rhaebo guttatus*	2	1	8.0	78.3	365	43.4
A	Bufonidae	*Rhinella marina*	2	1	3.5	79.3	362	43.9
A	Leptodactylidae	*Lithodytes lineatus*	4	2	2.5-3.0	81.4	366	44

A	Bufonidae	*Sclerophrys maculata**	2	1	3.0	86.7	359	44.1
A	Leptodactylidae	*Leptodactylus pentadactylus*	3	2	2.5-6.0	81.1	364	45.6
A	Craugastoridae	*Pristimantis chiastonotus*	1	1	1.0	74.6	364	46.3
A	Centrolenidae	*Vitreorana uranoscopa*	1	1	2.0	89.1	361	46.6
A	Ceratophryidae	*Lepidobatrachus laevis**	1	1	2.0	94.2	359	47.6
A	Centrolenidae	*Hyalinobatrachium cappellei*	5	4	1.0-1.5	80.7	359	47.9
A	Brachycephalidae	*Brachycephalus rotenbergae*	1	1	1.0	91.7	356	48.6
A	Eleutherodactylidae	*Eleutherodactylus marnockii*	1	1	2.0	76.0	355	49.3
A	Scaphiopodidae	*Spea bombifrons*	2	1	3.5	82.0	349	49.3
A	Microhylidae	*Gastrophryne olivacea*	1	1	1.0	76.2	349	49.4
A	Hylidae	*Itapotihyla langsdorffii*	1	1	2.0	98.6	368	49.9
A	Leptodactylidae	*Physalaemus cuvieri*	1	1	1.5	87.6	360	50.0
A	Scaphiopodidae	*Spea multiplicata*	1	1	4.0	89.9	354	50.6
A	Alytidae	*Alytes muletensis**	2	1	1.0	77.8	351	51.5
A	Hylidae	*Boana polytaenia*	1	1	2.5	91.6	365	51.5
A	Ceratobatrachidae	*Cornufer guentheri**	1	1	4.0	90.5	358	52.8
A	Pipidae	*Xenopus laevis**	5	3	2.0	89.4	352	52.9
A	Rhinophrynidae	*Rhinophrynus dorsalis*	4	3	1.0-1.5	81.9	349	53.5
A	Phyllomedusidae	*Phyllomedusa vaillantii*	2	1	4.0	85.1	348	54.6
A	Dendrobatidae	*Dendrobates leucomelas**	2	1	1.0	89.1	326	54.8
A	Phyllomedusidae	*Phyllomedusa distincta*	1	1	4.5	93.8	362	55.2
A	Microhylidae	*Phrynomantis microps**	2	1	2.0	85.2	343	56.7
C	Salamandridae	*Notophthalmus viridescens*	3	3	1.0	85.1	347	57.1
A	Hemisotidae	*Hemisus marmoratus**	1	1	1.5	84.9	345	57.3
A	Ascaphidae	*Ascaphus truei*	3	2	2.5-3.0	91.0	349	57.5
A	Microhylidae	*Chiasmocleis shudikarensis*	2	2	1.0	83.3	341	58.2
A	Microhylidae	*Kaloula pulchra**	4	3	2.0-3.0	84.0	339	58.5
A	Scaphiopodidae	*Scaphiopus couchii*	2	2	4.5-5.0	86.7	342	59.4

A	Alytidae	*Discoglossus galganoi*	2	1	2.0	92.0	346	59.6
C	Salamandridae	*Lissotriton vulgaris*	2	1	1.0	87.5	342	59.9
A	Hyperoliidae	*Kassina senegalensis**	1	1	3.0	97.0	348	60.1
A	Leptodactylidae	*Adenomera andreae*	1	1	1.0	86.8	337	63.4
A	Bufonidae	*Rhinella icterica*	1	1	6.5	92.0	338	63.5
A	Microhylidae	*Glyphoglossus guttulatus**	1	1	2.5	91.1	337	64.3
A	Bufonidae	*Bufo bufo*	1	1	3.5	98.0	341	66.8
A	Brachycephalidae	*Ischnocnema parva*	1	1	1.0	83.4	322	67.3
A	Odontophrynidae	*Proceratophrys boiei*	1	1	1.0	91.5	332	69.2
A	Microhylidae	*Dyscophus guineti**	1	1	2.0	93.4	331	69.9
C	Ambystomatidae	*Ambystoma mexicanum**	1	1	1.6	99.4	318	73.4
A	Pelobatidae	*Pelobates cultripes*	2	1	4.0	97.9	328	73.6
A	Craugastoridae	*Haddadus binotatus*	1	1	1.5	98.3	327	73.7
C	Salamandridae	*Salamandra salamandra**	2	1	1.9-2.0	97.1	317	73.9
C	Salamandridae	*Cynops pyrrhogaster**	2	1	0.8-0.9	97.2	318	74.0
A	Brachycephalidae	*Ischnocnema henselii*	1	1	2.0	98.2	314	91.5
A	Hylodidae	*Hylodes phyllodes* (small)	1	1	1.0	90.0	409	40.8
A	Hylidae	*Dendropsophus minutus* (tad)	2	1	1.0	74.0	359	48.2
A	Alytidae	*Alytes muletensis* (tad)	2	1	1.0	84.4	336	59.3

Table 2. Model comparisons for the evolution of lens transmission. AIC scores comparing models of the evolution of spectral lens transmission across binary ecological regimes, with lens diameter as a covariate. Models included neutral evolution with a single parameter across binary regimes (BM1), neutral evolution with multiple parameters (BMM), adaptive evolution with a single parameter across regimes (OU1) and adaptive evolution with different parameters across regimes (OUM). Best-fit model(s) are indicated in bold.

		t50				%UVA			
		BM1	BMM	OU1	OUM	BM1	BMM	OU1	OUM
Habitat	AIC	744.8	744.5	744.3	**731.8**	713.3	714.4	705.3	**695.8**
	ΔAIC	13	12.7	12.5	**0**	17.5	18.6	9.5	**0**
Activity period	AIC	744.8	**732.8**	744.3	**731.4**	713.3	711.1	705.2	**695.9**
	ΔAIC	13.4	**1.4**	12.9	**0**	17.4	15.2	9.3	**0**

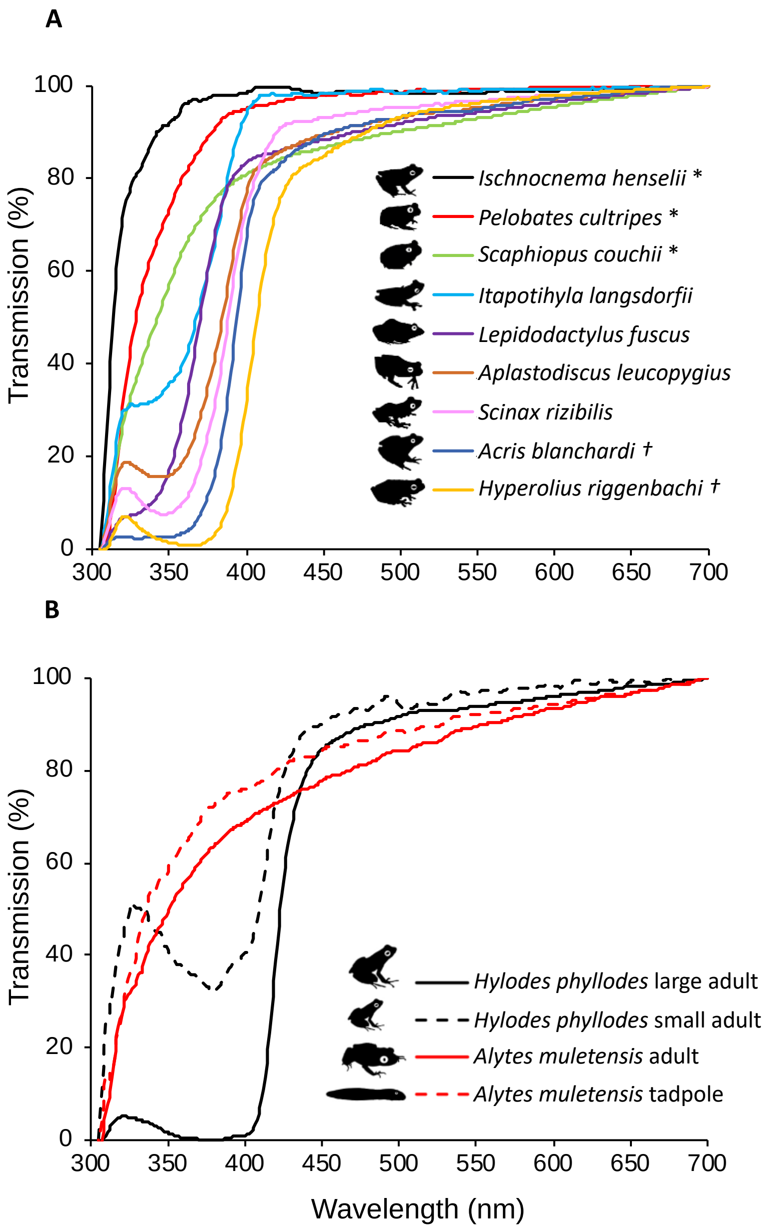

Figure 1. Representative examples of the spectral transmission of amphibian lenses. (A) Shortwave lens transmission varies across different species of anurans; * denotes an unpigmented (shortwave-transparent) lens, and † the presence of high concentrations of shortwave-absorbing pigment in the lens. The other species had lower levels of lens pigmentation. (B) Lens transmission in two *Hylodes phyllodes* males, one large adult (SVL = 34.4 mm) with a ca. 2 mm equatorial lens diameter, and one small adult (SVL = 29.1 mm) with a ca. 1 mm lens diameter, as well as a comparison of lens transmission in an adult and a tadpole of *Alytes muletensis*.

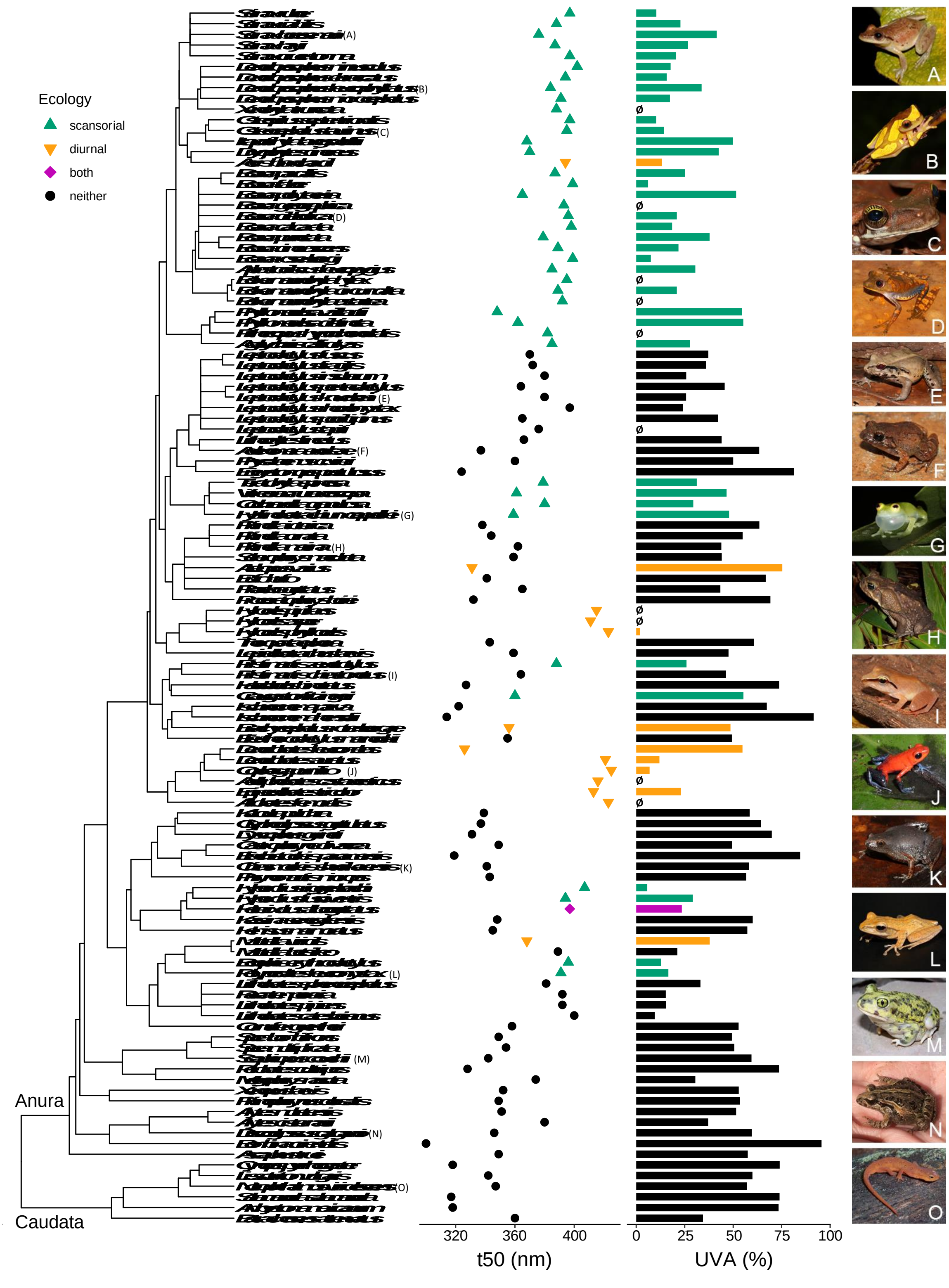

Figure 2. A phylogeny of frogs and salamanders used in tests for associations between ecology and lens transmission, with the dot plot depicting the wavelength of 50% transmission (t50) and the bar plot showing the proportion of UVA light transmitted (%UVA). Species taken from the literature (Yovanovich et al., 2019, 2020) and lacking data for %UVA because transmission spectra were not

measured to 315nm are denoted by Ø. Alphabetic annotations following species names correspond to photographs of specimens taken by J. Streicher (H, J, L-O) and C. Cox (A-G, I, K). Both scansorial and diurnal species show significantly lower shortwave transmission than non-scansorial or non-diurnal amphibians.

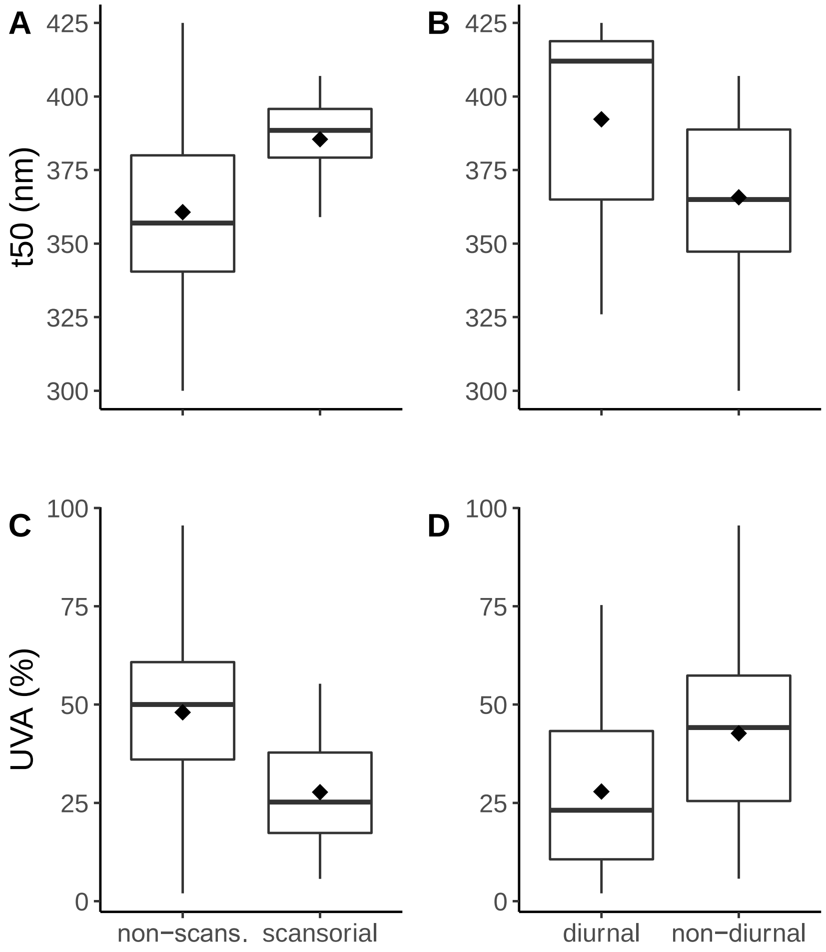

Figure 3. Boxplots of wavelength of 50% transmission (t50) and amount of UVA light transmitted (%UVA) for species across ecological categories for adult habitat (scansorial or non-scansorial; A,C) and activity pattern (diurnal or non-diurnal; B,D). Filled points indicate data collected for this study, and open points show data compiled from published literature. Black diamonds indicate the mean and black horizontal bars the median for each group.

Supporting Information

Additional supporting information may be found in the online version of this article.

S1. Spectrophotometry setup

S2. Artefactual decline in transmission from long to short wavelengths

S3. Effect of freezing on lens transmission

S4. Comparison of transmission data across studies

S5. Quantification of lens spectral transmission

S6. Lens size, ecology, and spectral transmission

S7. Ancestral state estimations of activity period and habitat

S8. Spectral transmission of lenses from all species examined

Table S1. Categorical assignment of adult species ecology

Supplementary References