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Visual pigments in a palaeognath bird, the emu *Dromaius novaehollandiae*: implications for spectral sensitivity and the origin of ultraviolet vision

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A comprehensive description of the spectral characteristics of retinal photoreceptors in palaeognaths is lacking. Moreover, controversy exists with respect to the spectral sensitivity of the short-wavelength-sensitive-1 (SWS1) opsin-based visual pigment expressed in one type of single cone: previous microspectrophotometric (MSP) measurements in the ostrich (*Struthio camelus*) suggested a violet-sensitive (VS) SWS1 pigment, but all palaeognath SWS1 opsin sequences obtained to date (including the ostrich) imply that the visual pigment is ultraviolet-sensitive (UVS). In this study, MSP was used to measure the spectral properties of visual pigments and oil droplets in the retinal photoreceptors of the emu (*Dromaius novaehollandiae*). Results show that the emu resembles most other bird species in possessing four spectrally distinct single cones, as well as double cones and rods. Four cone and a single rod opsin are expressed, each an orthologue of a previously identified pigment. The SWS1 pigment is clearly UVS (wavelength of maximum absorbance [λ_{max}] = 376 nm), with key tuning sites (Phe86 and Cys90) consistent with other vertebrate UVS SWS1 pigments. Palaeognaths would appear, therefore, to have UVS SWS1 pigments. As they are considered to be basal in avian evolution, this suggests that UVS is the most likely ancestral state for birds. The functional significance of a dedicated UVS cone type in the emu is discussed.

1. Introduction

The avian retina typically possesses four spectrally distinct single cone classes, a single double cone class, and a single rod class, together with their associated visual pigments [1]. A significant omission from the literature, however, is a comprehensive study of the photoreceptor complement and spectral characteristics in a member of the Palaeognathae (Order Struthioniformes), the large flightless birds that occur at the base of the avian radiation [2].

An early microspectrophotometric (MSP) study of the emu (*Dromaius novaehollandiae*) found a single type of rod, containing a visual pigment with a wavelength of maximum absorbance (λ_{max}) at 502 nm, and three different cone types, all with a λ_{max} at 567 nm but either lacking an oil droplet or possessing an oil droplet with differing wavelengths of half maximum transmission ($\lambda_{T0.5}$) [3]. As no other cone types were found in the emu retina, it was suggested that palaeognath colour discrimination might be limited when compared with neognaths such as the chicken (*Gallus gallus*) and pigeon (*Columba livia*), which are known to possess four spectrally distinct cone pigments with λ_{max} values at 413 nm, 467 nm, 507 nm, and 562 nm, in addition to a rod pigment [4,5]. A subsequent study of the ostrich (*Struthio camelus*) and rhea (*Rhea americana*), however, found four single cone classes containing visual pigments with λ_{max} values at 405 nm (ostrich only), 445 nm, 505 nm, and 570 nm, one double cone with a λ_{max} at 570 nm in both members,

and one rod (λ_{max} at 505 nm) [6]. Collectively, this suggested that palaeognaths are similar to neognaths in terms of their retinal photoreceptor complement.

The λ_{max} value (405 nm; $n = 1$) obtained for the most short-wavelength-sensitive (SWS) cone in the ostrich [6] would classify it as violet-sensitive (VS). VS SWS1 pigments are generally found in species with relatively large eyes (e.g. primates, dogs, cats, elephants, and horses), so a VS pigment might be expected to be present in palaeognaths. However, all palaeognaths studied to date, including the ostrich, have been shown to possess a SWS1 opsin with Cys90 [7,8], which in birds indicates a UV-sensitive (UVS) SWS1 pigment [9–11]. Although the available palaeognath SWS1 opsin sequence data are restricted to a few residues flanking site 90, this is clearly in contradiction to the reported presence of a VS SWS1 pigment in the ostrich [6].

In this study, MSP was used to measure the spectral absorption characteristics of emu retinal photoreceptors, and the coding sequences of the expressed visual pigments were determined by molecular analysis. These results provide, for the first time, a detailed assessment of palaeognath photoreceptor spectral sensitivities and address directly the issue of UVS/VS pigment evolution in birds.

2. Material and methods

(a) Animals
Two adult emus, reared in outdoor pens with exposure to full daylight and natural light : dark cycles, were dark adapted for 1 h prior to euthanization with an overdose of pentobarbitone (Lethabarb®, Virbac; 160 mg kg^{-1} delivered intravenously). Eyes were removed immediately under dim light and placed on ice until used.

(b) Microspectrophotometric measurements
Eyes were dissected under infrared illumination with the aid of an image converter and retinal tissue was prepared for MSP as described elsewhere [12]. Transverse absorbance spectra (330–800 nm) of individual photoreceptor outer segments and oil droplets were measured using a single-beam wavelength-scanning microspectrophotometer [13] and analysed using established methods [14].

(c) Spectral transmittance of the ocular media
Following removal of the posterior segment of the eye, the spectral transmittance (300–800 nm) of the combined ocular media (cornea, aqueous humour, and lens) in the anterior segment was measured along the optical axis. Light from a 175 W xenon broadband light source (Spectral Products) was delivered to the cornea via a 2 m, 200 μm diameter, quartz fibre-optic cable fitted with a quartz collimating lens. Light transmitted through the tissue fell on a Teflon diffuser placed in front of a second collimated quartz lens and was delivered to a charge-coupled device spectroradiometer (S2000; Ocean Optics) via a 2 m, 600 μm diameter, quartz fibre-optic cable. Each transmittance measurement comprised an average of 100 spectral scans. Three separate transmittance measurements were made from two eyes (taken from different emus) and averaged. The mean transmittance spectrum of each of the eyes was qualitatively similar, so both spectra were averaged together and normalized.

(d) Opsin sequences
Oligo-dT-primed retinal mRNA (2 μg) was converted to complementary DNA (cDNA) using the miScript polymerase chain reaction (PCR) Starter Kit (Qiagen) after extraction using the *mir*Vana™ RNA Isolation Kit (Life Technologies), according to the manufacturers' protocols. Opsin coding sequences were amplified using nested PCRs and degenerate primers targeting *LWS* (long-wavelength-sensitive), *SWS1*, *SWS2* (short-wavelength-sensitive-2), *RH2* (rhodopsin-like-2), and *RH1* (rod-opsin or rhodopsin-like-1) pigment genes (electronic supplementary material, table S1), as previously described [15]. All excised PCR products were purified using the FavorPrep Gel Purification Mini Kit (Fisher Biotech) and sequenced directly.

(e) Phylogenetic analysis
The MEGA 6 package [16] was used to generate maximum-likelihood trees from opsin amino acid sequences aligned in Clustal X [17]. Parameters used include 1 000 bootstraps, and the application of the Poisson model with uniform rates and partial deletion.

3. Results

(a) Microspectrophotometric measurements of retinal photoreceptors
MSP data for the pigments (figure 1; electronic supplementary material, figure S1) and oil droplets (figure 2) present in emu retinal photoreceptors are summarized in the electronic supplementary material, table S2. Based on the absorbance spectra fit to published templates [18], all emu pigments contain a retinal chromophore based on vitamin A$_1$. The retina contains a single medium-wavelength-sensitive (MWS) rod ($\lambda_{max} = 501$ nm) that is devoid of oil droplets. There are four spectrally distinct single cones: (i) a UVS cone ($\lambda_{max} = 367$ nm) with a transparent T-type oil droplet that has negligible absorptance between 330 and 800 nm, (ii) an SWS cone ($\lambda_{max} = 453$ nm) with a colourless/pale-green C-type oil droplet that has a cut-off wavelength λ_{cut} (the wavelength of the intercept at the value of maximum absorptance by a line tangential to the absorptance curve at half-maximum absorptance) at 408 nm, (iii) an MWS cone ($\lambda_{max} = 502$ nm) with a yellow Y-type oil droplet that has a λ_{cut} at 508 nm, and (iv) an LWS cone ($\lambda_{max} = 562$ nm) with a red R-type oil droplet that has a λ_{cut} at 559 nm. An asymmetric double cone is also present, with both principal and accessory cone members containing the same pigment found in LWS single cones ($\lambda_{max} = 562–563$ nm). Unlike the pigmented oil droplets in single cones, there is considerable variation in the λ_{cut} of the pale-green/yellow P-type oil droplet located in the double cone principal member. Two kinds of P-type oil droplet are present in the dorsal retina, with λ_{cut} values at 404 nm and 476 nm. By contrast, only a single P-type oil droplet with a λ_{cut} at 492 nm occurs in the ventral retina. The double cone accessory member is fairly uniform across the retina and does not contain an oil droplet; nonetheless, diffuse greenish-yellow pigmentation is present in the distal region of the inner segment with a λ_{cut} at 480 nm.

(b) Spectral transmittance of the ocular media
The transmittance of the combined ocular media is relatively high and uniform for wavelengths between 400 and 800 nm,
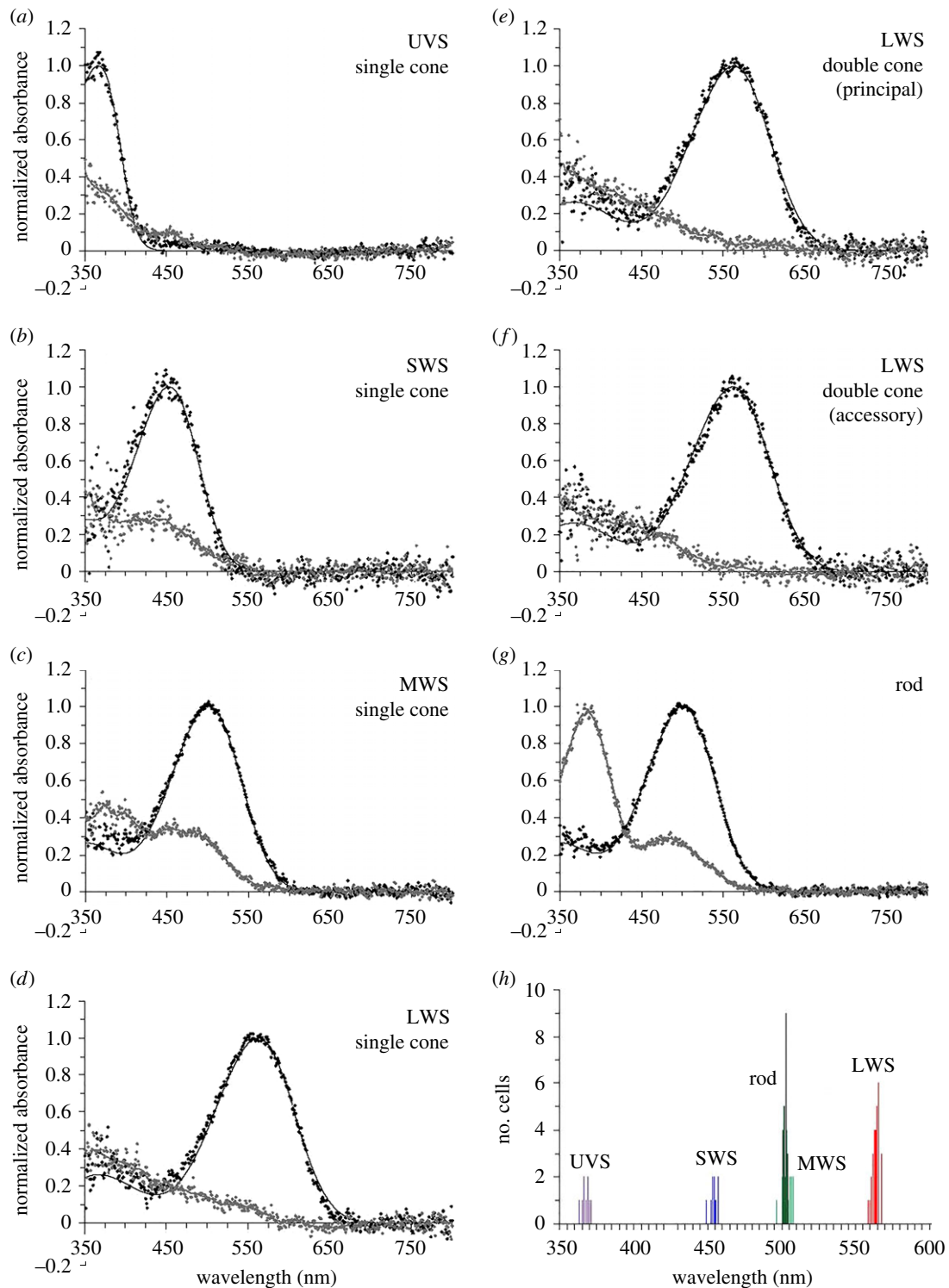
Figure 1. (a−g) Normalized mean pre- (black circles) and post-bleach (grey circles) spectra of pigments measured using MSP from emu retinal photoreceptor outer segments. Pre-bleach spectra are overlayed with best-fit rhodopsin (vitamin A_1) templates (black line). Post-bleach spectra are fitted with variable-point running average (grey line). (h) Histogram shows the spectral distribution of the wavelength of maximum absorbance (λ_{max}) values for individual photoreceptor outer segments that were used to generate the mean spectra. The data for the distribution of LWS pigment λ_{max} values includes measurements from LWS single cones, as well as both the principal and accessory members of double cones. λ_{max} values are UVS 367 nm, SWS 453 nm, MWS 502 nm, and LWS 562 nm. UVS, ultraviolet-sensitive; SWS, short-wavelength-sensitive; MWS, medium-wavelength-sensitive; LWS long-wavelength-sensitive.

but transmittance decreases sharply below 400 nm (electronic supplementary material, figure S2). The wavelength of 0.5 normalized transmittance ($\lambda_{T0.5}$) is 355 nm and the short wavelength cut-off, below which negligible light is transmitted to the retina, is approximately 335 nm.

(c) Opsin coding sequences

The coding sequences for five visual opsins were determined (GenBank Accession nos. LWS: KU568452, SWS1: KU568453,

SWS2: KU568454, RH2: KU568455, RH1: KU568456) and subjected to maximum-likelihood phylogenetic analysis, which confirmed them as orthologues of the four cone (*LWS*, *SWS1*, *SWS2*, and *RH2*) and single rod (*RH1*) opsin genes found in vertebrates (electronic supplementary material, figures S3−S5).

(i) Short-wavelength-sensitive-1

The MSP data clearly show that *SWS1*-expressing single cones are UVS ($\lambda_{max} = 367$ nm). This is consistent with the presence
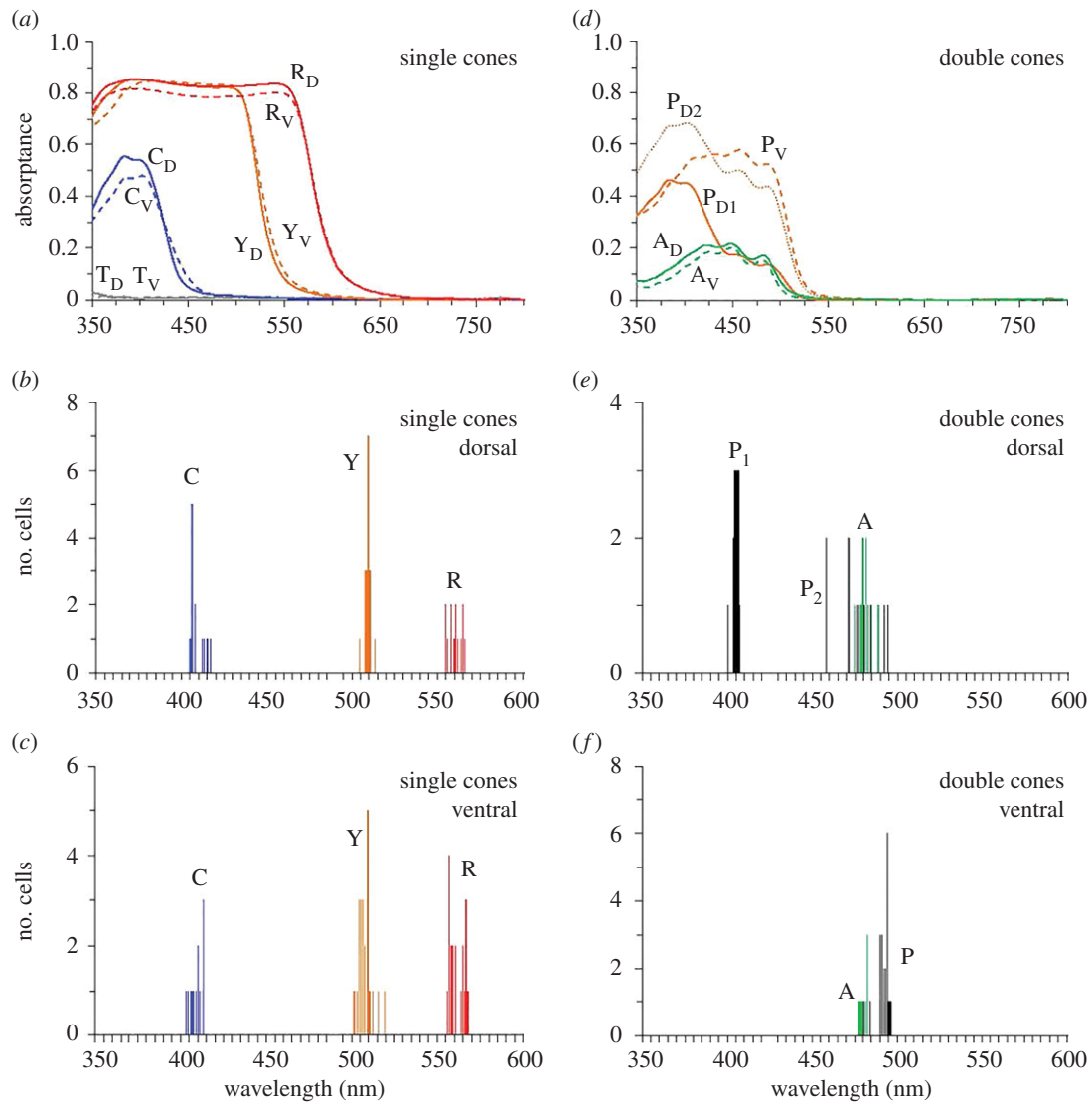
Figure 2. Mean spectral absorptance (a,d) and spectral distribution of cut-off wavelength (λ_{cut}) values (b,c,e,f) of oil droplets or diffuse spectral filters located in the inner segments of emu single (a–c) and double (d–f) cones. T, C, Y, R, and P, refer to T-, C-, Y-, R-, and P-type oil droplets located in UVS, SWS, MWS, LWS single cones and the principal members of LWS double cones, respectively, and A refers to the diffuse A-type oil droplet-like pigment located in the distal region of the inner segment of LWS double cone accessory members. D, dorsal retina (solid/dotted lines); V, ventral retina (dashed lines). Two distinct types of P-type oil droplet were present in the dorsal retina, which are identified by (arbitrary) subscripts 1 and 2.

of Phe86 and Cys90, as each is capable of generating a UVS pigment [9,10]. The presence of these residues in the emu SWS1 opsin has been reported previously [7]; however, since the sequenced region was restricted to amino acids 80–92, the potential impact of other tuning sites could not be ruled out. The emu SWS1 sequenced region reported here now extends to 90% of the coding region (with only the first 13 residues at the N-terminus and 23 residues at the C-terminus missing), thereby enabling a more complete study of amino acid changes across avian UVS and VS SWS1 pigments (electronic supplementary material, table S3). Unique changes in the emu pigment are present at three sites 49, 93, and 118, but for sites 49 and 118, the amino acid present in the emu sequence is also found in the UVS pigment of the green anole so it is unlikely that either is uniquely involved in spectral tuning of the pigment. Met93 would, however, appear to be unique to palaeognaths [7], so a functional role in spectral tuning cannot be ruled out.

(ii) Short-wavelength-sensitive-2

A number of residues are important for the spectral tuning of SWS2 pigments [19,20]. In particular, a Thr269Ala substitution results in a 10 nm short-wavelength shift [21]. The λ_{max} of the emu SWS2 pigment at 453 nm is similar to the majority of avian SWS pigments studied so far, including the chicken (λ_{max} = 453 nm) and pigeon (λ_{max} = 452 nm) [1]. In a few species, however, the λ_{max} of the SWS2 pigment is short-wavelength shifted to approximately 440 nm (e.g. rosella [22], canary [23], and zebra finch [24]). Significantly, in the emu (electronic supplementary material, figure S4), chicken [25], and pigeon pigments [26], the residue at site 269 is polar Ser (emu and pigeon) or polar Thr (chicken), whereas it is non-polar in the rosella (Ala [22]), canary (Cys), and zebra finch (Cys) pigments [23,24]. This polar to non-polar substitution appears sufficient, therefore, to account for the spectral shifts between avian species.

(iii) Rhodopsin-like-2

Five tuning sites (49, 52, 83, 86, and 97) were identified when spectrally distinct RH2 pigments of the Tokay gecko (Gecko gecko) and the green anole (Anolis carolinensis) were compared [27]. The sequenced region of the emu RH2 opsin (λ_{max} = 502 nm) does not extend to sites 49 and 52, but like other avian

RH2 sequences, tuning sites 83, 86, and 97 are identical to those of the spectrally similar green anole pigment ($\lambda_{\max} = 497$ nm).

(iv) Long-wavelength-sensitive pigments

Spectral tuning of LWS pigments depends largely on substitutions at five sites: 164, 181, 261, 269, and 292, which when occupied by Ser, His, Tyr, Thr, and Ala, respectively, result in a pigment with a $\lambda_{\max}$ at approximately 560–570 nm [28,29]. The residues at these sites in the emu LWS pigment are identical to those in other birds [22,23], with the exception of a Ser164Ala substitution. This change is predicted to cause a 7 nm short-wavelength shift in the $\lambda_{\max}$ of the pigment [30], which is consistent with the spectral peak of the emu LWS pigment at 562 nm compared with that of other birds at 566–571 nm.

(v) Rhodopsin-like-1

The key tuning residues for RH1 pigments at sites 83, 122, 207, 211, 261, 265, 269, 292, and 295 have been largely identified from studies of fish *RH1* gene sequences [31,32]. The only substitution across these sites in avian RH1 opsins is seen in the emu pigment, where Asn rather than Asp is found at site 83. This substitution is predicted to cause a 6 nm short-wavelength shift [28]; however, as the emu RH1 pigment ($\lambda_{\max} = 501$ nm) is spectrally almost identical to other birds ($\lambda_{\max} = 502–503$ nm) [25,26], it would appear that site 83 is not important for avian spectral tuning.

4. Discussion

The emu retina contains a similar photoreceptor complement to almost every other bird species studied to date (i.e. single rod, single double cone, and four spectrally distinct single cones) [1]. The four single cones generate a tetrachromatic visual system capable of excellent colour discrimination across the visible spectrum [33], and this is further refined by the presence of pigmented oil droplets that filter incoming light [34,35]. The only exceptions to this general format appear in some species of penguin, which have lost their MWS cone visual pigment through pseudogenization of the *RH2* opsin gene [36] and appear to possess only three spectral types of single cone [37].

Molecular analysis of the emu retina confirmed the expression of a single rod and four cone opsin genes, which accounts for the observed spectral characteristics of the different cone and rod photoreceptors. MSP has demonstrated unequivocally that the emu SWS cone is UVS: this establishes the SWS1 pigment in the emu as UVS and enables a re-assessment of the evolution of UVS/VS in birds. Key tuning sites for SWS1 opsins have been identified at positions 86, 90, and 93 [9,10,38]. Opsin gene sequencing in lampreys [39], a basal vertebrate, indicates that the ancestral SWS1 pigment in vertebrates was UVS with the presence of Phe86, Ser90, and Thr93. Spectral shifts from UVS to VS have occurred several times in vertebrate SWS1 pigment evolution as a result of substitutions at only two sites: 86 and 90. For most vertebrates, site 86 is critical for UVS tuning, with a shift to VS being mediated by Phe86Met in amphibians [40], Phe86Ser in birds [41], and Phe86Tyr, Phe86Ser, or Phe86Val in mammals [38,42,43]. In birds, however, an additional key change is at site 90, with Cys in UVS and Ser in VS pigments [9,10].

A previous study proposed that the ancestral avian SWS1 pigment was VS [41], which was largely based on the reported presence of Ser86 and Ser90 in the ostrich SWS1 opsin [44]. However, a recent report [7] showed that the SWS1 opsins in a number of palaeognath birds (including the ostrich) possess Phe86 and Cys90, a result that was unexpected, as both are capable of generating UVS pigments as single substitutions [9,10,38,45]. The SWS1 pigments in these basal birds all resemble, therefore, the emu opsin, and we conclude from this that all palaeognaths possess a UVS SWS1 pigment. Since palaeognaths occupy a basal position [2], this implies that UVS pigments were present at the start of the avian radiation.

For the palaeognath SWS1 sequences determined by Aidala *et al.* [7], the sequenced region of the SWS1 opsin was limited to amino acids 80–92 or 93; our extended emu SWS1 sequence not only confirms the presence of Phe86 and Cys90, but demonstrates that the only unique change at a potential tuning site [46] in palaeognath SWS1 pigments is Met93 (electronic supplementary material, table S3). Substitution at this site has been implicated in the evolution of VS SWS1 pigments in primates [47], but in this case, it is Pro that is present at site 93.

In extant diapsids (i.e. crocodiles, lizards, and snakes) that possess a common ancestor with birds, the amino acids present at sites 86 and 90 are Phe and Ala/Ser, respectively [15,48,49]. The phylogeny shown in figure 3 is based on a recent study of the relationships among 169 species of birds [2]. By superimposing residues 86 and 90 onto this phylogeny, it is evident that Phe86 is retained in the palaeognath lineage, with Ser90 replaced by Cys. By following the likely nucleotide changes for codons 86 and 90 that generated each amino acid substitution, it is apparent that just a single change in codon 90 of the SWS1 sequence is required to generate a Cys90 substitution as found in the palaeognaths, whereas in the neognaths, VS pigments arise from single changes in both codons to generate Phe86Ser and Cys90Ser substitutions. Subsequent changes in codon 86 give rise to additional changes (e.g. Ala, Cys, and Ser) in several avian orders in both the lower and upper parts of the phylogeny, including reverse mutations in the Pteroclidiformes and Trogoniformes to give Phe86 and presumably UVS pigments. It is only in the Ciconiiformes, Charadriiformes, Psittaciformes, and Passeriformes that Ser90 is frequently replaced by Cys to yield UVS pigments.

Sequencing of the emu SWS2 opsin has enabled further comparisons with the same pigment in other avian species. In all cases where the coding sequence is known, the longer wavelength shifted SWS2 pigments (including that of the emu) differ from the shorter wavelength shifted pigments at site 269, with polar Thr or Ser replaced by non-polar Cys or Ala. This site is located within helix VI in close proximity to the β-ionone ring of the chromophore and, therefore, in an ideal position to affect spectral tuning.

The peak sensitivities of avian RH2 pigments show relatively little variation, ranging from 497 nm in the rook (*Corvus frugileus*) to 507 nm in the chicken [1]. The emu is no exception with a $\lambda_{\max}$ at 502 nm, and consistent with this, does not differ at the three known tuning sites [27] included in the sequenced emu opsin.

Avian rod (RH1) pigments likewise show relatively little variation, with peaks ranging from 501 to 509 nm [50–52]. Not surprisingly, the RH1 amino acid sequences are also highly conserved across potential tuning sites, with the emu
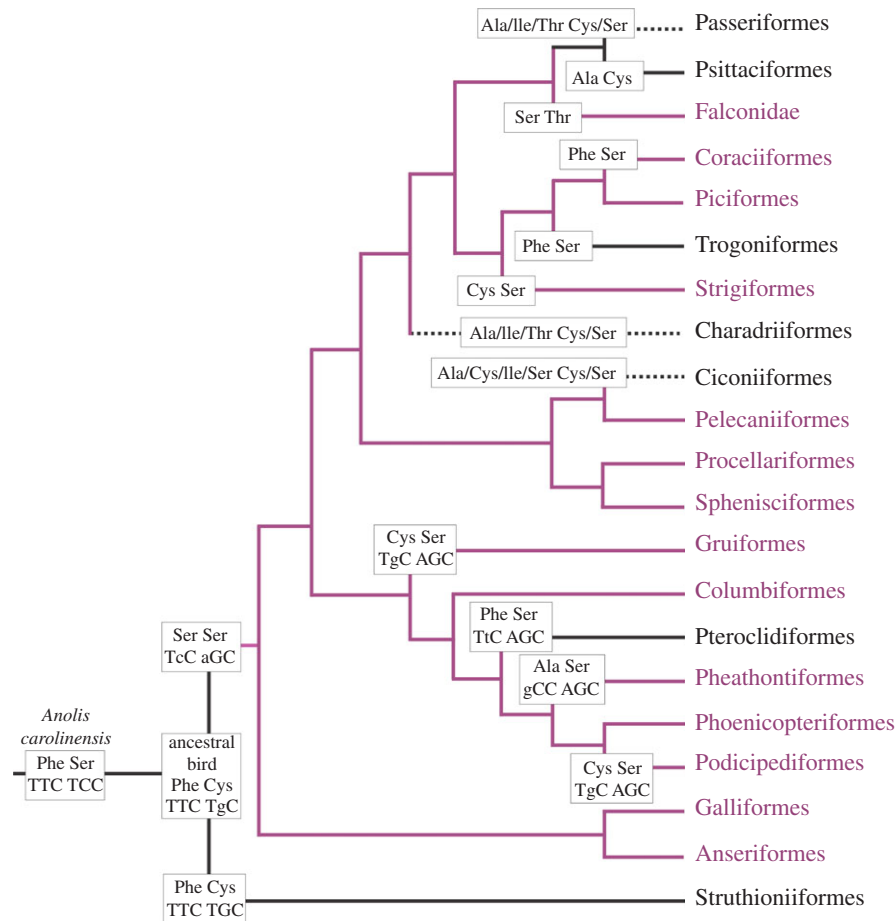
Figure 3. Evolution of UVS/VS SWS1 pigments in birds. The ancestral states of amino acids at sites 86 and 90 are shown [2]. For the branches in the lower part of the tree, codons 86 and 90 are also shown. Nucleotide changes in these codons are designated with lower case letters. The ancestral avian pigment is derived from that of the green anole (*Anolis carolinensis*). Where there is inter-Order variation at these sites, all alternatives are shown. Black lines indicate UVS pigments, violet lines represent VS pigments, and dotted lines designate the presence of both UVS and VS pigments within the Order. Adapted from [8,9].

RH1 opsin differing at just one site where Asp83 is replaced by Asn. This substitution has been implicated in relatively small spectral shifts in fish RH1 pigments [31], but does not appear to alter significantly the spectral sensitivity of emu rods. Finally, the small short-wavelength shift to 562 nm in the emu LWS pigment compared with orthologous pigments in other avian species (with λ_{max} values between 566 and 571 nm) can be accounted for adequately by the presence of Ala164, which is one of the five LWS tuning sites identified in vertebrates [28].

Optically, the presence of a UVS rather than a VS SWS1 pigment in the emu (and most likely other palaeognaths) is perhaps surprising given the large size of their eyes. In birds, eye axial length is inversely correlated with the amount of UV light transmitted by the ocular media, which can be defined by the $\lambda_{T0.5}$ value [53]. Based on the two-term exponential relationship between $\lambda_{T0.5}$ and $\log_{10}$ axial length (mm) [53], the predicted $\lambda_{T0.5}$ for the emu eyes measured in this study (eye axial lengths 32–34 mm) would be approximately 360 nm, which is consistent with the measured value of 355 nm. Both emu and ostrich have $\lambda_{T0.5}$ values that are characteristic of bird species that possess VS (mean $\lambda_{T0.5} = 358 \pm 20$ nm) rather than UVS SWS1 pigments (mean $\lambda_{T0.5} = 323 \pm 10$ nm) [53]. Thus, neither eye size nor ocular media $\lambda_{T0.5}$ are totally reliable predictors of the SWS1 λ_{max} value, although there remains a general relationship that warrants further investigation. Nonetheless, modelling in other bird species [53] suggests that, despite a $\lambda_{T0.5}$ at longer wavelengths than

would be expected, based on the λ_{max} value of the SWS1 pigment (and the consequent reduction in light transmission at wavelengths close to the peak sensitivity of the SWS1 pigment), the emu should still possess good detection and discrimination of UV wavelengths by virtue of a UVS rather than a VS SWS1 pigment.

Secondly, some of the most important optical aberrations that affect image quality in the vertebrate eye, by introducing blur (defocus) and reducing contrast (e.g. entoptic scatter [54], longitudinal [55] and transverse chromatic aberration [56]), are wavelength-dependent and increase markedly as the wavelength of light decreases. Although the actual magnitude of longitudinal chromatic aberration does not necessarily scale with eye size across species [55], its effects on image quality are theoretically worse in larger eyes (especially where the pupil is large relative to the focal length, i.e. a low *f*-number) as they have a shorter depth of focus (by virtue of their longer focal length) compared with small eyes [57].

In general, therefore, it might be expected that animals with larger eyes would have a more restricted range of pigment spectral sensitivities than animals with smaller eyes [58,59] and, in the case of the emu, to possess a VS rather than a UVS SWS1 pigment. Although the very largest vertebrate eyes (e.g. dichromatic elephants [43], monochromatic whales, seals [60], and sharks [13]) do contain relatively few spectrally distinct cones, this may be the result of phylogenetic inertia and/or adaptations to environmental light rather than optical factors. Indeed, many animals, including several birds, have multifocal

optical systems that compensate somewhat for longitudinal chromatic aberration through concentric zones of varying refractive power [61,62]. It is interesting to note, however, that while the ostrich appears to possess a multifocal optical system, the emu has a monofocal optical system, despite an apparently similar complement of photoreceptors [62].

The functional significance of a UVS rather than a VS SWS1 pigment in palaeognaths is unclear. Generally, their plumage has a low spectral reflectance in the UV region of the spectrum, with no notable peaks compared to other spectral regions [63], and at least some of the structural and optical properties of the mostly greyish-brown plumage in the emu may be adapted for thermoregulation [64] rather than intraspecific communication. Adult emus have patches of blue skin on their head and neck that are (subjectively to humans) 'darker' in females than males and absent from juveniles [65], which may be important in sexual selection. The blue colouration is a non-iridescent structural colour; similar integumentary colours in other birds tend to reflect UV wavelengths [66], although this cannot be confirmed for the emu as skin reflectance spectra are unavailable. Emus also have a varied diet that includes fruits, seeds, flowers, insects, and foliage [67], and it is well established that many of these food items have high UV reflectance or characteristic UV reflectance patterns that may be used by emus (as with other birds) to detect or discriminate them against other objects [68,69].

It is also possible that the emu and other palaeognaths retain an ancestral and perhaps 'generalist' form of colour vision that evolved to facilitate a wide variety of visual tasks under a range of light environments. These flightless birds might not have been subjected to selection pressures of sufficient magnitude to drive a shift in the spectral range of their visual system, as appears to have occurred in birds that have subsequently evolved a VS SWS1 pigment. Future work should concentrate on identifying such selection pressures, given that a large eye size in itself does not appear to necessitate the shift in spectral sensitivity from UVS to VS.

In conclusion, therefore, we have established that members of the Palaeognathae have not only retained the ancestral UVS SWS1 pigment but also possess ocular media that will allow transmission of UV light, thereby conferring UV sensitivity in these large flightless birds.

References

1. Hart NS, Hunt DM. 2007 Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169**, S7–S26. (doi:10.1086/510141)

2. Hackett SJ *et al.* 2008 A phylogenomic study of birds reveals their evolutionary history. *Science* **320**, 1763–1768. (doi:10.1126/science.1157704)

3. Sillman AJ, Bolnick DA, Haynes LW, Walter AE, Loew ER. 1981 Microspectrophotometry of the photoreceptors of palaeognathous birds—the emu and tinamou. *J. Comp. Physiol.* **144**, 271–276. (doi:10.1007/BF00612558)

4. Bowmaker JK. 1977 The visual pigments, oil droplets and spectral sensitivity of the pigeon. *Vision Res.* **17**, 1129–1138. (doi:10.1016/0042-6989(77)90147-X)

5. Bowmaker JK, Knowles A. 1977 The visual pigments and oil droplets of the chicken retina. *Vision Res.* **17**, 755–764. (doi:10.1016/0042-6989(77)90117-1)

6. Wright MW, Bowmaker JK. 2001 Retinal photoreceptors of paleognathous birds: the ostrich (*Struthio camelus*) and rhea (*Rhea americana*). *Vision Res.* **41**, 1–12. (doi:10.1016/S0042-6989(00)00227-3)

7. Aidala Z *et al.* 2012 Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines. *J. Comp. Physiol. A.* **198**, 495–510. (doi:10.1007/s00359-012-0724-3)

8. Odeen A, Hastad O. 2013 The phylogenetic distribution of ultraviolet sensitivity in birds. *BMC Evol. Biol.* **13**, 36. (doi:10.1186/1471-2148-13-36)

9. Wilkie SE, Robinson PR, Cronin TW, Poopalasundaram S, Bowmaker JK, Hunt DM. 2000 Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. *Biochemistry* **39**, 7895–7901. (doi:10.1021/bi992776m)

10. Yokoyama S, Radlwimmer FB, Blow NS. 2000 Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change. *Proc. Natl Acad. Sci. USA* **97**, 7366–7371. (doi:10.1073/pnas.97.13.7366)

11. Carvalho LS, Knott B, Berg ML, Bennett ATD, Hunt DM. 2011 Ultraviolet-sensitive vision in long-lived birds. *Proc. R. Soc. B* **278**, 107–114. (doi:10.1098/rspb.2010.1100)

12. Hart NS, Partridge JC, Cuthill IC. 1998 Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (*Sturnus vulgaris*). *J. Exp. Biol.* **201**, 1433–1446.

13. Hart NS, Theiss SM, Harahush BK, Collin SP. 2011 Microspectrophotometric evidence for cone monochromacy in sharks. *Naturwissenschaften* **98**, 193–201. (doi:10.1007/s00114-010-0758-8)

14. Hart NS. 2004 Microspectrophotometry of visual pigments and oil droplets in a marine bird, the wedge-tailed shearwater *Puffinus pacificus*: topographic variations in photoreceptor spectral characteristics. *J. Exp. Biol.* **207**, 1229–1240. (doi:10.1242/jeb.00857)

15. Davies WL, Cowing JA, Bowmaker JK, Carvalho LS, Gower DJ, Hunt DM. 2009 Shedding light on serpent sight: the visual pigments of henophidian snakes. *J. Neurosci.* **29**, 7519–7525. (doi:10.1523/JNEUROSCI.0517-09.2009)

16. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729. (doi:10.1093/molbev/mst197)

17. Larkin MA *et al.* 2007 Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948. (doi:10.1093/bioinformatics/btm404)

18. Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K. 2000 In search of the visual pigment

19. Chinen A, Matsumoto Y, Kawamura S. 2005 Spectral differentiation of blue opsins between phylogenetically close but ecologically distant goldfish and zebrafish. J. Biol. Chem. 280, 9460–9466. (doi:10.1074/jbc.M413001200)

20. Yokoyama S, Tada T. 2003 The spectral tuning in the short wavelength-sensitive type 2 pigments. Gene 306, 91–98. (doi:10.1016/S0378-1119(03)00424-4)

21. Cowing JA, Poopalasundaram S, Wilkie SE, Bowmaker JK, Hunt DM. 2002 Spectral tuning and evolution of short wave-sensitive cone pigments in cottoid fish from Lake Baikal. Biochemistry 41, 6019–6025. (doi:10.1021/bi025656e)

22. Knott B et al. 2013 How parrots see their colours: novelty in the visual pigments of Platycercus elegans. J. Exp. Biol. 216, 4454–4461. (doi:10.1242/jeb.094136)

23. Das D, Wilkie SE, Hunt DM, Bowmaker JK. 1999 Visual pigments and oil droplets in the retina of a passerine bird, the canary Serinus canaria: microspectrophotometry and opsin sequences. Vision Res. 39, 2801–2815. (doi:10.1016/S0042-6989(99)00023-1)

24. Yokoyama S, Blow NS, Radlwimmer FB. 2000 Molecular evolution of color vision of zebra finch. Gene 259, 17–24. (doi:10.1016/S0378-1119(00)00435-2)

25. Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T. 1992 Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc. Natl Acad. Sci. USA 89, 5932–5936. (doi:10.1073/pnas.89.13.5932)

26. Kawamura S, Blow NS, Yokoyama S. 1999 Genetic analyses of visual pigments of the pigeon (Columba livia). Genetics 153, 1839–1850.

27. Takenaka N, Yokoyama S. 2007 Mechanisms of spectral tuning in the RH2 pigments of Tokay gecko and American chameleon. Gene 399, 26–32. (doi:10.1016/j.gene.2007.04.036)

28. Yokoyama S. 2000 Molecular evolution of vertebrate visual pigments. Prog. Retin. Eye Res. 19, 385–419. (doi:10.1016/S1350-9462(00)00002-1)

29. Davies WI, Collin SP, Hunt DM. 2012 Molecular ecology and adaptation of visual photopigments in craniates. Mol. Ecol. 21, 3121–3158. (doi:10.1111/j.1365-294X.2012.05617.x)

30. Merbs SL, Nathans J. 1992 Absorption spectra of human cone pigments. Nature 356, 433–435. (doi:10.1038/356433a0)

31. Hunt DM et al. 2001 The molecular basis for spectral tuning of rod visual pigments in deep-sea fish. J. Exp. Biol. 204, 3333–3344.

32. Hunt DM, Fitzgibbon J, Slobodyanyuk SJ, Bowmakers JK. 1996 Spectral tuning and molecular evolution of rod visual pigments in the species flock of cottoid fish in Lake Baikal. Vision Res. 36, 1217–1224. (doi:10.1016/0042-6989(95)00228-6)

33. Goldsmith TH, Butler BK. 2005 Color vision of the budgerigar (Melopsittacus undulatus): hue matches, tetrachromacy, and intensity discrimination. J. Comp. Physiol. A 191, 933–951. (doi:10.1007/s00359-005-0024-2)

34. Govardovskii VI. 1983 On the role of oil drops in colour vision. Vision Res. 23, 1739–1740. (doi:10.1016/0042-6989(83)90192-X)

35. Vorobyev M, Osorio D, Bennett ATD, Marshall NJ, Cuthill IC. 1998 Tetrachromacy, oil droplets and bird plumage colours. J. Comp. Physiol. A 183, 621–633. (doi:10.1007/s003590050286)

36. Zhang G et al. 2014 Comparative genomics reveals insights into avian genome evolution and adaptation. Science 346, 1311–1320. (doi:10.1126/science.1251385)

37. Bowmaker JK, Martin GR. 1985 Visual pigments and oil droplets in the penguin, Spheniscus humboldti. J. Comp. Physiol. A 156, 71–77. (doi:10.1007/BF00610668)

38. Cowing JA, Poopalasundaram S, Wilkie SE, Robinson PR, Bowmaker JK, Hunt DM. 2002 The molecular mechanism for the spectral shifts between vertebrate ultraviolet- and violet-sensitive cone visual pigments. Biochem. J. 367, 129–135. (doi:10.1042/bj20020483)

39. Collin SP, Knight MA, Davies WL, Potter IC, Hunt DM, Trezise AEO. 2003 Ancient colour vision: multiple opsin genes in the ancestral vertebrates. Curr. Biol. 13, R864–R865. (doi:10.1016/j.cub.2003.10.044)

40. Starace DM, Knox BE. 1998 Cloning and expression of a Xenopus short wavelength cone pigment. Exp. Eye Res. 67, 209–220. (doi:10.1006/exer.1998.0507)

41. Carvalho LS, Cowing JA, Wilkie SE, Bowmaker JK, Hunt DM. 2007 The molecular evolution of avian ultraviolet- and violet-sensitive visual pigments. Mol. Biol. Evol. 24, 1843–1852. (doi:10.1093/molbev/msm109)

42. Parry JWL, Poopalasundaram S, Bowmaker JK, Hunt DM. 2004 A novel amino acid substitution is responsible for spectral tuning in a rodent violet-sensitive visual pigment. Biochemistry 43, 8014–8020. (doi:10.1021/bi049478w)

43. Yokoyama S et al. 2005 Elephants and human color-blind deuteranopes have identical sets of visual pigments. Genetics 170, 335–344. (doi:10.1534/genetics.104.039511)

44. Ödeen A, Håstad O. 2003 Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. Mol. Biol. Evol. 20, 855–861. (doi:10.1093/molbev/msg108)

45. Fasick JI, Applebury ML, Oprian DD. 2002 Spectral tuning in the mammalian short-wavelength sensitive cone pigments. Biochemistry 41, 6860–6865. (doi:10.1021/bi0200413)

46. Yokoyama S, Shi Y. 2000 Genetics and evolution of ultraviolet vision in vertebrates. FEBS Lett. 486, 167–172. (doi:10.1016/S0014-5793(00)02269-9)

47. Carvalho LS, Davies WL, Robinson PR, Hunt DM. 2012 Spectral tuning and evolution of primate short-wavelength-sensitive visual pigments. Proc. R. Soc. B 279, 387–393. (doi:10.1098/rspb.2011.0782)

48. Kawamura S, Yokoyama S. 1996 Phylogenetic relationships among short wavelength-sensitive opsins of American chameleon (Anolis carolinensis) and other vertebrates. Vision Res. 36, 2797–2804. (doi:10.1016/0042-6989(96)00034-X)

49. Yokoyama S, Blow NS. 2001 Molecular evolution of the cone visual pigments in the pure rod-retina of the nocturnal gecko, Gekko gekko. Gene 276, 117–125. (doi:10.1016/S0378-1119(01)00643-6)

50. Bowmaker JK, Heath LA, Wilkie SE, Hunt DM. 1997 Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. Vision Res. 37, 2183–2194. (doi:10.1016/S0042-6989(97)00026-6)

51. Hart NS, Partridge JC, Bennett ATD, Cuthill IC. 2000 Visual pigments, cone oil droplets and ocular media in four species of estrildid finch. J. Comp. Physiol. A 186, 681–694. (doi:10.1007/s003590000121)

52. Maier EJ, Bowmaker JK. 1993 Colour vision in the passeriform bird, Leiothrix lutea: correlation of visual pigment absorbance and oil droplet transmission with spectral sensitivity. J. Comp. Physiol. A 172, 295–301. (doi:10.1007/BF00216611)

53. Lind O, Mitkus M, Olsson P, Kelber A. 2014 Ultraviolet vision in birds: the importance of transparent eye media. Proc. R. Soc. B 281, 20132209. (doi:10.1098/rspb.2013.2209)

54. Coppens JE, Franssen L, van den Berg TJ. 2006 Wavelength dependence of intraocular straylight. Exp. Eye Res. 82, 688–692. (doi:10.1016/j.exer.2005.09.007)

55. Mandelman T, Sivak JG. 1983 Longitudinal chromatic aberration of the vertebrate eye. Vision Res. 23, 1555–1559. (doi:10.1016/0042-6989(83)90169-4)

56. Thibos LN, Bradley A, Still DL, Zhang X, Howarth PA. 1990 Theory and measurement of ocular chromatic aberration. Vision Res. 30, 33–49. (doi:10.1016/0042-6989(90)90126-6)

57. Green DG, Powers MK, Banks MS. 1980 Depth of focus, eye size and visual acuity. Vision Res. 20, 827–835. (doi:10.1016/0042-6989(80)90063-2)

58. Kröger RHH. 2000 Optical and developmental constraints on colour vision with lens eyes. J. Opt. A 2, R39. (doi:10.1088/1464-4258/2/6/203)

59. Rucker FJ, Osorio D. 2008 The effects of longitudinal chromatic aberration and a shift in the peak of the middle-wavelength sensitive cone fundamental on cone contrast. Vision Res. 48, 1929–1939. (doi:10.1016/j.visres.2008.06.021)

60. Newman LA, Robinson PR. 2005 Cone visual pigments of aquatic mammals. Vis. Neurosci. 22, 873–879. (doi:10.1017/S0952523805226159)

61. Kröger RHH, Fernald RD, Wagner H-J. 1999 Multifocal lenses compensate for chromatic defocus in vertebrate eyes. J. Comp. Physiol. A 184, 361–369. (doi:10.1007/s003590050335)

62. Lind OE, Kelber A, Kröger RHH. 2008 Multifocal optical systems and pupil dynamics in birds. J. Exp. Biol. 211, 2752–2758. (doi:10.1242/jeb.018630)

Partial reference at top left (continued from previous page):

template. Vis. Neurosci. 17, 509–528. (doi:10.1017/S0952523800174036)

63. Mullen P, Pohland G. 2008 Studies on UV reflection in feathers of some 1 000 bird species: are UV peaks in feathers correlated with violet-sensitive and ultraviolet-sensitive cones? *Ibis* **150**, 59 – 68. (doi:10.1111/j.1474-919X.2007.00736.x)

64. Maloney SK, Dawson TJ. 1995 The heat load from solar radiation on a large, diurnally active bird, the emu (*Dromaius novaehollandiae*). *J. Therm. Biol.* **20**, 381 – 387. (doi:10.1016/0306-4565(94)00073-R)

65. Schodde R, Tidemann SC. 1997 *Reader's digest complete book of Australian birds*. Sydney, Australia: Reader's Digest (Australia) Pty Ltd.

66. Prum RO, Torres R. 2003 Structural colouration of avian skin: convergent evolution of coherently scattering dermal collagen arrays. *J. Exp. Biol.* **206**, 2409 – 2429. (doi:10.1242/jeb.00431)

67. Davies SJJF. 1978 The food of emus. *Aust. J. Ecol.* **3**, 411 – 422. (doi:10.1111/j.1442-9993.1978.tb01189.x)

68. Burkhardt D. 1982 Birds, berries and UV. *Naturwissenschaften* **69**, 153 – 157. (doi:10.1007/BF00364887)

69. Stobbe N, Dimitrova M, Merilaita S, Schaefer HM. 2009 Chromaticity in the UV/blue range facilitates the search for achromatically background-matching prey in birds. *Phil. Trans. R. Soc. B* **364**, 511 – 517. (doi:10.1098/rstb.2008.0248)