Corrigendum

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In supplementary material Table S1, the data for kestrel are actually for sparrowhawk. The correct file is now available at http://jeb.biologists.org/content/216/10/1819/suppl/DC1. The authors apologise for any inconvenience that this error may have caused but assure readers that it does not affect the results or conclusions of the paper, or supplementary material Fig. S1.

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RESEARCH ARTICLE

Ultraviolet sensitivity and colour vision in raptor foraging

Olle Lind*, Mindaugas Mitkus, Peter Olsson and Almut Kelber
Department of Biology, Lund University, Sölvegatan 35, 22362 Lund, Sweden
*Author for correspondence (olle.lind@biol.lu.se)

SUMMARY

Raptors have excellent vision, yet it is unclear how they use colour information. It has been suggested that raptors use ultraviolet (UV) reflections from vole urine to find good hunting grounds. In contrast, UV plumage colours in songbirds such as blue tits are assumed to be 'hidden' communication signals, inconspicuous to raptors. This ambiguity results from a lack of knowledge about raptor ocular media transmittance, which sets the limit for UV sensitivity. We measured ocular media transmittance in common buzzards (*Buteo buteo*), sparrowhawks (*Accipiter nisus*), red kites (*Milvus milvus*) and kestrels (*Falco tinnunculus*) so that, for the first time, raptor UV sensitivity can be fully described. With this information, and new measurements of vole urine reflectance, we show that (i) vole urine is unlikely to provide a reliable visual signal to hunting raptors and (ii) blue tit plumage colours are more contrasting to blue tits than to sparrowhawks because of UV reflectance. However, as the difference between blue tit and sparrowhawk vision is subtle, we suggest that behavioural data are needed to fully resolve this issue. UV cues are of little or no importance to raptors in both vole and songbird interactions and the role of colour vision in raptor foraging remains unclear.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/10/1819/DC1

Key words: ultraviolet reflectance, ocular media transmittance, bird vision, raptor hunting behaviour, visual modelling, vole urine.

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INTRODUCTION

Birds are sensitive to ultraviolet (UV) light and use UV cues to guide behaviour (reviewed in Bennett and Cuthill, 1994). In raptors, UV sensitivity has been ascribed two conflicting roles in foraging behaviour. First it was suggested that raptors detect UV reflections of vole urine and use this cue to confine hunting behaviour to areas with high densities of prey (Viitala et al., 1995; Koivula and Viitala, 1999) (see also Härmä et al., 2011). Later, it was discovered that even though raptors are sensitive to UV light, some of their prey (songbirds) are sensitive to UV light of even shorter wavelengths (Ödeen and Håstad, 2003). Many songbirds have plumage colours with strong UV components used as signals in sexual communication, and with the new understanding of how UV sensitivity varies among birds, it was suggested that songbird UV signalling provides a 'private' communication channel inconspicuous to eavesdropping predators (Håstad et al., 2005) (see also Guilford and Harvey, 1998). These two hypotheses about UV vision in raptor foraging co-exist because of incomplete knowledge about the spectral range and amplitude of raptor UV sensitivity.

Bird colour vision is mediated by four photoreceptor types, each with a spectrally distinct visual pigment; the ultraviolet or violet-sensitive cone (UVS/VS, sws1 pigment), the short-wavelength-sensitive cone (SWS, sws2-pigment), the medium-wavelength-sensitive cone (MWS, rh2 pigment) and the long-wavelength-sensitive cone (LWS, m/lws pigment) (reviewed in Hart, 2001). Birds also have double cones and rod photoreceptors, which are believed to mediate achromatic information in bright and dim light, respectively (Campenhausen and Kirschfeld, 1998; Goldsmith and Butler, 2003; Goldsmith and Butler, 2005; Osorio et al., 1999; Vorobyev and Osorio, 1998; Lind and Kelber, 2011) (reviewed in Martin and Osorio, 2008).

Each cone is equipped with a pigmented oil droplet that filters the incident light before it reaches the light-sensitive pigment in the cone outer segments. The oil droplets act as short-wavelength cut-off filters that narrow the spectral sensitivity of cones and shift them to longer wavelengths. Theoretically, this enhances spectral resolution and colour constancy, although experimental validation of these effects is still lacking (Vorobyev et al., 1998). The oil droplets of the UVS/VS cones absorb very little light at wavelengths longer than 330 nm and do not shift pigment sensitivity (Hart and Hunt, 2007). Instead, the ocular media act as short-wavelength cut-off filters and change the sensitivity of the UVS/VS cones, similar to the way in which the pigmented oil droplets change the sensitivity of the other cone types (e.g. Hart et al., 2000; Lind and Kelber, 2009) (see also Hart and Hunt, 2007).

The spectral sensitivity of SWS, MWS and LWS cones varies little among terrestrial bird species while the variation in UVS/VS cone sensitivity divides birds into two groups: birds with UVS cones that have a maximum sensitivity at wavelengths below 400 nm and birds with VS cones that have a maximum sensitivity above 400 nm (Hart, 2001). Conveniently, it is possible to estimate the peak wavelength of sws1 pigments (of UVS/VS cones) from the pigment's amino acid sequence (Ödeen et al., 2009). From such studies, it is clear that raptors have the VS cone type with maximal absorbance of the sws1 pigments at 405 or 406 nm, while songbirds have the UVS type with a sensitivity peak around 370 nm (Ödeen and Håstad, 2003).

However, it is not the spectral position of the sws1 pigment that sets the short-wavelength limit of photoreception but the transmittance of the ocular media (Hart and Hunt, 2007). The ocular media transmittance in raptors was not known when the hypothesized roles of UV reception in raptors were formulated (Viitala et al., 1995; Koivula and Viitala, 1999; Håstad et al., 2005).

Here, we present data on the ocular media transmittance in the common buzzard, *Buteo buteo* (Linnaeus 1758), the sparrowhawk, *Accipiter nisus* (Linnaeus 1758), the red kite, *Milvus milvus* (Linnaeus 1758) and the kestrel, *Falco tinnunculus*, Linnaeus 1758, and describe, for the first time, the spectral range of raptor UV sensitivity. We used a few widely accepted rules of generalization of bird cone sensitivity to estimate the sensitivity of all cones used for colour vision in the common buzzard (a vole-hunting species) (Reif et al., 2001) and the sparrowhawk (a songbird-hunting species) (Heintzelman, 1964). This allowed us to evaluate the roles of colour vision and UV reception in two model systems of visual signalling in raptor foraging: (i) the detection of bank vole, *Myodes glareolus* (formerly *Clethrionomys glareolus*) (Schreber 1780) urine by common buzzards and (ii) the detection of blue tit, *Cyanistes caeruleus* (Linnaeus 1758) plumage colours by sparrowhawks. Our study provides new insights into visual aspects of raptor–prey interactions and highlights important considerations for the assessment of similar questions about UV sensitivity and visual modelling.

MATERIALS AND METHODS

Measurement of ocular media transmittance

We received one adult common buzzard, one sparrowhawk, one red kite and one kestrel directly after they had been euthanized (measurements started within 1 h of the point of death). All animals were wild specimens taken care of by a bird rescue station in southern Sweden as a result of injuries and were killed for reasons unrelated to this study. The collection of specimens was approved by the Swedish Environmental Protection Agency (permit no. NV-00160-12).

We enucleated the eyes and cut a circular window (diameter 8–10 mm) in the back of the eye (removing the sclera, choroid and retina) making sure that the vitreous humour was left intact. The eye was placed, with the lens facing down, in a custom-made matte black plastic container (35 mm diameter, 32 mm height, for the common buzzard and the red kite, and 25 mm diameter, 22 mm height, for the sparrowhawk and the kestrel) with a circular (5 mm) fused silica window in the bottom. Metal washers kept the eyes positioned within the container. The container was filled with 340 mOsmol kg^{-1} phosphate-buffered saline (PBS) solution to prevent the eyes from drying out. A light guide (1000 μm in diameter, Ocean Optics, Dunedin, FL, USA) connected to a PX2-Xenon lamp (Ocean Optics) illuminated the eyes from below through the fused silica window, and the light that passed through the eye was collected using another light guide (600 μm in diameter, Ocean Optics) at the top, and sent to a spectrometer (Maya, Ocean Optics). We aligned the light guides and the eye using an optomechanical system (microbench, LINOS, Göttingen, Germany). This secured the collection of predominantly axial light that was only refracted or scattered a little. As a reference, we measured the transmittance of the container with washers and PBS solution before we inserted the eyes. All light guides had a numerical aperture of 0.22.

We measured the transmittance of both eyes in each specimen with 1 nm resolution, and each eye was measured three times. The second eye was measured 30–60 min after the first eye (meanwhile, it was kept intact within the scull). The spectrometer was controlled by Spectrasuit software (v 1.0, Ocean Optics) and all transmittance measurements were processed in MATLAB (R2011a, The MathWorks, Natick, MA, USA) by calculating the average for each eye, smoothing the data by an 11-point running average to reduce noise and normalizing the transmittance spectrum to the highest value within the range 300–700 nm.

Spectral reflectance data

We received urine from bank voles and field voles [*Microtus agrestis* (Linnaeus 1761)] that were trapped at Stensoffa field station in southern Sweden (55.7°N, 13.4°E). The voles were trapped for the collection of faeces for other research purposes and the urine was saved as a by-product by placing the voles on metal net in containers that separated the faeces on top of the net from the urine at the container bottom. Vole urine was supplied to us several times during a period of approximately 4 weeks and we kept the urine in a freezer at −20°C until we measured its reflectance. At the end of this 4 week period, we compared frozen urine with fresh urine to make sure the freezing process did not change its reflectance properties. In this study, we focused on bank voles, which are more common in the collection area.

We measured the reflectance of bank vole urine on different substrates in an open matte black box with 10 compartments (Fig. 1). Two compartments were filled with sand, two with fresh green grass, two with dry grass, two with white filter paper and another two with white filter paper used for reference measurements (Munktell Filter AB, Grycksbo, Sweden). One compartment with each substrate was used for urine treatments and one for water treatments (Fig. 1). The reflectance of the white filter paper was compared with a white ceramic standard (TOP Sensor Systems WS-2, Ocean Optics) and found to be flat and above 95% in the region between 300 and 700 nm.

We measured the reflectance of the substrates at midday in the shadow of a building in full daylight. Measurements were taken at a 45 deg angle against the substrate with a light guide (1000 μm in diameter, Ocean Optics) connected to a spectrometer (Maya, Ocean Optics). First, we measured the untreated substrates. Then, we carefully applied 0.5 ml distilled water or vole urine to the central region of the substrates (a circular area with a radius of 13 mm, resulting in a very high concentration of vole urine per unit area compared with earlier studies) (cf. Koivula and Viitala, 1999) and measured the reflectance of these treated regions. Following this first measurement, the box was covered with a Perspex window (transparent to light between 300 and 700 nm) to prevent disturbance from wind and rain, and placed outside, exposed to sunlight, until the next day (day 2) when we again pipetted urine and water upon the substrates and took measurements. The same procedure was repeated on day 3. On day 4, we took new measurements of the untreated substrates (this time at the peripheral region of each compartment) and measurements of the dried treated substrates. Finally, we again added 0.5 ml urine or water, and measured the freshly treated substrates again.

We measured all substrates for each treatment three times and calculated the average. Reference measurements were taken between samples measurements. The interval between reference and sample measurements was typically 5 s and never longer than 10 s to ensure stable ambient light conditions. Recording noise of the spectroradiometer changes with temperature, especially at shorter wavelengths, such as in the UV region. For this reason, we made sure that the equipment attained the outside temperature (about 15°C) before measurements were taken.

From these measurements we calculated the chromatic contrast between the treated and the untreated substrates on day 1 (fresh treatments) and on day 4 (dry and fresh treatments). The chromatic contrast between urine samples and water samples was calculated for each day.

To model the interaction between sparrowhawks and blue tits, we used the reflectance measurements of male blue tit plumage from earlier publications (Hunt et al., 1998) and calculated the chromatic

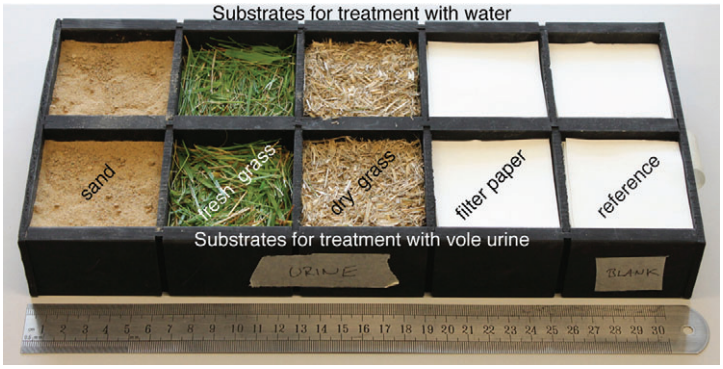

Fig. 1. Substrate box for reflectance measurements of vole urine and distilled water on different substrates. The treatments (urine and distilled water) were applied in the central 13 mm region of each compartment. The reference is white filter paper (see Materials and methods for details). The markings on the ruler values indicate centimetres.

contrast against a background of green grass measured in this study (untreated substrate day 1). For this analysis, we used data at wavelengths between 300 and 700 nm to which blue tits are sensitive.

Estimating photoreceptor sensitivity

The spectral sensitivity of a raptor cone, R, of type i depends on the spectral sensitivity of its visual pigment, r, the transmittance of its oil droplet, p, and the transmittance of the ocular media, o:

$$R_i(\lambda) = r_i(\lambda)p_i(\lambda)o(\lambda)\ . \quad (1)$$

The peak wavelength (λ_{max}) of the sws1 pigment (VS cones) in common buzzards and sparrowhawks is 405 nm (Ödeen and Håstad, 2003). The λ_{max} of the sws2 pigment (449 nm, SWS cone), the rh2 pigment (504 nm, MWS cone) and the m/lws pigment (567 nm, LWS cone) were predicted using generalizations about the correlation between the λ_{max} of the sws1 pigment and the λ_{max} of other cone pigments in other bird species (Hart and Vorobyev, 2005). The λ_{max} values were used to calculate the full spectral sensitivity of the cone pigments using the pigment template suggested elsewhere (Govardovskii et al., 2000). The λ_{max} of the sws1 pigments in the red kite and the kestrel are not known; the red kite and the kestrel were therefore excluded from the analyses of chromatic contrast although conclusions about UV sensitivity in these species are still possible (see Results and Discussion).

The pigmented oil droplets of cones are assumed to function as cut-off filters that are completely transparent at the long-wavelength part of the visible spectrum (Hart and Vorobyev, 2005) and it is assumed that oil droplets share a common spectral profile that is characterized by two parameters, the oil droplet cut-off wavelength (λ_{cut}) and the wavelength at which 50% of the light is transmitted (λ_{mid}) (Lipetz, 1984). It is possible to predict λ_{mid} from λ_{cut} with a high accuracy, and the λ_{cut} values of the SWS, MWS and LWS cone oil droplets can be predicted from the spectral position of the sws1 pigment (Hart and Vorobyev, 2005). These generalizations together with Hart and Vorobyev's oil droplet template (Hart and Vorobyev, 2005) were used to calculate the transmittance of the oil droplets.

To model cone sensitivity in the ultraviolet range, we used our new data of ocular media transmittance (Fig. 2A,B, Fig. 3B; Eqn 1). The effects of possible inaccuracies in these estimations were assessed by a sensitivity analysis (Lind and Kelber, 2009). We considered cone-based vision in bright light conditions and assumed that colour vision is based upon single cones only while achromatic vision is driven by input from the double cones (reviewed in Martin and Osorio, 2008) (but see Lind and Kelber, 2011).

The sensitivity of cones in the blue tit was calculated using the visual pigment and oil droplet templates (Govardovskii et al., 2000; Hart and Vorobyev, 2005) together with published data of λ_{max} of the visual pigments, λ_{cut} and λ_{mid} of the oil droplets and the transmittance of the ocular media (Hart et al., 2000).

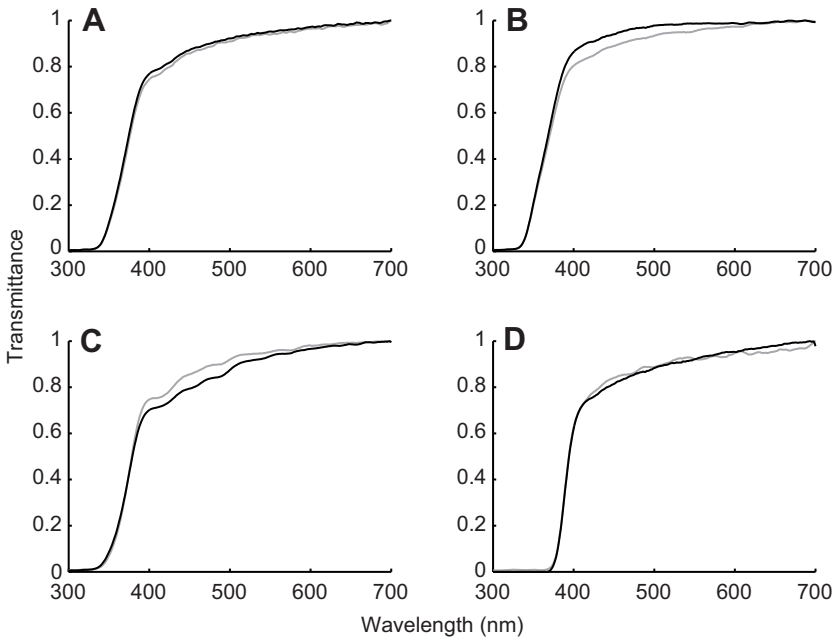

Fig. 2. The ocular media transmittance in four raptor species: (A) common buzzard, (B) sparrowhawk, (C) kestrel and (D) red kite. Measurements from left eyes (grey lines) and right eyes (black lines) are shown for each species. The measurements were taken from whole-eye preparations and each curve is the 11-point running average normalized to the highest value within the range 300–700 nm (see Materials and methods for details). Average ocular media transmittance data and the variation between individual measurements for each species are available in supplementary material Fig. S1 and Table S1.

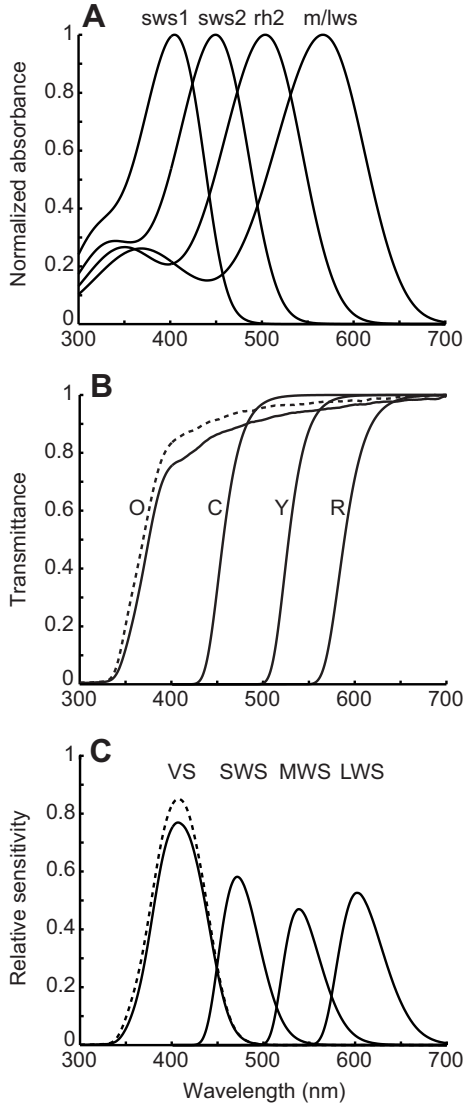

Fig. 3. The absorbance of visual pigments (A), transmittance of oil droplets and ocular media (B) and sensitivity of cones (C) in common buzzards (solid lines) and sparrowhawks (dashed lines). The ocular media transmittance O (see B) is the average for both eyes in each specimen (see Fig. 2A,B); the relative sensitivity of cones (VS, SWS, MWS, LWS; see C) is a function of the predicted absorbance of the visual pigments (sws1, sws2, rh2, m/lws; see A), the predicted transmittance of the oil droplets (C, Y, R; see B) and the measured transmittance of the ocular media (O; see B). Visual pigment absorbance and oil droplet transmittance are identical in common buzzards and sparrowhawks, while the sensitivity of the VS cone differs as a result of variation in ocular media transmittance (see B). Peak wavelength position, λ_{max}, of cone sensitivities (C) is as follows: VS, 407 nm; SWS, 471 nm; MWS, 538 nm; and LWS, 602 nm. Tabulated ocular media transmittance data are available in supplementary material Table S1.

Modelling chromatic contrast

The first step in calculating the chromatic contrast between two stimuli, such as green grass with and without bank vole urine, is to determine the quantum catch of the cones. This is given by:

$$Q_i = \int R_i(\lambda) S(\lambda) I(\lambda) \mathrm{d}\lambda \,, \quad (2)$$

where Q is the quantum catch of receptor type i, S denotes the reflectance spectrum of the stimulus and I is the illuminating spectrum (Kelber et al., 2003). We used standard daylight (D65) as the illuminating light spectrum (Wyszecki and Stiles, 1982).

Quantum catch, as calculated using Eqns 1 and 2, does not account for self-screening, which tends to broaden the spectral sensitivity of photoreceptors (Warrant and Nilsson, 1998). However, self-screening is negligible in photoreceptors with short outer segments. Inspection of retina sections of the common buzzard with transmission electron microscopy indicated cone outer segment lengths of less than 10 μm (O.L., M.M., P.O. and A.K., unpublished data). The change in our predicted chromatic contrasts when including self-screening was less than 5% even for unusually long cone outer segments of 30 μm (cf. Rojas et al., 1999; McNeil et al., 2005; Emond et al., 2006) using an absorption coefficient of $0.035\,\mu\mathrm{m}^{-1}$ (cf. Warrant and Nilsson, 1998).

The receptor quantum catches were compared to first determine the contrast between the stimuli, Δf, for each receptor type i:

$$\Delta f_i = \ln\left(\frac{Q_{i,\mathrm{stimulus1}}}{Q_{i,\mathrm{stimulus2}}}\right) . \quad (3)$$

The contrast values from Eqn 3 were calculated assuming logarithmic scaling of photoreceptor responses and thus are independent of how receptors are adapted (Schaefer et al., 2007).

The receptor contrast values were then compared to determine the chromatic distance between the stimuli for all receptors using a receptor noise-limited model of colour discrimination proposed previously (Vorobyev and Osorio, 1998). In this model, colour discrimination thresholds are set by receptor noise that is propagated into higher order mechanisms. Spatial summation can improve signal strength (signal-to-noise ratio) so that the limiting Weber fraction in each receptor mechanism can be estimated from Eqn 4 (Vorobyev and Osorio, 1998):

$$\omega = \frac{v_i}{\sqrt{\eta_i}} , \quad (4)$$

where ω is the Weber fraction, v is the standard deviation of the noise in an individual cone of type i, and η is the number of this cone type per receptive field. The absolute noise levels of bird photoreceptors are not known but a Weber fraction of 0.1 in the LWS mechanism of the pekin robin (*Leiothrix lutea*) has been derived from behavioural data of photopic spectral sensitivity (Maier, 1992; Vorobyev et al., 1998). We thus used a Weber fraction of the LWS mechanism of 0.1 and the assumption that noise is independent of receptor type and proportional to the relative abundance of each receptor type in the retina (Eqn 4). We assumed a cone abundance ratio of 1:2:2:4 (VS:SWS:MWS:LWS) (Vorobyev and Osorio, 1998), which results in higher Weber fractions in the VS, SWS and MWS mechanisms compared with that in the the LWS mechanism.

The chromatic contrast, ΔS, between stimuli was calculated assuming that receptor signals are compared in colour opponent mechanisms, which in birds are unknown. However, it is not necessary to specify these mechanisms as thresholds are set by receptor noise rather than by how receptor signals combine in retinal processes, and therefore the following equation applies (Vorobyev and Osorio, 1998):

$$\Delta S^2 = \frac{\begin{array}{l}(\omega_1\omega_2)^2(\Delta f_4 - \Delta f_3)^2 + (\omega_1\omega_3)^2(\Delta f_4 - \Delta f_2)^2 \\ +(\omega_1\omega_4)^2(\Delta f_3 - \Delta f_2)^2 + (\omega_2\omega_3)^2(\Delta f_4 - \Delta f_1)^2 \\ +(\omega_2\omega_4)^2(\Delta f_3 - \Delta f_1)^2 + (\omega_3\omega_4)^2(\Delta f_2 - \Delta f_1)^2\end{array}}{(\omega_1\omega_2\omega_3)^2 + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2} , \quad (5)$$

The unit of ΔS is JND (just noticeable difference), and the discrimination threshold is 1 JND.

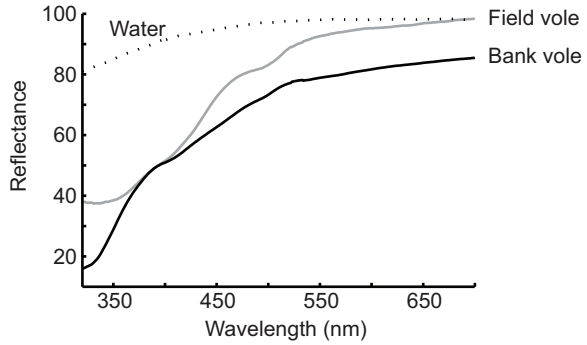

Fig. 4. The reflectance of bank vole urine, field vole urine and water on filter paper. Reflectance was measured from fresh samples, i.e. shortly after the samples were applied to the filter paper.

Sensitivity analysis

Our analyses are based upon generalizations about cone spectral sensitivities and noise levels. Inaccuracies in these estimations can change the predicted chromatic contrasts substantially and need to be accounted for (Lind and Kelber, 2009). In this study, we considered a 50% decrease and a 100% increase in the general cone noise level (Weber fraction of 0.05 and 0.2, respectively, for the LWS mechanism). We did not consider inaccuracies in relative noise levels (by changing cone abundance ratios) as the effect of this variation is relatively small and is masked by the effect of shifting the general noise level (Lind and Kelber, 2009). The effects of inaccuracies in the predicted sensitivity of the VS, SWS, MWS and LWS cones were estimated by repeating the calculations of chromatic contrast using all 81 possible combinations of the originally predicted pigment absorbance spectra (Fig. 3A), and pigment absorbance spectra with λ_{max} shifted either −10 nm or +10 nm. This range of deviation was determined based on the variation in pigment sensitivity among birds (Hart and Vorobyev, 2005; Hart and Hunt, 2007; Lind and Kelber, 2009). Original data of ocular media transmittance were used (Fig. 3B) and for the SWS, MWS and LWS cones, the oil droplet transmittance spectra were shifted together with the pigment absorbance spectra (−10 nm or +10 nm).

RESULTS

Ocular media transmittance

The ocular media transmittance was similar in common buzzards, sparrowhawks and kestrels; the spectral position of 50% transmittance, $\lambda_{0.5}$, was 375, 369 and 379 nm, respectively (Fig. 2A–C). This similarity results in almost identical predictions of cone sensitivity in the common buzzard and the sparrowhawk (Fig. 3C). The ocular media in red kites transmit less UV light than those of the other raptors and the spectral position of $\lambda_{0.5}$ was 394 nm (Fig. 2D). The delay of 30–60 min between measurements of the two eyes in each specimen did not affect the results substantially; the difference in $\lambda_{0.5}$ between the left and right eye was less than 2 nm in all examined raptor species (Fig. 2). Tabulated ocular media transmittance data are available in supplementary material Table S1.

Vole urine reflectance

Raptors are not sensitive to light of wavelengths below 320 nm (Fig. 3C). Vole urine reflectance and chromatic contrast are therefore shown and analysed for wavelengths between 320 and 700 nm (Figs 4–6). We could not detect any distinct UV reflectance peaks in the urine of bank voles on filter paper (Fig. 4) or on any of the other substrates (Fig. 5). This was true also for field vole urine (Fig. 4). In contrast, we found a strong decrease in urine reflectance within the UV range, 320–400 nm (Figs 4, 5).

Chromatic contrast of vole urine

The chromatic contrast of bank vole urine on a sandy substrate was low and consistently below 1 JND (Fig. 6A,B). The contrast was higher for urine on grass, and urine showed a higher contrast to green grass than to dry grass (Fig. 6A,B). There were only small differences in contrast between day 1 and day 4 on sand and green grass, while urine reflectance on dry grass changed more over the test period (Fig. 6A). Water generally had a lower contrast to the substrate compared with urine (Fig. 6A,B) but the contrast between urine and water was close to or below 1 JND (Fig. 6C).

We also used the urine samples with the highest contrast against the background (fresh sample on day 1; Fig. 6A) to compare model predictions including data in the UV range (320–400 nm) with predictions for which we set the spectral reflectance in the UV range to zero in both treated and untreated substrates. This difference was small, with maximally 0.43 JND for vole urine on green grass indicating that most contrast is not in the UV range (Fig. 6D).

Chromatic contrast of blue tit plumage coloration

The chromatic contrasts between blue tit plumage colours and the green background (green grass; Fig. 5A) were all above the detection threshold of 1 JND (Fig. 7). This is true also for calculations assuming the highest value of cone noise (Weber fraction of 0.2). The contrast between plumage and green background was higher for blue tits than for sparrowhawks and when removing the spectral information in the UV (by assuming zero reflectance of stimuli at wavelengths between 300 and 400 nm), contrast was decreased by 8% to 24% for sparrowhawks and by 20% to 45% for blue tits (Fig. 7).

Model sensitivity to parametric error

Calculations assuming different noise levels to half or double the original value resulted in a 100% increase and a 50% decrease in predicted JND values, respectively.

To analyse the sensitivity of the model predictions to inaccuracies in estimated cone sensitivities, we calculated the average change in chromatic contrasts between treated and untreated substrates shown in Fig. 6A,B (N=18) for all 81 possible combinations of manipulated cone sensitivities (see Materials and methods). Compared with the original predictions (Fig. 3C and Fig. 6A,B), the largest change was an increase by 16±12% (mean ± s.d.), which occurred when the VS and SWS cones were shifted −10 nm, the MWS cone was not changed and the LWS cone was shifted +10 nm. This effect is small compared with the effect of changing the general noise level and does not change our conclusions.

We also determined how the predicted contrast changed when illumination was altered from a daylight spectrum (D65) to a blue-shifted spectrum of the sky when the sun is at the horizon. The corresponding change in contrast did not exceed 4% of its original value.

DISCUSSION

Can common buzzards detect bank vole urine?

Vole urine on filter paper clearly absorbs UV light more strongly than light at wavelengths above 400 nm (Fig. 4). This is in agreement with recent studies (Huitu et al., 2008; Kellie et al., 2004), while it is in contrast to older data collected in Finland (Viitala et al., 1995; Koivula and Viitala, 1999; Koivula et al., 1999) (see also Chávez et al., 2003). Possible reasons for this discrepancy, such as different

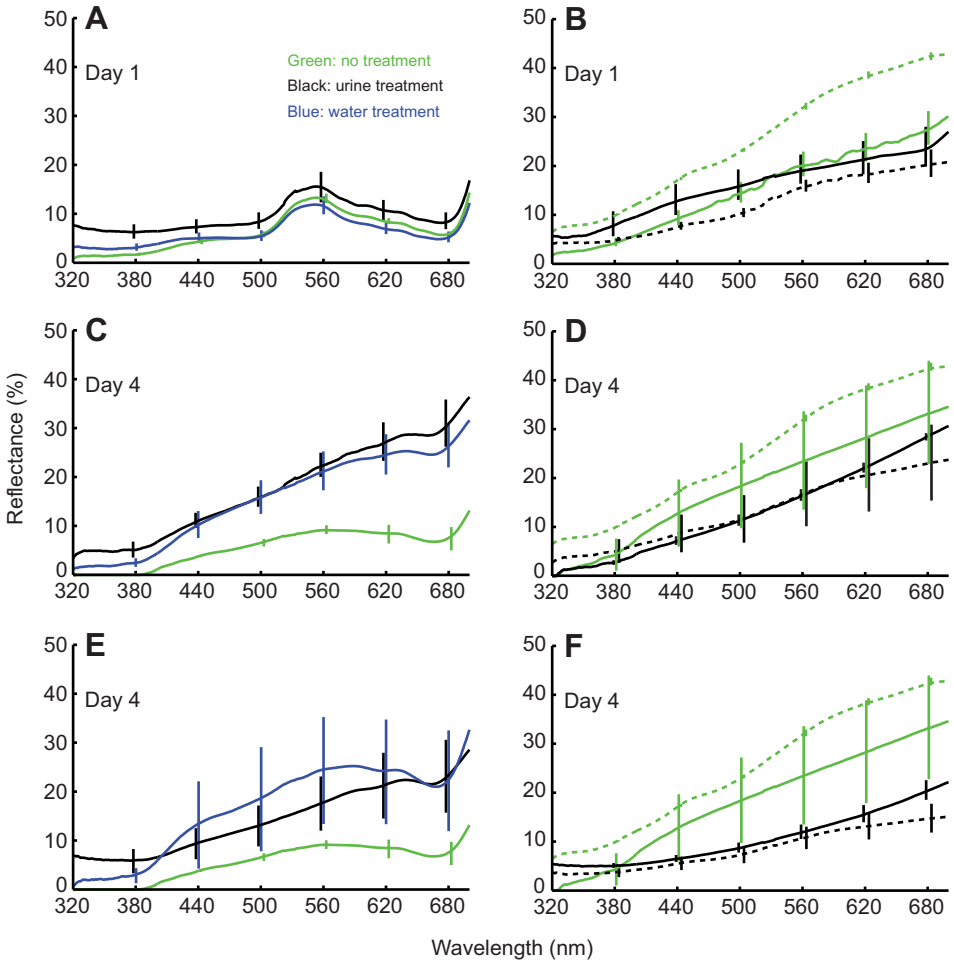

Fig. 5. The reflectance of untreated and urine-treated substrates [green grass in A, C and E; dry grass (solid lines) and sand (dashed lines) in B, D and F]. Examples of the reflectance from water-treated green grass substrates are given in A, C and E. Error bars indicate the standard deviation for all individual measurements (the error bars are slightly displaced laterally to enhance visibility). Measurements were taken when the substrate was still wet, shortly after the treatments were applied (fresh treatment: A, B, E and F) and when the treated substrates had been left drying overnight (dry samples: C and D) on the indicated days. Each curve represents the mean of three measurements smoothed with an 11-point running average. See Materials and methods for details.

diets, have been discussed earlier (Kellie et al., 2004) but the issue will remain unsolved until new measurements of the rodent populations of Finland are compared with other populations to ensure consistent methods of obtaining and analysing spectral data.

Most natural materials, including grass and sand, reflect little UV light, and applying vole urine on these substrates changes the substrate's reflectance very little (Fig. 5). The comparison of model predictions with and without information in the UV (Fig. 6D) shows that spectral information in the UV range adds little or nothing to the chromatic contrast between bank vole urine and the substrate.

Common buzzards might detect a chromatic contrast between grass with and without vole urine because of spectral differences between 400 and 700 nm (Fig. 6A) However, it is unlikely that raptors can discriminate between urine markings and water (Fig. 6D). The chromatic contrast between substrates treated with urine and water is close to threshold even when considering very low receptor noise levels.

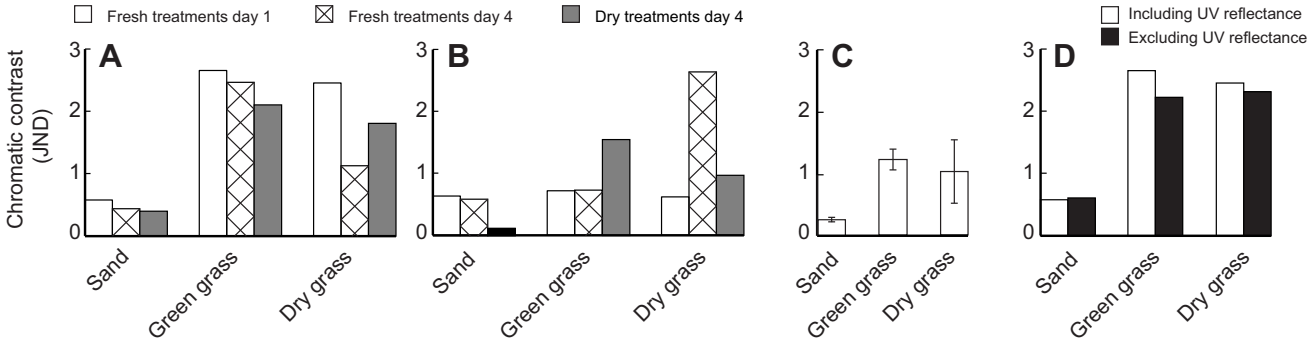

Fig. 6. The chromatic contrast for common buzzards viewing (A) substrate treated with bank vole urine against untreated substrate, (B) substrate treated with distilled water against untreated substrate and (C) substrate treated with vole urine against substrate treated with distilled water. (D) The chromatic contrast between fresh urine treatments on day 1 and substrate (from A, analysed with and without spectral information in the UV range, 320–400 nm). Values in C are means (±s.e.m.) for measurements taken over 4 days (N=4). Chromatic contrast predictions are based on raptor cone sensitivities (Fig. 3C) used in a receptor noise-limited model of colour discrimination (Vorobyev and Osorio, 1998) (see Materials and methods for details). The threshold of colour discrimination is 1 JND (just noticeable difference).

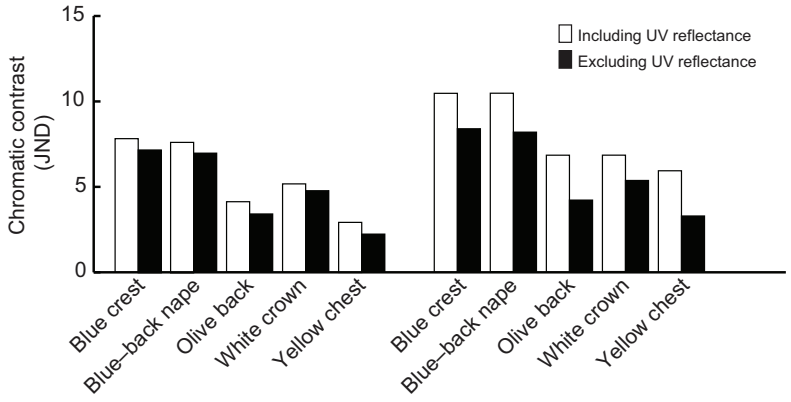

Fig. 7. The chromatic contrast between plumage colours of male blue tits (Hunt et al., 1998) and a green background (green grass in Fig. 5A) with and without spectral information in the UV region of the spectrum (300–400 nm) as viewed by a sparrowhawk (left) and a blue tit (right). Model predictions are as in Fig. 6 and are described in detail in Materials and methods.

Thus, it seems unlikely that vole urine can provide a reliable visual cue under natural circumstances where weak signals are confounded by urine from other mammals, destroyed by rain and wind, and seen from a distance where the low spatial resolution of chromatic vision has to be considered (Lind and Kelber, 2011).

Our conclusions relate to the interactions between common buzzards and voles, while the original hypothesis was formulated for another vole-hunting species, the kestrel (Viitala et al., 1995). Kestrels and common buzzards share a similar ocular media transmittance (Fig. 2A,C) and they probably also share spectral tuning of the sws1 pigment as all raptors investigated so far have sws1 pigments with sensitivity peaks at 405–406 nm (Ödeen and Håstad, 2003). We suggest that our conclusions about the detection of vole urine by common buzzards apply to kestrels as well.

We do not consider achromatic cues in this study, but the lack of any pronounced peaks or characteristics of the shape and amplitude of urine reflectance spectra on grass and sand (Fig. 5) suggests that no achromatic cues are available. We therefore suggest that visual cues other than urine, such as vole trails and voles running in these trails, or odour cues, may be more reliable for detecting areas with high vole densities.

Does the plumage coloration of blue tits represent a hidden communication channel?

Sparrowhawks can detect the plumage colours of blue tits against a green background even with very high cone noise levels (Fig. 7). However, the chromatic contrast of the colours is higher for blue tits than for sparrowhawks (Fig. 7). These differences are too large to be explained by inaccuracies in the estimates of cone sensitivity in sparrowhawks (Fig. 7). Removing the spectral information in the UV range (300–400 nm) affects the chromatic contrast for blue tits more than for sparrowhawks, rendering plumage discriminability similar for the two species (Fig. 7).

Our results are thus in agreement with the hypothesis about a ‘hidden’ communication channel in UV among blue tits (Håstad et al., 2005) (see also Guilford and Harvey, 1998) and this study adds information about its general applicability. We used green grass as the contrasting background (Fig. 5A, Fig. 7) while earlier studies used green foliage of deciduous and coniferous trees (Håstad et al., 2005). The hypothesis remains valid when changing the illuminant from a standard daylight spectrum to a blue-shifted irradiance spectrum measured at sunset or changing the adapting background from green grass to brown leaves (data not shown). Furthermore, the hypothesis applies to raptors other than sparrowhawks, such as common buzzards and kestrels, which have similar cone sensitivities to sparrowhawks (Fig. 3) as well as red kites (*M. milvus*), which have ocular media that transmit only a little more UV light than those of the human eye (Fig. 2D) (Wyszecki and Stiles, 1982). Common buzzards, kestrels and red kites all have diets that include songbirds (Davis and Davis, 1981; Korpimäki, 1985; Reif et al., 2001).

Still, we cannot exclude the possibility that general cone noise levels are higher in blue tits than in raptors (50% more noise in blue tit cones would make their plumage colours more contrasting to raptors than to themselves). Moreover, the lack of data on how supra-threshold chromatic contrast affects detection makes it difficult to understand exactly how much ‘more conspicuous’ a colour distance of 10 JND is compared with a distance of 8 JND (Fig. 7). This issue represents perhaps the greatest challenge for more informative visual modelling besides the lack of data on bird photoreceptor noise levels.

There is also a need to investigate the relative importance of chromatic and achromatic cues for raptor hunting behaviour. For raptors hunting on the wing, motion signals are very important for detection, and these are probably mediated by achromatic rather than chromatic mechanisms [see Campenhausen and Kirschfeld (Campenhausen and Kirschfeld, 1998) and references therein].

The role of colour vision in raptor hunting

We have shown that the ocular media of raptor eyes transmit only a little UV light. With this new information, we used visual modelling to quantify the chromatic signals involved in two model systems of raptor–vole and raptor–songbird interactions. It has been suggested that UV signals have a predominant role in these interactions, either as cues for raptor hunting behaviour or as ‘hidden’ songbird signals concealed from raptors (Viitala et al., 1995; Koivula and Viitala, 1999; Håstad et al., 2005) (and see Zampiga et al., 2006; Zampiga et al., 2008). Our analyses show that raptors do not use UV cues for detection in these interactions, and our data suggest that it is unlikely that bank vole and field vole urine provide any visual cue that raptors can use. Raptors can detect the plumage colours of blue tits as a result of chromatic contrast in the visual spectrum between 400 and 700 nm, although it remains unclear whether they utilize this potential. The role of colour vision and UV reception in raptors therefore remains unclear.

LIST OF SYMBOLS AND ABBREVIATIONS

I	illuminating spectrum
JND	just noticeable difference
LWS	long-wavelength-sensitive cone
m/lws	visual pigment of the LWS cone
MWS	medium-wavelength-sensitive cone
o	transmittance of ocular media
p	transmittance of oil droplets
Q	quantum catch of photoreceptor
r	spectral sensitivity of visual pigments

R	spectral sensitivity of cone photoreceptors
rh2	visual pigment of the MWS cone
S	reflectance spectrum
SWS	short-wavelength-sensitive cone
sws1	visual pigment of the UVS/VS cone
sws2	visual pigment of the SWS cone
UV	ultraviolet light
UVS/VS	ultraviolet- or violet-sensitive cone
v	standard deviation of noise in an individual cone
Δf	contrast in receptors
η	number of cone types within a receptive field
$\lambda_{0.5}$	wavelength of 50% transmittance (of ocular media)
λ_{cut}	cut-off wavelength (of oil droplet)
λ_{max}	peak wavelength (of visual pigments or cones)
λ_{mid}	wavelength of 50% transmittance (of oil droplet)
ω	Weber fraction

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AUTHOR CONTRIBUTIONS

All authors contributed equally to all parts of the article.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

Bennett, A. T. D. and Cuthill, I. C. (1994). Ultraviolet vision in birds: what is its function? *Vision Res.* **34**, 1471-1478.
Campenhausen, M. v. and Kirschfeld, K. (1998). Spectral sensitivity of the accessory optics system of the pigeon. *J. Comp. Physiol. A* **183**, 1-6.
Chávez, A. E., Bozinovic, F., Peichl, L. and Palacios, A. G. (2003). Retinal spectral sensitivity, fur coloration, and urine reflectance in the genus *Octodon* (rodentia): implications for visual ecology. *Invest. Ophthalmol. Vis. Sci.* **44**, 2290-2296.
Davis, P. E. and Davis, J. E. (1981). The food of the red kite in Wales. *Bird Study* **28**, 33-40.
Emond, M. P., McNeil, R., Cabana, T., Guerra, C. G. and Lachapelle, P. (2006). Comparing the retinal structures and functions in two species of gulls (*Larus delawarensis* and *Larus modestus*) with significant nocturnal behaviours. *Vision Res.* **46**, 2914-2925.
Goldsmith, T. H. and Butler, B. K. (2003). The roles of receptor noise and cone oil droplets in the photopic spectral sensitivity of the budgerigar, *Melopsittacus undulatus*. *J. Comp. Physiol. A* **189**, 135-142.
Goldsmith, T. H. and Butler, B. K. (2005). Color vision of the budgerigar (*Melopsittacus undulatus*): hue matches, tetrachromacy, and intensity discrimination. *J. Comp. Physiol. A* **191**, 933-951.
Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.
Guilford, T. and Harvey, P. H. (1998). The purple patch. *Nature* **392**, 867-869.
Härmä, O., Kareksela, A. S., Siitari, H. and Suhonen, J. (2011). Pygmy owl *Glaucidium passerinum* and the usage of ultraviolet cues of prey. *J. Avian Biol.* **42**, 89-91.
Hart, N. S. (2001). The visual ecology of avian photoreceptors. *Prog. Retin. Eye Res.* **20**, 675-703.
Hart, N. S. and Hunt, D. M. (2007). Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169 Suppl. 1**, S7-S26.
Hart, N. S. and Vorobyev, M. (2005). Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *J. Comp. Physiol. A* **191**, 381-392.
Hart, N. S., Partridge, J. C., Cuthill, I. C. and Bennett, A. T. D. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). *J. Comp. Physiol. A* **186**, 375-387.
Håstad, O., Victorsson, J. and Ödeen, A. (2005). Differences in color vision make passerines less conspicuous in the eyes of their predators. *Proc. Natl. Acad. Sci. USA* **102**, 6391-6394.
Heintzelman, D. S. (1964). Spring and summer sparrow hawk food habits. *Wilson Bull.* **76**, 323-330.
Huitu, O., Helander, M., Lehtonen, P. and Saikkonen, K. (2008). Consumption of grass endophytes alters the ultraviolet spectrum of vole urine. *Oecologia* **156**, 333-340.
Hunt, S., Bennett, A. T. D., Cuthill, I. C. and Griffiths, R. (1998). Blue tits are ultraviolet tits. *Proc. Biol. Sci.* **265**, 451-455.
Kelber, A., Vorobyev, M. and Osorio, D. (2003). Animal colour vision – behavioural tests and physiological concepts. *Biol. Rev. Camb. Philos. Soc.* **78**, 81-118.
Kellie, A., Dain, S. J. and Banks, P. B. (2004). Ultraviolet properties of Australian mammal urine. *J. Comp. Physiol. A* **190**, 429-435.
Koivula, M. and Viitala, J. (1999). Rough-legged buzzards use vole scent marks to assess hunting areas. *J. Avian Biol.* **30**, 329-332.
Koivula, M., Koskela, E. and Viitala, J. (1999). Sex and age-specific difference in ultraviolet reflectance of scent marks of bank voles (*Clethrionomys glareolus*). *J. Comp. Physiol. A* **185**, 561-564.
Korpimäki, E. (1985). Diet of the kestrel *Falco tinnunculus* in the breeding season. *Ornis Fenn.* **62**, 130-137.
Lind, O. and Kelber, A. (2009). Avian colour vision: effects of variation in receptor sensitivity and noise data on model predictions as compared to behavioural results. *Vision Res.* **49**, 1939-1947.
Lind, O. and Kelber, A. (2011). The spatial tuning of achromatic and chromatic vision in budgerigars. *J. Vis.* **11**, 1-9.
Lipetz, L. E. (1984). A new method for determining peak absorbance of dense pigment samples and its application to the cone oil droplets of *Emydoidea blandingii*. *Vision Res.* **24**, 567-604.
Maier, E. J. (1992). Spectral sensitivities including the ultraviolet of the passeriform bird *Leiothrix lutea*. *J. Comp. Physiol. A* **170**, 709-714.
Martin, G. R. and Osorio, D. (2008). Vision in birds. In *The Senses: A Comprehensive Reference*, Vol. 1 (ed. D. Masland and R. M. Albright), pp. 25-52. San Diego, CA: Academic Press.
McNeil, R., McSween, A. and Lachapelle, P. (2005). Comparison of the retinal structure and function in four bird species as a function of the time they start singing in the morning. *Brain Behav. Evol.* **65**, 202-214.
Ödeen, A. and 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.
Ödeen, A., Hart, N. S. and Håstad, O. (2009). Assessing the use of genomic DNA as a predictor of the maximum absorbance wavelength of avian SWS1 opsin visual pigments. *J. Comp. Physiol. A* **195**, 167-173.
Osorio, D., Miklósi, A. and Gonda, Z. (1999). Visual ecology and perception of coloration patterns by domestic chicks. *Evol. Ecol.* **13**, 673-689.
Reif, V., Tornberg, R., Jungell, S. and Korpimäki, E. (2001). Diet variation of common buzzards in Finland supports the alternative prey hypothesis. *Ecography* **24**, 267-274.
Rojas, L. M., McNeil, R., Cabana, T. and Lachapelle, P. (1999). Behavioral, morphological and physiological correlates of diurnal and nocturnal vision in selected wading bird species. *Brain Behav. Evol.* **53**, 227-242.
Schaefer, H. A. M., Schaefer, V. and Vorobyev, M. (2007). Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? *Am. Nat.* **169 Suppl. 1**, S159-S169.
Viitala, J., Korpimäki, E., Palokangas, P. and Koivula, M. (1995). Attraction of kestrels to vole scent marks visible in ultraviolet light. *Nature* **373**, 425-427.
Vorobyev, M. and Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proc. Biol. Sci.* **265**, 351-358.
Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J. and Cuthill, I. C. (1998). Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**, 621-633.
Warrant, E. J. and Nilsson, D.-E. (1998). Absorption of white light in photoreceptors. *Vision Res.* **38**, 195-207.
Wyszecki, G. and Stiles, W. S. (1982). *Color Science: Concepts and Methods, Quantitative Data and Formulae*, 2nd edn. New York, NY: Wiley.
Zampiga, E., Gaibani, G., Csermely, D., Frey, H. and Hoi, H. (2006). Innate and learned aspects of vole urine UV-reflectance use in the hunting behavior of the common kestrel *Falco tinnunculus*. *J. Avian Biol.* **37**, 318-322.
Zampiga, E., Gaibani, G. and Csemely, D. (2008). Ultraviolet reflectance and female mating preferences in the common kestrel (*Falco tinnunculus*). *Can. J. Zool.* **86**, 479-483.