J Comp Physiol A (2000) 186: 375–387

ORIGINAL PAPER

N. S. Hart · J. C. Partridge · I. C. Cuthill
A. T. D. Bennett

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.)

Accepted: 8 January 2000

Abstract The spectral absorption characteristics of the retinal photoreceptors of the blue tit (*Parus caeruleus*) and blackbird (*Turdus merula*) were investigated using microspectrophotometry. The retinae of both species contained rods, double cones and four spectrally distinct types of single cone. Whilst the visual pigments and cone oil droplets in the other receptor types are very similar in both species, the wavelength of maximum sensitivity (λ_{max}) of long-wavelength-sensitive single and double cone visual pigment occurs at a shorter wavelength (557 nm) in the blackbird than in the blue tit (563 nm). Oil droplets located in the long-wavelength-sensitive-single cones of both species cut off wavelengths below 570–573 nm, theoretically shifting cone peak spectral sensitivity some 40 nm towards the long-wavelength end of the spectrum. This raises the possibility that the precise λ_{max} of the long-wavelength-sensitive visual pigment is optimised for the visual function of the double cones. The distribution of cone photoreceptors across the retina, determined using conventional light and fluorescence microscopy, also varies between the two species and may reflect differences in their visual ecology.

Key words Colour vision · Microspectrophotometry · Avian retina

Abbreviations *AD* anterior dorsal · *AV* anterior ventral · *C* central · *D* dorsal · *LWS* long-wavelength-sensitive · *MSP* microspectrophotometer · *MWS* medium-wavelength-sensitive · *PBS* phosphate-buffered saline · *PD* posterior dorsal · *PV* posterior ventral · *SWS* short-wavelength-sensitive · *UVS* ultraviolet-sensitive · *V* ventral · *VS* violet-sensitive · λ wavelength · λ_{max} wavelength of maximum sensitivity · λ_{cut} cut-off wavelength · λ_{mid} wavelength of 50% maximum measured absorptance

N. S. Hart (✉)[1] · J. C. Partridge · I. C. Cuthill
A. T. D. Bennett
School of Biological Sciences, University of Bristol,
Woodland Road, Bristol BS8 1UG, UK

Present address:
[1]Vision, Touch and Hearing Research Centre,
The University of Queensland, Brisbane,
QLD 4072, Australia,
e-mail: n.hart@vthrc.uq.edu.au
Tel.: +61-7-3365-4098; Fax: +61-7-3365-4522

Introduction

In addition to a single class of medium-wavelength-sensitive (MWS) rod and a single class of long-wavelength-sensitive (LWS) double cone, the retinae of most birds contain four classes of single cone with maximum sensitivity to different regions of the spectrum. The use of spectral filters (oil droplets) to narrow cone spectral sensitivity functions (Baylor and Hodgkin 1973; Neumeyer and Jäger 1985; Kawamuro et al. 1997) suggests that the maximum number of spectrally distinct single cone types, and the spectral location of their wavelength of maximum sensitivity (λ_{max}), are constrained in birds to some extent by (1) the range of relevant wavelengths useable in vision reaching the retina, (2) the need to minimise potentially disadvantageous excessive overlap between adjacent chromatic channels (Barlow 1982), and (3) the need to space receptor sensitivities evenly across the visible spectrum (Vorobyev and Menzel 1999). Indeed, the spectral absorption characteristics of retinal photoreceptors measured in the few species of bird studied to date using microspectrophotometry are broadly similar (see Bowmaker et al. 1997 for review; also Hart et al. 1998, 1999; Das et al. 1999).

Nevertheless, there is considerable variation between species in the λ_{max} values of the visual pigments expressed in certain cone types. For example, the visual pigment found in cones with a transparent (T-type) oil droplet, which shows no detectable absorption of wavelengths between at least 330 nm and 750 nm, has a λ_{max} value between 403 nm and 426 nm (violet sensitive, VS) in the duck *Anas platyrhynchos* (Jane and

Bowmaker 1988), domestic turkey *Meleagris gallopavo* (Hart et al. 1999), pigeon *Columba livia* (Bowmaker et al. 1997), chicken *Gallus gallus* (Fager and Fager 1981; Yoshizawa and Fukada 1993; Bowmaker et al. 1997), penguin *Spheniscus humboldti* (Bowmaker and Martin 1985) and Japanese quail *Coturnix coturnix japonica* (Bowmaker et al. 1993), but between about 355 nm and 380 nm (ultraviolet sensitive, UVS) in the Pekin robin *Leothrix lutea* (Maier and Bowmaker 1993), zebra finch *Taeniopygia guttata* and budgerigar *Melopsittacus undulatus* (Bowmaker et al. 1997), European starling *Sturnus vulgaris* (Hart et al. 1998) and canary *Serinus canaria* (Das et al. 1999).

Whilst this UVS/VS dichotomy constitutes the greatest variation observed to date within a single cone type, other more subtle variations exist. Understanding why these variations exist depends entirely on complete and high quality data from more species. Here, we provide microspectrophotometric data for two species of passerine, the blue tit (*Parus caeruleus* L. 1758) and the blackbird (*Turdus merula* L. 1758). These two species were chosen mainly because they exhibit different foraging strategies: blue tits are generally arboreal (Lack 1971; Moreno 1981), whereas blackbirds are ground feeders (Hillstead 1944; Cramp et al. 1988). We show that, whilst the spectral absorption characteristics of the other photoreceptors are very similar, there is a clear difference in the λ_{max} values of the LWS cone visual pigment between species. Furthermore, we provide measurements of the transmission of the ocular media and the relative abundance of the different cone photoreceptors across the retina of these two species.

Materials and methods

Animals

Wild-caught adult blue tits and blackbirds were kept in unheated outdoor aviaries illuminated with natural skylight filtered by translucent Perspex roofing. Their diet consisted of a proprietary insect-based feed (Orlux soft bill, Belgium), daily supplements of two to three mealworms and, in the case of blue tits, sunflower seeds and peanuts. Captive birds are susceptible to the depletion of carotenoids in the coloured oil droplets of retinal cone photoreceptors (Hart et al. 1998), so all microspectrophotometric recordings were made within two months of the capture date.

Microspectrophotometry

Birds were held in darkness overnight and killed by approved humane methods (cervical dislocation followed by decapitation). Preparation of retinal tissue for analysis using a microspectrophotometer (MSP) was as described elsewhere (Hart et al. 1998, 1999). Photoreceptors were mounted in a solution of phosphate-buffered saline (PBS; Dulbecco 'A' tabletised PBS made to a concentration of 340 mosmol kg^{-1}, Oxoid, Basingstoke, UK) diluted 1: 3 with pure glycerol (BDH) and adjusted to pH 7.3 using 1 mol l^{-1} NaOH. Separate retinal preparations were made for the measurement of oil droplet absorption spectra and these samples were mounted in pure glycerol. Absorption spectra (330–750 nm) of individual photoreceptors were measured using a computer-controlled, wavelength-scanning, single-beam MSP, the construction and operation of which has been described in detail elsewhere (Hart et al. 1998).

Dimensions of the measuring beam were adjusted according to the size of the photoreceptors and varied from approximately 1 μm × 1 μm for UVS cones and oil droplets to 2 μm × 10 μm for rods. Rod outer segments were typically 3–4 μm in diameter and 15–20 μm in length. Dimensions of measured cone outer segments ranged from about 2 μm × 2 μm up to 5 μm × 5 μm, UVS cones being the smallest and double cones the biggest. Cone outer segments were often folded over upon themselves or otherwise distorted and, consequently, it was difficult to estimate either true length or transverse pathlength. For this reason, specific absorbances per micrometer of cone outer segment were not calculated and absorbances measured at the λ_{max} of the mean difference spectrum are given instead (Tables 1 and 2, which also list diameters of the different oil droplet types).

Analysis of visual pigment absorbance spectra

Baseline and sample scans were made from tissue-free and cellular samples, respectively. Data were recorded at each odd wavelength on the 'downward' long-wavelength to short-wavelength spectral pass and at each interleaved even wavelength on the 'upward' short-wavelength to long-wavelength spectral pass. A single scan consisted of two downward and two upward spectral passes in alternate succession, and spectral passes of the same 'direction' were averaged together.

In order to minimise in-scan bleaching, only one sample scan of each outer segment was made, but this was combined with two separate measurements of the baseline absorbance spectrum. Averaging the two absorbance spectra obtained in this way improved the signal-to-noise ratio of the spectra used for determining visual pigment λ_{max} values (Bowmaker et al. 1997). Because of their smaller diameter, and the resultant reduction in signal-to-noise ratio of the spectra obtained, three pairs of sample and baseline scans were made from outer segments containing UVS visual pigment. In-scan bleaching of UVS visual pigments was less of a problem because of the reduced light flux from the monochromator light source at ultraviolet wavelengths.

Having recorded absorbance spectra from outer segments, the visual pigments were bleached with white light for 5 min and an identical number of sample and baseline scans made subsequently. The post-bleach average spectrum created in this way was deducted from the pre-bleach average to create a difference spectrum for each outer segment.

Baseline and sample data were converted into absorbance values at 1-nm intervals. Absorbance is a logarithmic measure of light absorption and is defined as the $\log_{10}$ of the ratio of the intensity of the light incident upon a sample to the intensity of the light transmitted by the sample or, alternatively, as $-\log_{10}(T)$ where T is transmission (1-absorptance). Absorbances are additive (doubling the pathlength doubles the absorbance) but when normalised, the shape of an absorbance spectrum remains unchanged regardless of concentration or pathlength. Absorbance is not to be confused with absorptance, which is the ratio of the intensity of light absorbed by a sample to the intensity of light incident upon it. The shape of an absorptance spectrum will be dependent upon the concentration and pathlength of the sample. Upward and downward scans were averaged by fitting a weighted (delta function), three-point running average to the absorbance data, i.e. the two absorbance values on either side of a datum were averaged and this mean averaged with the datum. This eliminated separation of the upward and downward spectral passes caused by in-scan bleaching, resulting in a more accurate estimate of the λ_{max} whilst increasing the apparent full-width at half-maximum (FWHM) bandwidth by only ca. 1 nm (Hart 1998).

Pre- and post-bleach absorbance spectra were then normalised to the peak and long-wavelength offset absorbances obtained by fitting a variable-point unweighted ('box-car') running average, the number of points being determined by the signal-to-noise ratio of the data (Hart et al. 1998). The λ_{max} was determined using the polynomial of Partridge and DeGrip (1991) or, for UVS visual

pigments, that derived from the data of Stavenga et al. (1993) and Palacios et al. (1996) by Hart et al. (1998). Specifically, each point on the long-wavelength limb of the absorbance spectrum with an absorbance between 80% and 20% of the normalised maximum was used to estimate the λ_{max}, the average of all these estimates being taken as the best estimate of the λ_{max} of the visual pigment (Partridge and DeGrip 1991).

Spectra were retained for further analysis only if they satisfied the acceptance criteria described and justified elsewhere (Levine and MacNichol 1985; Hart et al. 1998, 1999). Criteria were relaxed for UVS cones, these being so rarely encountered, and all spectra from cells that were shown to be photolabile were included. Acceptable spectra from each photoreceptor type were averaged and reanalysed.

Analysis of oil droplet absorption spectra

A single sample scan was made from each oil droplet and combined with a single baseline scan. Each scan consisted of only one downward and one upward spectral pass, which were not averaged together. Sample and baseline data were converted to absorptance and normalised to the maximum and long-wavelength-offset absorptances obtained by fitting an 11-point, unweighted running average to the data (Hart et al. 1998). Oil droplets are described by their 'cut-off wavelength' (λ_{cut}), which is the wavelength of the intercept at the value of maximum measured absorptance by the line tangent to the oil droplet absorptance curve at half maximum measured absorptance (Lipetz 1984). For comparison with other studies (e.g. Partridge 1989), the wavelength corresponding to 50% maximum measured absorptance (λ_{mid}) (Lipetz 1984) is also given (see Tables 1, 2).

Spectrophotometry of ocular media

Absorbance measurements (200–800 nm) of the ocular media (cornea, aqueous humour, lens and vitreous humour) were made from whole eyes using a Shimadzu 2101 PC UV-VIS scanning spectrophotometer (Shimadzu, Japan) fitted with a Shimadzu ISR-260 integrating sphere assembly. Following enucleation, a small circular 'window' was created in the posterior pole of the eye by removing a portion of the sclera opposite to, and of approximately the same size as, the cornea. Careful dissection ensured that a negligible amount of vitreous remained attached to the section of retina removed along with the sclera.

The intact eye of the blue tit was placed in a rectangular aluminium insert, designed to fit inside a standard (10-mm pathlength) quartz cuvette, in which a 6.8-mm-diameter hole (the same diameter as the eye) had been drilled to coincide with the measuring beam of the spectrophotometer and in which the eye could be positioned in its normal orientation relative to the incident light. Thin plastic rings were lodged inside the insert hole in front of and behind the eye to prevent movement.

The eye of the blackbird was measured in a similar fashion, but using a modified 'cuvette'. This comprised a Perspex cylinder of length 17.0 mm and internal diameter 15.8 mm, sealed at either end by glass coverslips (19 mm diameter, no. 0 thickness) held in place with silicone sealant. The internal diameter of the cylinder was reduced, using concentric rings of flexible plastic, to ensure that eyes were held perpendicular to the path of the measuring beam and did not move during measurement. Care was taken to avoid compression of the eye which would alter it's length along the optic axis. Identical inserts and cuvettes were placed in the reference channel of the spectrophotometer. Eyes were bathed in, and measured relative to, 340 mosmol kg^{-1} PBS.

Topographical spatial distribution of cone photoreceptors

One bird of each species was dark adapted for at least 1 h prior to sacrifice. Eyes were removed under infra-red illumination, hemisected and left to soak in 340 mosmol kg^{-1} PBS for 1 h to encourage detachment of the pigment epithelium from the neural retina. Retinae were subsequently fixed for 1–2 min in 10% paraformaldehyde in 0.1 mol l^{-1} phosphate buffer and divided into four quadrants (posterior dorsal, PD; anterior dorsal, AD; posterior ventral, PV and anterior ventral, AV) using the pecten for orientation. Counts of oil droplets from ten fields of view from each quadrant were made from both the left and right eyes. Observations were made using an Olympus BHS microscope (Olympus, Japan), fitted with a reflected light fluorescence attachment (mercury lines isolated at 334 nm and 365 nm) and a long-pass filter for viewing wavelengths longer than 435 nm. The 'colour' and duration of oil droplet autofluorescence when irradiated with near-ultraviolet wavelengths is a particularly useful criterion when distinguishing droplets located in the UVS and short-wavelength-sensitive (SWS) single cones and the principal member of double cones, which may appear 'colourless' to the human eye but differ in spectral transmission, chemical composition and fluorescent properties (Goldsmith et al. 1984; Hart et al. 1998).

Results

Microspectrophotometry

Microspectrophotometric results from the blue tit and blackbird are summarised in Tables 1 and 2. The retinae of both species displayed an identical complement of photoreceptors: a single class of MWS rod, a single class of LWS double cone and four types of spectrally distinct single cones. Mean absorbance spectra (and difference spectra) of the visual pigments measured in these photoreceptors are displayed in Figs. 1 and 2 for the blue tit and blackbird, respectively. Each spectrum is overlaid with an appropriate (λ_{max}/λ)-transformed rhodopsin template generated using the equations given by Stavenga et al. (1993). In each case, the β-peak of the template absorbance spectrum is shifted linearly with respect to the α-peak, as suggested by Palacios et al. (1996) and the relative extinction coefficients of the α- and β-peaks are those proposed by Stavenga et al. (1993). The absorbance spectra of UVS visual pigment have narrower FWHM bandwidths than those of visual pigments with λ_{max} in the human visible spectrum, even when transformed on a scale of λ_{max}/λ (Hawryshyn and Hárosi 1994; Palacios et al. 1996). Consequently, for UVS visual pigments the coefficients given by Palacios et al. (1996) were substituted into the equations of Stavenga et al. (1993). Mean absorptance spectra of the oil droplets found in the different cone types are displayed in Figs. 4 and 5.

Blue tit

Rod photoreceptors, which in birds do not possess an oil droplet, contained a rhodopsin with a mean λ_{max} of 503 nm. UVS single cones, with a 'transparent' or T-type oil droplet in their inner segment that showed no detectable absorption of wavelengths between 330 nm and 750 nm, contained a rhodopsin of mean λ_{max} 372 nm. SWS single cones with a 'colourless' or C-type

Table 1 Characteristics of visual pigments and oil droplets in the retinal photoreceptors of the blue tit (*Parus caeruleus*). Values are plus/minus one standard deviation. Standard deviations for the wavelength of maximum sensitivity (λ_{max}) values of mean visual pigment absorbance spectra refer to the error in estimating the visual pigment λ_{max} using the method described in the text. Standard deviations for the mean λ_{max}, cut-off wavelength (λ_{cut}) and wavelength of 50% maximum measured absorptance (λ_{mid}) values represent the variance of the individual records used to create the mean spectra. Rods do not contain oil droplets; no oil droplet was observed in the accessory member of the double cone pair (*D* dorsal, *V* ventral, *UVS* ultraviolet-sensitive, *SWS* short-wavelength-sensitive, *MWS* medium-wavelength-sensitive, *LWS* long wavlength-sensitive)

	Rod	Single cones				Double cones	
		UVS	SWS	MWS	LWS	Principal	Accessory
Visual pigments							
λ_{max} of mean prebleach spectrum (nm)	503.3 ± 1.0	371.2 ± 2.1	448.0 ± 3.4	502.9 ± 1.6	563.1 ± 2.1	564.7 ± 2.1	563.4 ± 3.0
Mean λ_{max} of prebleach spectra (nm)	503.4 ± 1.1	372.4 ± 3.8	448.6 ± 2.5	502.3 ± 3.0	562.7 ± 1.5	565.1 ± 1.8	563.4 ± 2.2
λ_{max} of mean difference spectrum (nm)	506.6 ± 1.9	372.0 ± 3.8	448.5 ± 7.7	508.6 ± 3.8	563.8 ± 3.2	565.0 ± 2.0	561.4 ± 4.8
Mean λ_{max} of difference spectra (nm)	506.3 ± 1.3	367.3 ± 7.2	446.5 ± 8.0	509.1 ± 2.6	563.8 ± 1.3	565.1 ± 1.7	563.0 ± 3.5
Absorbance at λ_{max} (mean difference spectrum)	0.023	0.006	0.007	0.010	0.018	0.017	0.012
Number of outer segments	6	5	5	10	7	14	4
		T-type	C-type	Y-type	R-type	P-type	A-type
						Dorsal / Ventral	
Oil droplets							
λ_{cut} of mean absorptance spectrum (nm)	–	< 330	413.5	507.7	572.6	417.4 / 417.9	–
λ_{mid} of mean absorptance spectrum (nm)	–	< 330	426.6	528.0	595.9	431.1 / 436.4	–
Maximum corrected absorptance of mean absorptance spectrum	–	< 0.01	0.25	0.83	0.87	0.72 / 0.58	–
Mean λ_{cut} (nm)	–	< 330	412.9 ± 3.1	507.6 ± 4.3	572.5 ± 1.7	416.8 ± 3.2 / 417.9 ± 0.5	–
Mean λ_{mid} (nm)	–	< 330	426.2 ± 2.2	527.5 ± 4.3	595.9 ± 1.8	430.6 ± 3.5 / 437.0 ± 3.4	–
Mean diameter (μm)	–	2.0 ± 0.5	2.2 ± 0.4	2.7 ± 0.3	3.1 ± 0.2	3.2 ± 0.2 / 3.0 ± 0.3	–
Number of oil droplets	–	8	12	13	14	7 / 9	–

Table 2 Characteristics of visual pigments and oil droplets in the retinal photoreceptors of the blackbird (*Turdus merula*). Values are plus/minus one standard deviation. Standard deviations for the λ_{max} values of mean visual pigment absorbance spectra refer to the error in estimating the visual pigment λ_{max} using the method described in the text. Standard deviations for the mean λ_{max}, λ_{cut} and λ_{mid} values represent the variance of the individual records used to create the mean spectra. Rods do not contain oil droplets; no oil droplet was observed in the accessory member of the double cone pair. For abbreviations see Table 1

	Rod	Single cones				Double cones			
		UVS	SWS	MWS	LWS	Principal			Accessory
Visual pigments									
λ_{max} of mean prebleach spectrum (nm)	504.5 ± 0.6	373.0 ± 2.3	453.5 ± 1.5	504.3 ± 2.0	557.2 ± 1.2	556.8 ± 1.0			555.8 ± 2.1
Mean λ_{max} of prebleach spectra (nm)	504.4 ± 0.9	372.5 ± 5.0	453.6 ± 2.2	503.8 ± 2.7	557.0 ± 0.8	556.9 ± 1.3			556.0 ± 1.8
λ_{max} of mean difference spectrum (nm)	506.6 ± 1.1	367.2 ± 4.9	455.7 ± 3.1	507.9 ± 3.4	557.6 ± 2.0	557.3 ± 1.4			555.8 ± 3.0
Mean λ_{max} of difference spectra (nm)	506.8 ± 1.8	365.2 ± 5.7	456.8 ± 4.3	508.2 ± 2.3	557.6 ± 2.4	557.4 ± 1.8			556.1 ± 1.5
Absorbance at λ_{max} (mean difference spectrum)	0.038	0.007	0.008	0.011	0.018	0.023			0.015
Number of outer segments	15	4	12	8	9	35			7
		T-type	C-type	Y-type	R-type	P-type			A-type
						Dorsal	Central	Ventral	
Oil droplets									
λ_{cut} of mean absorptance spectrum (nm)	–	< 330	414.3	514.2	569.9	414.7	439.1	472.3	–
λ_{mid} of mean absorptance spectrum (nm)	–	< 330	429.1	532.5	593.0	431.0	455.5	494.2	–
Maximum corrected absorptance of mean absorptance spectrum	–	< 0.01	0.29	0.88	0.88	0.78	0.79	0.90	–
Mean λ_{cut} (nm)	–	< 330	413.8 ± 3.2	514.8 ± 2.4	570.0 ± 1.8	414.3 ± 1.1	438.8 ± 2.1	470.4 ± 7.5	–
Mean λ_{mid} (nm)	–	< 330	428.8 ± 5.1	532.3 ± 3.9	592.9 ± 2.0	431.0 ± 1.5	455.5 ± 1.5	493.4 ± 4.0	–
Mean diameter (μm)	–	2.3 ± 0.6	2.5 ± 0.6	3.2 ± 0.4	3.3 ± 0.4	4.0 ± 0.3	3.2 ± 0.2	3.9 ± 0.2	–
Number of oil droplets	–	5	23	33	61	11	13	23	–

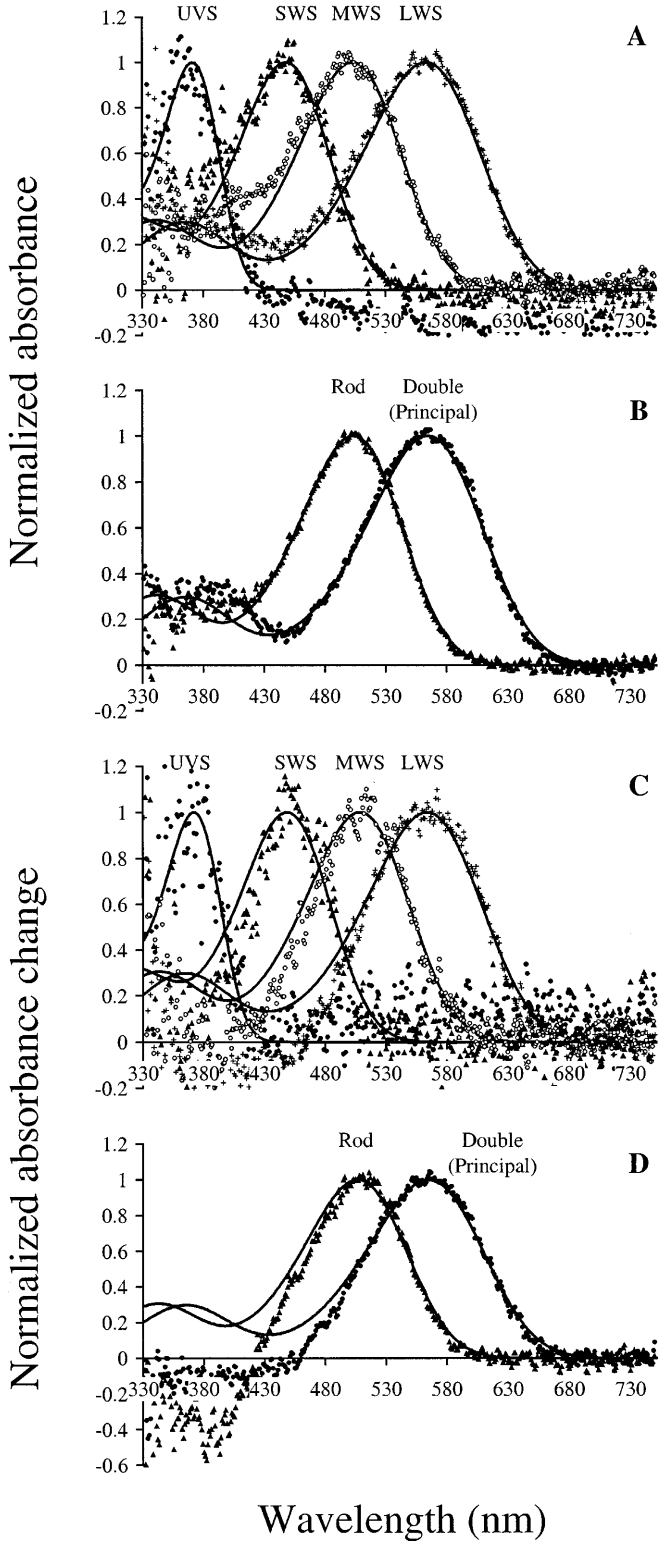

Fig. 1A–D Normalised mean prebleach absorbance (**A, B**) and difference (**C, D**) spectra of visual pigments from the blue tit (*Parus caeruleus*). **A**, **C** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B, D** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). *UVS* ultraviolet-sensitive; *SWS* short-wavelength-sensitive; *MWS* medium-wavelength-sensitive; *LWS* long-wavelength-sensitive

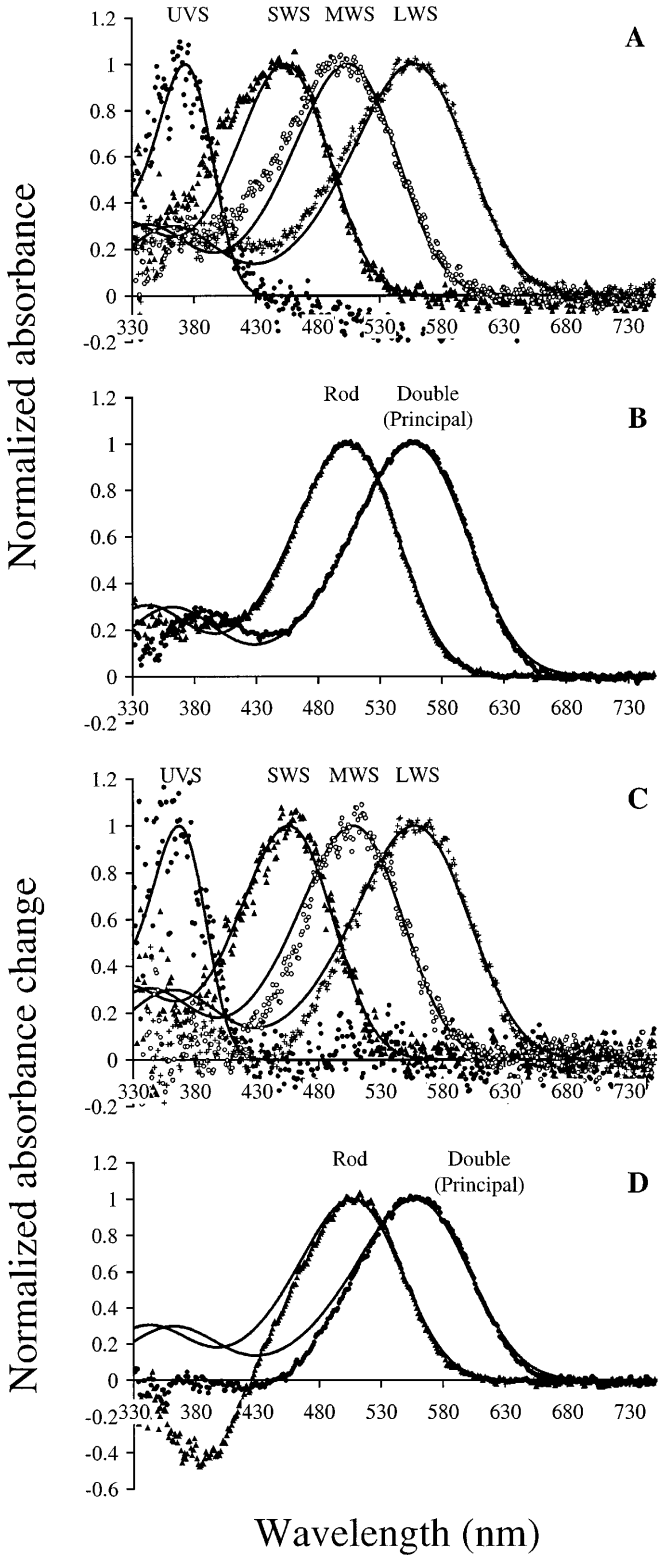

Fig. 2A–D Normalised mean prebleach absorbance (**A, B**) and difference (**C, D**) spectra of visual pigments from the blackbird (*Turdus merula*). **A, C** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B, D** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). For abbreviations see Fig. 1

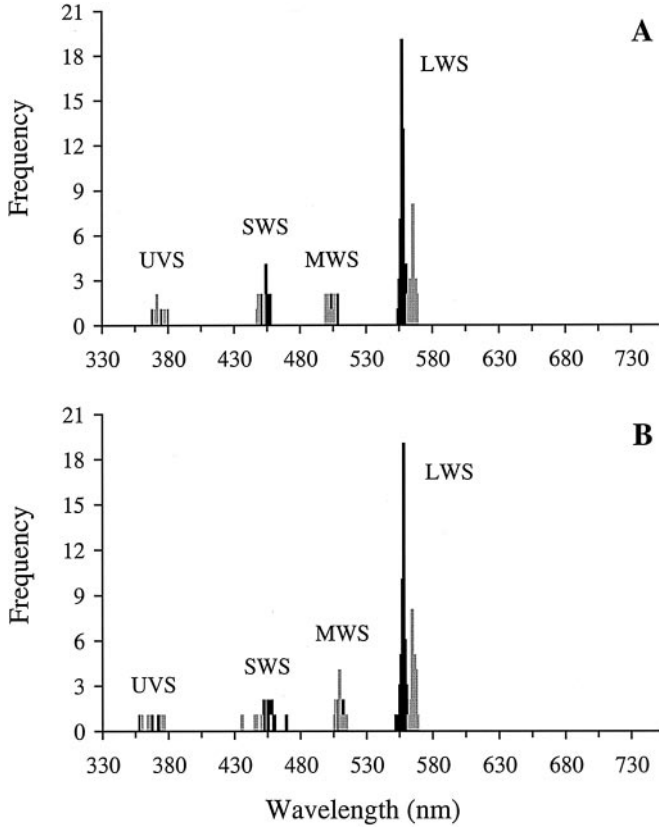

Fig. 3A, B Histograms showing the spectral distribution of cone visual pigment wavelength of maximal sensitivity (λ_{max}) values determined from prebleach absorbance (**A**) and difference (**B**) spectra for the blue tit (*grey bars*) and blackbird (*black bars*). In the case of the LWS cone visual pigment, values from LWS single cones and both members of the double cone pair are displayed. For abbreviations see Fig. 1

oil droplet (mean λ_{cut} 413 nm), MWS single cones with a yellow or Y-type oil droplet (mean λ_{cut} 508 nm) and LWS single cones with a red or R-type oil droplet (mean λ_{cut} 573 nm) contained rhodopsins with mean λ_{max} at 449 nm, 502 nm and 563 nm, respectively. The principal and accessory members of the double cone pair both contained the LWS rhodopsin (mean λ_{max} values measured at 565 nm and 563 nm, respectively). Only the principal members contained an oil droplet (P-type) in their inner segment. The spectral absorptance characteristics of this droplet type varied only marginally across the retina, those droplets occurring in the ventral half of the retina having a very similar cut-off wavelength (mean λ_{cut} 418 nm) to those found in the dorsal half (mean λ_{cut} 417 nm) but with a slightly more pronounced shoulder in the absorptance spectrum at about 490 nm (Fig. 4C).

Blackbird

Rod photoreceptors in the blackbird contained a rhodopsin of mean λ_{max} 504 nm. UVS single cones contained a visual pigment that was similar to that of the blue tit (mean λ_{max} 373 nm). The spectral location (mean λ_{max} 454 nm) of the SWS visual pigment found in cones with a C-type oil droplet (mean λ_{cut} 414 nm) was at a longer wavelength than that measured in the blue tit. Whilst the visual pigment measured in the MWS single cones was similar to that found in the blue tit (mean λ_{max} 504 nm), the cut-off wavelength of the Y-type oil droplet with which it was associated occurred at a longer wavelength (mean λ_{cut} 515 nm), although this may be an artefact of the increased average diameter of this droplet type in the blackbird (Table 1). Furthermore, the LWS single cones, whilst containing R-type oil droplets that were spectrally similar to those found in the blue tit (mean λ_{cut} 570 nm) contained a visual pigment whose spectral location occurred at a noticeably shorter wavelength (mean λ_{max} 557 nm).

Both members of the double cone also contained this short-wavelength-shifted LWS visual pigment (mean λ_{max} values measured at 557 nm and 556 nm for the principal and accessory members, respectively). Once again, only the principal member contained an oil droplet, the λ_{cut} value of which increased in a dorsal to ventral manner (Fig. 5C, D).

The histograms displayed in Fig. 3 demonstrate the variation in visual pigment λ_{max} values between the two species. It is readily apparent that there is a difference in the spectral location of the LWS, and possibly the SWS, cone visual pigments, although the quality of the microspectrophotometric data obtained from the LWS single and double cones is considerably better than that of the SWS cones.

Ocular media

Absorbance measurements of the pre-retinal ocular media of the blue tit and blackbird were converted into transmission and are displayed in Fig. 6. On the assumption that apparent absorbance by the combined ocular media at 800 nm was due only to light scattering (Geeraets et al. 1960; van den Berg and Spekreijse 1997), transmission was assumed to be unity at this wavelength, and all measurements were scaled relative to an absorbance of zero at 800 nm. All spectra were fitted with an 11-point, unweighted running average to smooth random noise in the data. A number of smoothed spectra obtained from multiple scans from the same individuals were averaged together for display. Both species investigated in this study have ocular media that transmit well into the near ultraviolet. Wavelengths of 0.5 transmission were 317 nm and 343 nm for the blue tit and blackbird, respectively.

Topographical distribution of cones

LWS and MWS single cones were readily identified by their red and yellow oil droplets respectively. Trans-

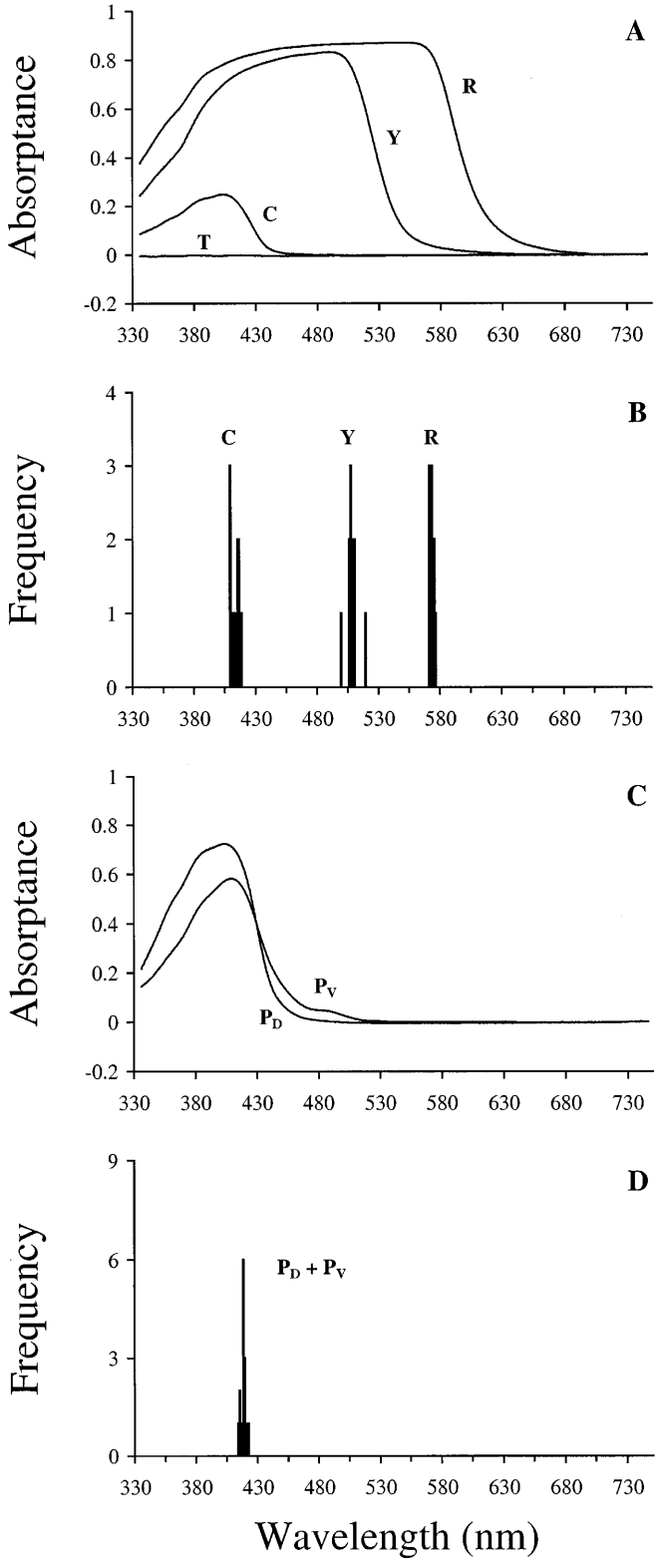

Fig. 4 Mean absorptance spectra of oil droplets found in the single cones (**A**) and the principal member of the double cones (**C**) in the blue tit. Also shown are histograms illustrating the spectral distribution of the cut-off wavelength (λ_{cut}) values determined for each of the single cone (**B**) and double cone (**D**) oil droplets used to create the mean spectra. *T* transparent or T-type; *C* 'colourless' or C-type; *Y* yellow or Y-type; *R* red or R-type; *P* principal member of double cone or P-type. P_D and P_V refer to P-type oil droplets in the principal member of the double cones measured in the dorsal and ventral regions of the retina, respectively

parent (T-type) oil droplets were the smallest observed and, as they contained no detectable carotenoid, did not fluoresce on stimulation with ultraviolet light. The principal oil droplets associated with the double cones were generally largest and displayed a long-lasting, faint, greenish fluorescence. By contrast, the C-type oil droplets associated with SWS single cones were generally smaller in diameter than all but the T-type droplets and displayed a brighter, more transient, bluish fluorescence.

Because of potential variation in the degree to which the retina spreads out on mounting, percentages of each cell type were calculated and used in preference to actual cell densities. Consequently, data were converted to proportions and arcsine-square-root transformed prior to statistical analysis (Sokal and Rohlf 1995). Significance was assessed using balanced repeated-measures multivariate analyses of variance (MANOVA, Minitab 10.51, Minitab), with the transformed proportions of all the measured cell types as the dependent variables and within-subjects factors 'side' (left/right eye) and 'quadrant' (eye region).

Pooling the results for all four quadrants from both the left and right eyes, the mean relative abundance of each cone type in both the blue tit and blackbird is summarised in Table 3. In general, it is evident that the blue tit retina displays a higher single cone to double cone ratio than the blackbird. In contrast, the ratios of single cone types, considered in isolation, were quite similar in the two species (Table 3).

Blue tit

Multivariate analysis of the blue tit data revealed a significant effect of quadrant (Wilk's $F_{15,188} = 6.74$, $P < 0.001$) and left or right side (Wilk's $F_{5,68} = 12.28$, $P < 0.001$) on cone distribution. There was also a significant interaction of quadrant and left/right side (Wilk's $F_{15,188} = 4.69$, $P < 0.001$) which suggests that the distribution of cones across the retina differs between left and right eyes.

Inspection of the mean relative abundance of each cone type displayed in Fig. 7A reveals that there are no consistent trends in retinal distribution, despite significant univariate test statistics for the effect of quadrant on LWS ($F_{1,72} = 16.82$, $P < 0.001$), MWS ($F_{1,72} = 22.03$, $P < 0.001$), UVS ($F_{1,72} = 6.89$, $P < 0.001$) and double cones ($F_{1,72} = 30.26$, $P < 0.001$). The lack of comparable retinal distributions between the two eyes explains the significant univariate test statistics for the quadrant × left/right eye interaction (LWS $F_{3,72} = 6.62$, $P = 0.001$; MWS $F_{3,72} = 11.52$, $P < 0.001$; UVS $F_{3,72} = 5.16$, $P = 0.003$; double cones $F_{3,72} = 18.96$, $P < 0.001$). It is conceivable that the small size of the blue

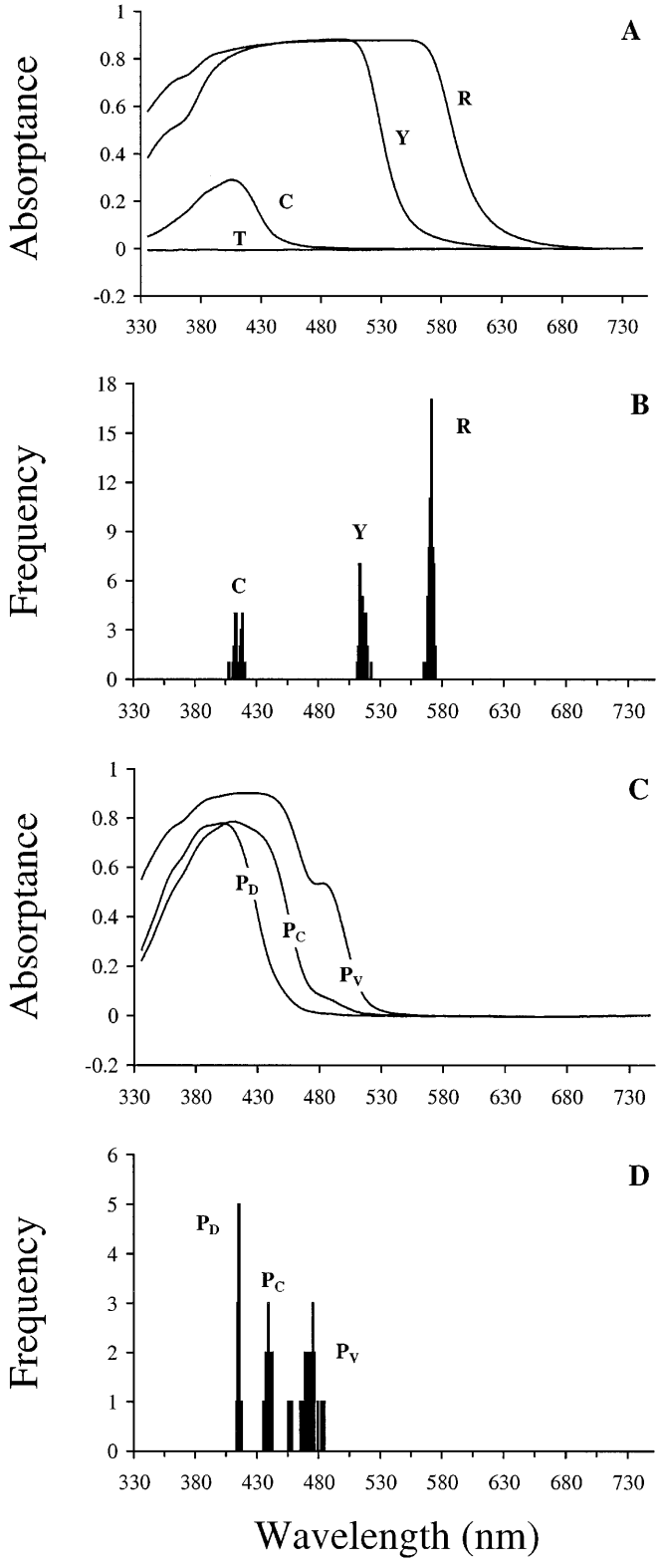

Fig. 5 Mean absorptance spectra of oil droplets found in the single cones (**A**) and the principal member of the double cones (**C**) in the blackbird. Also shown are histograms illustrating the spectral distribution of the λ_{cut} values determined for each of the single cone (**B**) and double cone (**D**) oil droplets used to create the mean spectra. For abbreviations see Fig. 4. P_D, P_C and P_V refer to P-type oil droplets in the principal member of the double cones measured in the dorsal, central and ventral regions of the retina, respectively

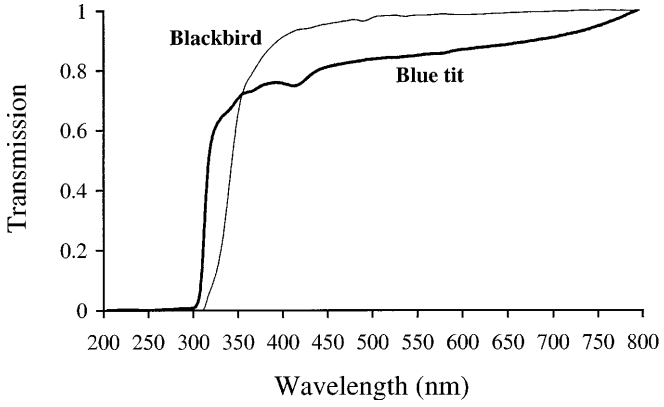

Fig. 6 Mean transmission spectra of the ocular media of the blue tit (*thick trace*) and blackbird (*thin trace*). Each line represents an 11-point, unweighted running average fitted to the mean spectrum created from multiple scans of the same eye (blue tit $n = 4$; blackbird $n = 3$). Wavelengths of 0.5 transmission were 317 nm and 343 nm for the blue tit and blackbird, respectively

tit eyes resulted in an uneven dissection of the left and right retinae. Errors of this type are exaggerated in smaller eyes, and will have a large influence with such a limited sample size.

As reported elsewhere for the starling (Hart et al. 2000), LWS and MWS single cones were significantly more abundant in the left eye (univariate: LWS $F_{1,72} = 10.30$, $P = 0.002$; MWS $F_{1,72} = 22.09$, $P < 0.001$), and double cones were significantly more abundant in the right eye (univariate: $F_{1,72} = 17.10$, $P < 0.001$).

Blackbird

Results of the blackbird MANOVA indicated a significant effect of quadrant (Wilk's $F_{15,188} = 6.97$, $P < 0.001$) and left or right side (Wilk's $F_{5,68} = 4.77$, $P = 0.001$) on cone distribution. Furthermore, there was a significant interaction of quadrant and left/right side (Wilk's $F_{15,188} = 3.40$, $P < 0.001$) which suggests that the pattern of regional variations is different for left and right eyes.

The univariate tests revealed that the distribution of all of the different cone types differed depending on retinal location (LWS $F_{1,72} = 18.50$, $P < 0.001$; MWS $F_{1,72} = 7.63$, $P < 0.001$; SWS $F_{1,72} = 3.43$, $P = 0.021$; UVS $F_{1,72} = 11.72$, $P < 0.001$; double cones $F_{1,72} = 7.12$, $P < 0.001$). The mean relative abundance of each cone type in each retinal quadrant of each eye is displayed in Fig. 7B. LWS single cones were most abundant in the PD quadrant, and least abundant in the PV quadrant. MWS cones, like the LWS cones, were relatively more abundant in the PD quadrant. SWS cones were most abundant in the AV quadrant, whilst UVS cones were least abundant in this region. Double cones were most abundant in the PV quadrant and least abundant in the PD quadrant. Furthermore, these trends were identical for both the left and right eyes.

Table 3 Mean relative abundance and ratios of each type of cone photoreceptor in the retinae of the blue tit (*Parus caeruleus*) and blackbird (*Turdus merula*). Count data were pooled across all regions of both left and right eyes. Only one specimen of each species was used. For abbreviations see Table 1

Species		Single cones (%)				Double cones (%)
		UVS	SWS	MWS	LWS	
Blue tit	% of all cones	7.6	14.6	20.4	20.5	36.9
	Single cone ratios	1	1.92	2.68	2.70	–
	Single:double cone ratio			1.71		1
Blackbird	% of all cones	6.8	12.1	15.0	13.3	52.9
	Single cone ratios	1	1.78	2.21	1.96	–
	Single:double cone ratio			1		1.12

Although the results of the MANOVA revealed a significant difference between left and right eyes, none of the univariate tests were significant. It is possible that, whilst not significant on their own, some cumulative effect of the different cone types resulted in a significant multivariate test statistic. Variations in overall cone proportions between the two eyes are small. LWS single cones and double cones are slightly more abundant in the left eye, whereas the remaining single cone types are relatively more abundant in the right eye.

The significant interaction between quadrant and left/right side revealed by the MANOVA was explained only by the differing distribution of UVS (univariate:

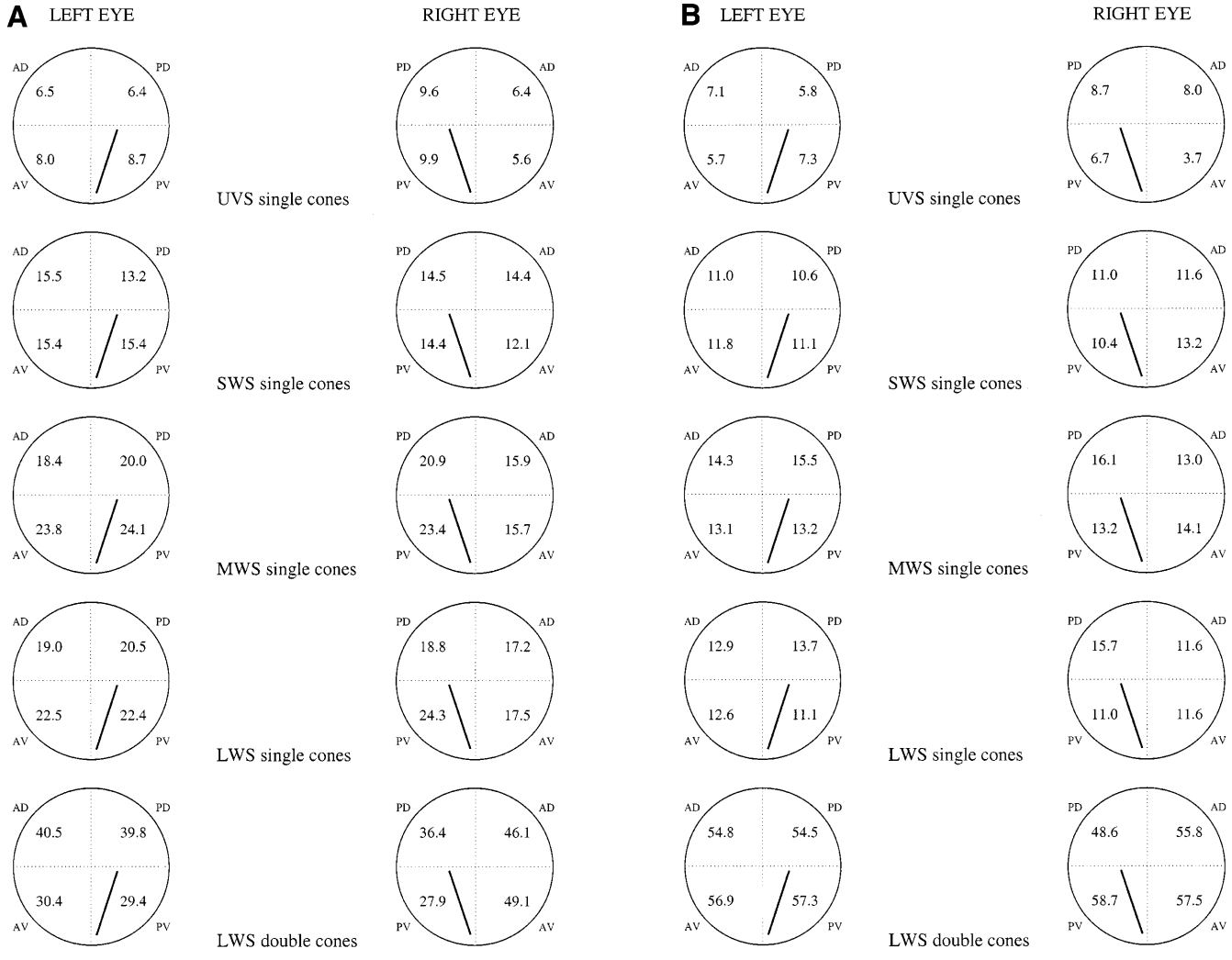

Fig. 7 Distribution of the different classes of cone photoreceptor in the retinae of blue tit (**A**) and blackbird (**B**). Each diagram represents the fundus of an eye as observed from the corneal aspect of the appropriate side of the birds head. The *horizontal and vertical dashed lines* represent dissection cuts which separated the retina into four quadrants. The *oblique solid line* indicates the position of the pecten. Values represent the mean percentage of each particular cone type relative to the mean total number of cones in each quadrant. *AD* anterior dorsal; *PD* posterior dorsal; *AV* anterior ventral; *PV* posterior ventral; *UVS* ultraviolet-sensitive; *VS* violet-sensitive; *SWS* short-wavelength-sensitive; *MWS* medium-wavelength-sensitive; *LWS* long-wavelength-sensitive

$F_{1,72} = 7.49$, $P < 0.001$) and LWS single cones (univariate: $F_{1,72} = 3.93$, $P = 0.012$) between the left and right eyes. The existence of such differences is difficult to interpret. With such small sample sizes, the chances of committing a Type I-statistical error are relatively high, and hence our conclusions of retinal asymmetry in both the blue tit and blackbird remain tentative.

Discussion

The spectral characteristics of the photoreceptors of the blue tit and blackbird are very similar to those described in the other passeriform and psittaciform species studied to date (Maier and Bowmaker 1993, Bowmaker et al. 1997; Hart et al. 1998; Das et al. 1999), the similarities including the presence of a cone visual pigment with a λ_{max} below 380 nm. That many birds show considerable sensitivity to wavelengths in the ultraviolet spectral region is now well established (Bennett and Cuthill 1994; Cuthill et al. 2000) and the ultraviolet components of certain visual signals are integral to mate choice decisions (Andersson et al. 1998; Hunt et al. 1998) and foraging performance (Church et al. 1998) in blue tits.

Whilst the visual pigments measured in the rods, UVS and MWS single cones of the blue tit are spectrally very similar to those of the blackbird, there are small inter-specific differences in the λ_{max} values of the SWS and LWS cone visual pigments. The difference (ca. 5 nm) in SWS visual pigment λ_{max} may well be an artefact of the poorer quality of the microspectrophotometric data for this cone type: smaller outer segments and lower visual pigment density (Tables 1, 2) give records with a lower signal-to-noise ratio than those obtained for LWS single and double cones (Figs. 1, 2). Nevertheless, the visual pigments located in the SWS single cones of birds investigated using microspectrophotometry range in λ_{max} from 430 nm in the zebra finch, *Taeniopygia guttata* (Bowmaker et al. 1997) to 463 nm in the tawny owl, *Strix aluco* (Bowmaker and Martin 1978) and multiple spectral locations for SWS visual pigment λ_{max}, which may be interpreted as λ_{max} cluster points (Dartnall and Lythgoe 1965; Partridge et al. 1989), are therefore likely.

The higher quality of microspectrophotometric data for LWS cones (both single and double) makes us more confident of a genuine difference in the spectral location of the λ_{max} of this visual pigment type between the two species, although the magnitude of the difference (ca. 5 nm) is similar to that seen in the case of the SWS pigments. Furthermore, the blackbird is not the only avian species with a LWS cone visual pigment whose λ_{max} occurs at shorter wavelengths than has been found usually in birds. Similar visual pigments (both with a λ_{max} at 555 nm) were measured microspectrophotometrically in the tawny owl (Bowmaker and Martin 1978) and inferred from electrophysiological data in the great horned owl, *Bubo virginianus* (Jacobs et al. 1987) and a LWS visual pigment with a λ_{max} at 543 nm was measured microspectrophotometrically in the Humboldt penguin, *Spheniscus humboldti* (Bowmaker and Martin 1985).

Interestingly, these three spectral locations for LWS visual pigments in birds are identical to those produced by the three different allelic forms of the single polymorphic middle- to long-wavelength-sensitive cone visual pigment opsin gene in marmosets, *Callithrix jacchus jacchus*, and tamarins, *Saguinus mystax* and *S. fuscicollis* (543 nm, 556 nm and 563 nm; Neitz et al. 1991; Tovée et al. 1992; Williams et al. 1992; Hunt et al. 1993). It is thought that only three non-homologous amino acid substitutions in the opsin protein are responsible for the differences between these monkey LWS pigment types (Shyue et al. 1998). The substitution of serine by alanine at position 180 (human LWS numbering) and glycine by serine at position 233 are thought to shift the λ_{max} of the LWS visual pigment from 563 nm to 556 nm, whilst the 543-nm λ_{max} form of the pigment is created by a further substitution of threonine by alanine at position 285.

Both chicken and canary LWS opsins, which create visual pigments with λ_{max} at 571 nm and 567 nm, respectively (Bowmaker et al. 1997; Das et al. 1999), contain serine at position 180 and alanine (a non-polar amino acid like glycine) at position 233 (Okano et al. 1992; Das et al. 1999). We predict that, whilst the amino acid sequence of the blue tit LWS opsin will be more similar to that of the canary and chicken, the blackbird LWS opsin sequence will display similar substitutions to the marmoset and tamarin 556-nm λ_{max} opsin variants at these tuning sites. Furthermore, it is not inconceivable that avian LWS visual pigments with λ_{max} above 560 nm fall into more than one spectral type: λ_{max} values measured microspectrophotometrically range from 563 nm to 571 nm (Sillman et al. 1981; Jane and Bowmaker 1988; Bowmaker et al. 1993; Maier and Bowmaker 1993; Bowmaker et al. 1997; Hart et al. 1998, 1999, this study; Das et al. 1999). Whilst some of this observed variation may be due to noisy data, or even differences in the accuracy of the wavelength calibration of the different MSPs used in each study, subtle variations in opsin amino acid sequence may also explain the spread of λ_{max}.

Consideration of the interplay between oil droplet and visual pigment suggests the hypothesis that, at least in birds, the LWS visual pigment is spectrally tuned to optimise the visual function of the double cones rather than the LWS single cones. LWS single cones have their effective spectral sensitivity shifted towards longer wavelengths by approximately 40 nm due to the long-pass spectral character of the R-type oil droplet with which they are associated (Bowmaker 1977; Bowmaker and Knowles 1977; Maier and Bowmaker 1993). Consequently, the λ_{cut} of the R-type droplet determines the precise peak effective spectral sensitivity of the LWS single cone (601 nm and 605 nm, respectively, in the blackbird and blue tit) rather than the visual pigment. However, in the double cones the same LWS visual pigment is associated with an oil droplet (often only

present in the principal member) which absorbs strongly at short wavelengths but does not shift the peak sensitivity of the double cone away from the λ_{max} of the visual pigment. In summary, selection pressure is more likely to act on the λ_{max} of the LWS visual pigment in the double cones rather than in the LWS single cones (which are effectively tuned by the absorption of the oil droplet).

Single cones are thought to subserve colour vision whilst double cones represent an achromatic channel (Maier and Bowmaker 1993; Vorobyev and Osorio 1998) and have been implicated in movement detection (Campenhausen and Kirschfeld 1998). Thus, the location of the double cone λ_{max} value in birds may be selected so as to optimise spatial contrasts for each species. The background radiance of many terrestrial habitats is dominated by the reflectance spectrum of green vegetation, which peaks at around 550 nm, and may explain the convergence in the shape of the electroretinogram (ERG)-determined photopic spectral sensitivity functions measured in many terrestrial vertebrates, despite differences in absolute radiance and irradiance and the shape of the irradiance spectrum in different habitats (Fleishman et al. 1997). A thorough investigation of the spectral environments encountered by each of the two species examined here will be necessary to determine whether subtle variations in the shape of the background radiance spectrum are responsible for the differences in λ_{max} observed.

Blackbirds occupy extremely diverse habitats, including dense woodland, most types of farmland, heaths, moors and some wetlands, and forage largely on the ground (Hillstead 1944; Cramp et al. 1988). Much of their time during feeding is actually spent with the head in an upright position, scanning the vicinity for sources of disturbance (Smith 1974; Lawrence 1985). Examination of Fig. 7B reveals that the blackbird has a higher proportion of double cones in the ventral half of the retina, which views the celestial visual hemisphere, a feature consistent with a role in detecting aerial predators that was also noted in another ground-feeding species, the European starling (Feare 1984; Hart et al. 1998).

Blue tits, on the other hand, are generally arboreal in habit (Lack 1971) and display quite the opposite trend in dorso-ventral double cone distribution (Fig. 7A). Furthermore, the blue tit also lacks the pronounced dorso-ventral gradient in P-type oil droplet pigmentation seen in the blackbird (cf. Figs. 4C and 4D with Figs. 5C and 5D) and starling (Hart et al. 1998). A higher carotenoid concentration in the ventral P-type droplets, which results in a λ_{cut} at longer wavelengths, will reduce the amount of short-wavelength radiation reaching the double cone outer segments relative to those in the dorsal retina. This feature of some avian retinae presumably has an analogous function to the dorsal 'eye shades' observed in the corneas of certain fishes, that is to even out the intensity of the incident light across the retina (Douglas and Marshall 1999). One reason for the absence of this adaptation in the blue tit may be that, when foraging, they spend up to 44% of their time hanging upside down, inspecting the undersides of leaves and twigs for prey items (Moreno 1981).

It appears, therefore, that the relative abundance, retinal distribution and spectral absorption properties of the double cones may reflect differences in the visual ecology of these two species. Whilst the other inter- and intra-retinal differences described herein remain to be explained, it is evident that there is much variation in avian photoreceptors to be investigated.

Acknowledgements Work undertaken for this project was divided among the co-authors as follows: NSH – all data acquisition and analysis, manuscript preparation, bird capture and maintenance; JCP – MSP construction, analysis software and manuscript preparation; ICC – statistical advice and manuscript preparation; ATDB – bird catching and maintenance. This research was supported by the BBSRC (Research Grant 7/S05042 to ICC and JCP and Research Studentship 95/RS/ASP/099 to NSH). Birds were caught under English Nature licences to ATDB and ICC and all experiments complied with the "Principals of animal care", publication No. 86–23, revised 1985, of the National Institute of Health and also with the current laws of the United Kingdom.

References

Andersson S, Örnborg J, Andersson M (1998) Ultraviolet sexual dimorphism and assortive mating in blue tits. Proc R Soc Lond Ser B 265: 445–450

Barlow HB (1982) What causes trichromacy? A theoretical analysis using comb-filtered spectra. Vision Res 22: 635–643

Baylor DA, Hodgkin AL (1973) Detection and resolution of visual stimuli by turtle photoreceptors. J Physiol (Lond) 234: 163–198

Bennett ATD, Cuthill IC (1994) Ultraviolet vision in birds: what is it's function? Vision Res 34: 1471–1478

Berg TJTP van den, Spekreijse H (1997) Near infrared light absorption in the human eye media. Vision Res 37: 249–253

Bowmaker JK (1977) The visual pigments, oil droplets and spectral sensitivity of the pigeon. Vision Res 17: 1129–1138

Bowmaker JK, Knowles A (1977) The visual pigments and oil droplets of the chicken retina. Vision Res 17: 755–764

Bowmaker JK, Martin GR (1978) Visual pigments and colour vision in a nocturnal bird, *Strix aluco* (tawny owl). Vision Res 18: 1125–1130

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

Bowmaker JK, Kovach JK, Whitmore AV, Loew ER (1993) Visual pigments and oil droplets in genetically manipulated and carotenoid deprived quail: a microspectrophotometric study. Vision Res 33: 571–578

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

Campenhausen Mv, Kirschfeld K (1998) Spectral sensitivity of the accessory optic system of the pigeon. J Comp Physiol A 183: 1–6

Cramp S, Brooks DJ, Dunn E, Gillmor R, Hall-Craggs J, Hollom PAD, Nicholson EM, Ogilvie MA, Roselaar CS, Sellar PJ, Simmons KEL, Voous KH, Wallace DIM, Wilson MG (1988) Handbook of the birds of Europe, the Middle East and North Africa, vol V. Oxford University Press, Oxford

Church SC, Bennett ATD, Cuthill IC, Partridge JC (1998) Ultraviolet cues affect the foraging behaviour of blue tits. Proc R Soc Lond Ser B 265: 1509–1514

Cuthill IC, Partridge JC, Bennett ATD, Church SC, Hart NS, Hunt S (2000) Ultraviolet vision in birds. Adv Study Behav 28 (in press)

Dartnall HJA, Lythgoe JN (1965) The spectral clustering of visual pigments. Vision Res 5: 81–100

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

Douglas RH, Marshall NJ (1999) A review of vertebrate and invertebrate optical filters. In: Archer SN, Djamgoz MBA, Loew ER, Partridge JC, Vallerga S (eds) Adaptive mechanisms in the ecology of vision. Kluwer, Dordrecht, pp 95–162

Fager LY, Fager RS (1981) Chicken blue and chicken violet, short wavelength visual pigments. Vision Res 21: 581–586

Feare C (1984). The starling. Oxford University Press, Oxford

Fleishman LJ, Bowman M, Saunders D, Miller WE, Rury MJ, Loew ER (1997) The visual ecology of Puerto Rican anoline lizards: habitat light and spectral sensitivity. J Comp Physiol A 181: 446–460

Geeraets WJ, Williams RC, Chan G, Ham WT Jr, Gerry D III, Schmidt FH (1960) The loss of light energy in retina and choroid. Arch Ophthalmol 64: 606–615

Goldsmith TH, Collins JS, Licht S (1984) The cone oil droplets of avian retinas. Vision Res 24: 1661–1671

Hart NS (1998) Avian photoreceptors. PhD Thesis, University of Bristol, UK

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

Hart NS, Partridge JC, Cuthill IC (1999) Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey (*Meleagris gallopavo*). Vision Res 39: 3321–3328

Hart NS, Partridge JC, Cuthill IC (2000) Retinal asymmetry in birds. Current Biology 10: 115–117

Hawryshyn CW, Hárosi FI (1994) Spectral characteristics of visual pigments in rainbow trout (*Oncorhynchus mykiss*). Vision Res 34: 1385–1392

Hillstead AFC (1944) The blackbird. Faber and Faber, London

Hunt DM, Williams AJ, Bowmaker JK, Mollon JD (1993) Structure and evolution of the polymorphic photopigment gene of the marmoset. Vision Res 33: 147–154

Hunt S, Bennett ATD, Cuthill IC, Griffiths R (1998) Blue tits are ultraviolet tits. Proc R Soc Lond Ser B 265: 451–455

Jacobs GH, Crognale M, Fenwick J (1987) Cone pigment of the great horned owl. Condor 89: 434–436

Jane SD, Bowmaker JK (1988) Tetrachromatic colour vision in the duck (*Anas platyrhynchos* L.): microspectrophotometry of visual pigments and oil droplets. J Comp Physiol A 162: 225–235

Kawamuro K, Irie T, Nakamura T (1997) Filtering effect of cone oil droplets detected in the P-III response spectra of Japanese quail. Vision Res 37: 2829–2834

Lack D (1971) Ecological isolation in birds. Blackwell, Oxford

Lawrence ES (1985) Evidence for search image in blackbirds (*Turdus merula* L.): short-term learning. Anim Behav 33: 929–937

Levine JS, MacNichol EF Jr (1985) Microspectrophotometry of primate photoreceptors: art, artefact and analysis. In: Fein A, Levine JS (eds) The visual system. Liss, New York, pp 73–87

Lipetz LE (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: 597–604

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

Moreno J (1981) Feeding niches of woodland birds in a montane coniferous forest in central Spain during winter. Ornis Scand 12: 148–159

Neitz M, Neitz J, Jacobs GH (1991) Spectral tuning of pigments underlying red-green color vision. Science 252: 971–974

Neumeyer C, Jäger J (1985) Spectral sensitivity of the freshwater turtle *Pseudemys scripta elegans*: evidence for the filter-effect of colored oil droplets. Vision Res 25: 833–838

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

Palacios AG, Goldsmith TH, Bernard GD (1996) Sensitivity of cones from a cyprinid fish (*Danio aequipinnatus*) to ultraviolet and visible light. Vis Neurosci 13: 411–421

Partridge JC (1989) The visual ecology of avian cone oil droplets. J Comp Physiol A 165: 415–426

Partridge JC, DeGrip WJ (1991) A new template for rhodopsin (vitamin A_1 based) visual pigments. Vision Res 31: 619–630

Partridge JC, Shand J, Archer SN, Lythgoe JN, Groningen-Luyben WAM van (1989) Interspecific variation in the visual pigments of deep-sea fishes. J Comp Physiol A 164: 513–529

Shyue S-K, Boissinot S, Schneider H, Sampaio I, Schneider MP, Abee CR, Williams L, Hewett-Emmett D, Sperling HG, Cowing JA, Dulai KS, Hunt DM, Li W-H (1998) Molecular genetics of spectral tuning in New World monkey color vision. J Mol Evol 46: 697–702

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

Smith JNM (1974) The food search behaviour of two European thrushes. I. Description and analysis of search paths. Behaviour 48: 276–302

Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York

Stavenga DG, Smits RP, Hoenders BJ (1993) Simple exponential functions describing the absorbance bands of visual pigment spectra. Vision Res 33: 1011–1017

Tovée MJ, Bowmaker JK, Mollon JD (1992) The relationship between cone pigments and behavioural sensitivity in a New World monkey (*Callithrix jacchus jacchus*). Vision Res 32: 867–878

Vorobyev M, Menzel R (1999) Flower advertisement for insects: bees, a case study. In: Archer SN, Djamgoz MBA, Loew ER, Partridge JC, Vallerga S (eds) Adaptive mechanisms in the ecology of vision. Kluwer, Dordrecht, pp 537–553

Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond Ser B 265: 351–358

Williams JW, Hunt DM, Bowmaker JK, Mollon JD (1992) The polymorphic photopigments of the marmoset: spectral tuning and genetic basis. EMBO J 11: 2039–2045

Yoshizawa T, Fukada Y (1993) Preparation and characterisation of chicken rod and cone pigments. In: Hargrave PA (ed) Photoreceptor cells. Academic Press, New York, pp 161–179