

VISUAL PIGMENTS, OIL DROPLETS AND CONE PHOTORECEPTOR DISTRIBUTION IN THE EUROPEAN STARLING (*STURNUS VULGARIS*)

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Summary

Microspectrophotometric measurements of retinal photoreceptors from the European starling (*Sturnus vulgaris*) revealed four classes of single cone, containing visual pigments with wavelengths of maximum absorbance (λ_{\max}) at 563, 504, 449 and close to 362 nm. The two longer-wave-sensitive single cones contained brightly coloured oil droplets which cut off light below 572 and 514 nm, respectively. The 449 nm λ_{\max} pigment was associated with a 'colourless' oil droplet with peak measured absorbance below 400 nm. The ultraviolet-sensitive visual pigment was paired with a transparent oil droplet which showed no significant absorption above 350 nm. A single class of double cone was identified, both members of which contained the longwave-sensitive (λ_{\max} 563 nm) visual pigment. The principal member of the double cone contained an oil droplet with a topographically variable cut-off wavelength below 471 nm; the oil droplet found in

the accessory member was only measured in the ventral retina and displayed three distinct peaks of absorption at approximately 430, 450 and 480 nm. Rod photoreceptors had a λ_{\max} at 503 nm. A new polynomial for fitting visual pigment templates to ultraviolet-sensitive visual pigment data is given. Topographic density measurements of the different cone classes were made using Nitroblue-tetrazolium chloride to label selectively bleached photoreceptors. The two classes of shortwave-sensitive single cone were more abundant in the dorsal retina, and longwave-sensitive single cones were notably less abundant in the dorso-temporal region of the retina, which subserves binocular vision.

Key words: microspectrophotometry, MSP, visual pigments, cone photoreceptors, oil droplets, bird, vision, colour vision, European starling, *Sturnus vulgaris*.

Introduction

Vision is the primary sense for most birds, and neognathus species have among the most complex retinæ of any vertebrate (Meyer, 1977). Until recently, most hypotheses concerning the role of colour in avian behavioural ecology (e.g. crypsis, aposematic coloration and sexual signalling) have relied on human assessments of colour rather than the perceptual system of the natural receivers of the signals (for a review, see Bennett *et al.* 1994). Such oversights on the part of evolutionary and behavioural biologists are partly due to the paucity of relevant physiological data.

Spectrophotometric analyses of visual pigment extracts (e.g. Fager and Fager, 1981; Yoshizawa and Fukada, 1993), electrophysiological techniques (e.g. Chen *et al.* 1984; Govardovskii and Zeuva, 1977; Parrish *et al.* 1984; Wortel *et al.* 1984) and operant tests of spectral sensitivity (e.g. Emmerton and Delius, 1980; Maier, 1992; Palacios and Varela, 1992) have contributed greatly to our understanding of avian visual systems. Nevertheless, microspectrophotometry (MSP), a technique first adapted by Hanoaka and Fujimoto (1957) for the measurement of the absorption properties of vertebrate photoreceptors and further developed for use in birds (Bowmaker, 1984; Liebman, 1972), remains the only method

for obtaining absorption spectra of visual pigments and oil droplets in single cells.

Single-cell microspectrophotometry involves passing a very narrow beam of light through a cell, scanning through the spectrum, and measuring the transmission at each wavelength. In this way, absorption spectra of photoreceptor visual pigments and oil droplets can be recorded *in situ*. However, microspectrophotometry of avian photoreceptors is complicated by their small size (cone outer segments are generally less than 2 μm in diameter) and the loss of cone outer segments during cell preparation. The time-consuming nature of the technique, as well as the specialised equipment required, has severely limited the number of species studied in detail (Bowmaker *et al.* 1997).

In addition to a single class of rod, the retinæ of most diurnal birds contain a single class of longwave-sensitive (LWS) double cone and four classes of single cone (Bowmaker *et al.* 1997). Each of the cone classes is always associated with a particular type of oil droplet. Oil droplets are highly refractile lipid-based globules situated at the distal end (ellipsoid) of the inner segments of cone photoreceptors. Most contain carotenoid pigments (Goldsmith *et al.* 1984; Wald and

Zussman, 1937), which alter the spectral transmission characteristics of the oil droplets and are generally considered to act as long-pass 'cut-off' filters (Bowmaker, 1977; Liebman and Granda, 1975; Partridge, 1989). The spectral sensitivity of a cone photoreceptor is determined jointly by the spectral transmission of the oil droplet (and that of other ocular media, including the lens and cornea) and the spectral absorbance of the visual pigment (Baylor and Hodgkin, 1973; Kawamuro *et al.* 1997; Neumeier and Jäger, 1985).

The ability of birds to detect 'near' ultraviolet wavelengths (300–400 nm) was first demonstrated some 25 years ago in hummingbirds (Huth and Burkhardt, 1972) and pigeons (Wright, 1972), and since then it has been shown in over 30 species of bird (for a review, see Bennett and Cuthill, 1994). However, there appears to be a physiological dichotomy in short-wavelength photoreception, which may reflect phylogeny. In addition to cone visual pigments maximally sensitive in the longwave (LWS), mediumwave (MWS) and shortwave (SWS) regions of the human-visible spectrum, bird retinæ contain single cones with a visual pigment maximally sensitive to either violet light, as in the duck *Anas platyrhynchos* (Jane and Bowmaker, 1988), chicken *Gallus gallus* (Fager and Fager, 1981; Yoshizawa and Fukada, 1993), penguin *Spheniscus humboldti* (Bowmaker and Martin, 1985), Japanese quail *Coturnix coturnix japonica* (Bowmaker *et al.* 1993) and pigeon *Columba livia* (Bowmaker *et al.* 1997), or to ultraviolet wavelengths, as in the Pekin robin *Leothrix lutea* (Maier and Bowmaker, 1993), zebra finch *Taeniopygia guttata* and budgerigar *Melopsittacus undulatus* (Bowmaker *et al.* 1997).

Many studies have suggested a relationship between photoreceptor spectral sensitivity and the spectral distribution of the ambient light (e.g. Lythgoe and Partridge, 1989), and there have been several attempts to correlate the distribution of retinal oil droplets with photic environment (Begin and Handford, 1987; Goldsmith *et al.* 1984; Lythgoe, 1979; Muntz, 1972; Partridge, 1989). However, only Goldsmith *et al.* (1984) and Partridge (1989) attempted this using an objective measure of oil droplet colour (MSP), although neither of these studies paired oil droplets with visual pigments or considered the role of single and double cones. Consequently, the physiological understanding of avian visual ecology remains largely speculative. The information in this study, concerning the characteristics and distribution of visual pigments and oil droplets in the retina of the European starling, will help to augment the limited physiological data available for drawing such ecological correlates.

The European starling, *Sturnus vulgaris* (Passeriformes), is a diurnal, ground-feeding bird. Capable of migrating both by night and during the day, its vast geographical distribution, varied range and omnivorous diet make it one of the most versatile generalist bird species in the world (Feare, 1984). In addition, because its visual fields, eye movements and optical morphology have been described in great detail (Martin, 1986), and because so much work on its behavioural ecology has been

undertaken, the starling is an excellent candidate for a detailed investigation of visual physiology.

Materials and methods

Animals

Spectrophotometric, microspectrophotometric and topographical spatial distribution measurements were conducted on the retinal photoreceptors of adult European starlings *Sturnus vulgaris*. Procedures were carried out on both reproductively inactive birds, which had been held in captivity for approximately 18 months, and freshly caught birds, which were analysed within 1 month of the capture date. Subjects were housed in environmentally controlled conditions and allowed water and food, in the form of 404 Gold Start crumbs ACS (Dalgety Agriculture Ltd, Bristol, UK), *ad libitum*.

Microspectrophotometry

Birds were held in darkness overnight and killed by approved humane methods. The eyes were removed under infrared light (Kodak Wratten filter no. 87C over a standard 6 V tungsten source) with the aid of an infrared image converter (FJW Industries, USA) attached to one ocular of a low-power stereomicroscope. Subsequent dissections of the eyes and retinæ were performed under dim red light from a head torch [3.8 V tungsten lamp; filtered by double-thickness Lee no. 182 filter; wavelength at 50% transmission (λ_{T50}) 677 nm]. One eye was used immediately, the other being stored overnight on ice. Each eye was hemisected using a fine razor blade and the posterior hemisphere placed immediately in cold (3°C) phosphate-buffered saline (PBS; Dulbecco 'A' tabletised PBS made to a concentration of 340 mosmol kg⁻¹, pH 7.3; Oxoid Ltd, Basingstoke, UK).

Small sections of retina, typically approximately 2 mm in diameter and usually still attached to the pigmented epithelium, were cut away using fine scissors and transferred in a wide-bored Gilson pipette tip to a 22 mm×64 mm no. 0 coverslip. Excess saline was blotted away and replaced with PBS containing 7.5% dextran (Sigma 242,000RMM) to reduce movement of the tissue. The preparation was then covered with a circular no. 0 coverslip (19 mm diameter) and pressed gently under filter paper to express excess saline. The edges of the top coverslip were then sealed with clear nail varnish, using a fine paint-brush, to prevent dehydration and movement of the top coverslip.

The microspectrophotometer (MSP) used to measure both visual pigment and oil droplet absorption spectra is a single-beam, wavelength-scanning, computer-controlled instrument similar in design to one described in detail by Partridge (1989). The MSP measuring beam is derived from light produced by a 100 W quartz-halogen bulb powered by a stabilised 12 V d.c. power supply (Oriel Corporation, USA). The filament of the bulb is focused onto the entrance slit (1 mm) of a Jobin Yvon H-1061 UV-VIS grating monochromator (Instruments S.A. Ltd, Middlesex, UK), the output of which (8 nm full width at

half-maximum FWHM bandwidth) illuminates an adjustable aperture. This consists of two sets of opposable slits which control the vertical and horizontal dimensions of the beam in the plane of the specimen. The aperture housing also contains a calcite crystal which linearly polarises the light passing through the aperture, a feature that enables the dichroic absorption properties of rod and cone outer segments to be exploited (Hárosi and MacNichol, 1974; Hárosi and Malerba, 1975). The measuring beam is directed into the plane of the specimen on a microscope stage and demagnified by a Zeiss Ultrafluor $\times 32$ objective used as a condenser lens (numerical aperture, NA, 0.4). Above the stage, an Olympus $\times 100$ DApO 100UV objective (set to an NA of 1.3) focuses the beam either onto a small area of the photocathode of a Hamamatsu R928 photomultiplier or, by the use of a sliding prism, towards a far-red-sensitive video camera (75 series miniature CCTV camera; Insight Vision Systems Ltd, Malvern, UK) which is connected to a monochrome video monitor for viewing the specimen. Background illumination is provided by an infrared light-emitting diode (LED), the light from which is introduced to the light path by a thin glass beam-splitter positioned below the condenser lens. The passage of the measuring beam can be interrupted before reaching the specimen by an electric shutter controlled either manually or automatically *via* the computer.

Because avian photoreceptor outer segments and oil droplets are very small, never greater than approximately $4\ \mu\text{m}$ in diameter, axial chromatic aberration from the condenser objective can present a considerable problem. Left uncorrected, this results in unacceptable levels of light 'leakage' around outer segments and oil droplets due to the wavelength-dependence of the plane of focus, which (with the Ultrafluor $\times 32$ objective) exceeds $10\ \mu\text{m}$ between 510 and 800 nm. To eliminate this wavelength-dependent aberration, the focus of the condenser is automatically adjusted during a scan by a computer-driven piezo-electric translator (PIFOC P-720.00, Physik Instrumente, Germany) which moves the condenser objective rapidly, with a resolution of approximately 10 nm, to maintain the plane of focus.

To measure an absorption spectrum, the photoreceptor outer segment or oil droplet was first focused using the microscope's conventional focus control which moved the specimen stage vertically. The measuring beam, typically $1\text{--}2\ \mu\text{m}$ in diameter, was then focused (usually at 730 nm) in an area adjacent to the photoreceptor, the infrared background illumination extinguished, and the light path was directed to the photomultiplier. A 'baseline' scan was recorded as the computer-controlled stepper motor drove the monochromator from 730 nm to 350 nm and back again. Light hitting the photocathode induced a nanoamp current in the photomultiplier which was converted to a voltage in the headstage amplifier. This voltage was amplified and fed into an analogue multiplier together with the voltage from a digital-to-analogue converter, which received a variable digital 'gain' signal from the computer, to give an approximately constant voltage at all wavelengths. This voltage was then amplified, inverted and low-pass-filtered with a Butterworth two-stage

active filter, and fed to a 2 MHz voltage-to-frequency (V/F) converter. After a short delay at each wavelength to allow the filtered signal to settle, the frequency output at each 'odd' wavelength on the 'downward' longwave-to-shortwave spectral pass and at each 'even' wavelength on the 'upward' shortwave-to-longwave spectral pass was integrated over 10 ms using a CTM-05 counter/timer board (MetraByte Co., Taunton, MA, USA) in the computer. A single scan took approximately 15 s and, like all other automated procedures, was controlled by a Microsoft QuickBASIC program. Having recorded a baseline scan, the photoreceptor outer segment or oil droplet was moved into the measuring beam and a 'sample' scan was made in the same way. Outer segments containing visual pigments were 'bleached' for 10 min using monochromatic (8 nm FWHM bandwidth) light from the monochromator at a wavelength corresponding to the wavelength of maximum absorbance, λ_{max} , of the pigment measured, and a 'post-bleach' sample scan was made in order to create difference spectra and to confirm photolability. Because of the reduced light flux from the monochromator light source at ultraviolet wavelengths, putative ultraviolet-sensitive visual pigments were bleached with 'white' light, emitted by the monochromator at its blaze angle, for 10 min.

Analysis of visual pigment absorption spectra

MSP data files were read into the analysis program, a Microsoft Excel 5.0c macro, and the baseline and sample frequencies recorded from the V/F converter at 1 nm intervals were converted into absorbance values. A 23-point 'box-car' running average was passed through the absorbance values and the peak value noted with its corresponding wavelength. MSP sample scans are frequently offset from the baseline scans, due to optical effects (e.g. scattering or focusing of the measuring beam by the sample; Lipetz, 1984; Young and Martin, 1984; Levine and MacNichol, 1985), and it was therefore necessary to introduce an offset when normalising the absorbances. This was calculated at the longwave end of the spectrum where there was no detectable absorbance due to the visual pigment. The maximum corrected absorbance was taken as the peak absorbance minus the longwave offset absorbance, and the data were normalised to this range for subsequent calculations and display. The bandwidths of absorbance spectra were calculated as the difference between the wavelength corresponding to half-maximum absorbance on the longwave and shortwave limbs of the running average absorbance spectrum.

In order to determine the wavelength of peak absorbance (λ_{max}), the analysis used the polynomial derived by Partridge and DeGrip (1991) to fit the data with a rhodopsin template spectrum. For display, the $\lambda_{\text{max}}/\lambda$ -transformed template of Stavenga *et al.* (1993) was used, but with the β -peak of the absorbance spectrum shifted linearly with respect to the α -peak, as suggested by Palacios *et al.* (1996), and utilising the relative extinction coefficients of the α - and β -peaks proposed by Stavenga *et al.* (1993). Each point on the longwave limb with an absorbance between 80% and 20% of the normalised maximum was used to estimate the λ_{max} (Partridge and

DeGrip, 1991), the average of all these estimates being taken as the best estimate of the λ_{\max} of the visual pigment. Only the longwave limb of a visual pigment absorbance spectrum was used to estimate the λ_{\max} because this region of the curve is least affected by photoproduct build-up, short-wavelength light-scattering and distortion due to high concentrations of visual pigment (Bowmaker *et al.* 1975; Levine and MacNichol, 1985).

Ultraviolet-sensitive (UVS) visual pigments have absorbance spectra that are narrower than those of visual pigments with λ_{\max} values in the human visible spectrum, even when transformed on a scale of λ_{\max}/λ (Hawryshyn and Hárosi, 1994; Palacios *et al.* 1996). It is evident that UVS visual pigments require their own template, and Palacios *et al.* (1996) have produced coefficients to be used in conjunction with the visual pigment templates of Stavenga *et al.* (1993) for the analysis of UVS visual pigment data. A sixth-degree polynomial describing the relationship of absorbance between 80% and 20% longwave-normalised absorbance and template λ_{\max} was calculated from this modified model and used to fit ultraviolet templates to our data in preference to the polynomial of Partridge and DeGrip (1991). The polynomial relating normalised longwave absorbance (D) at a given wavelength (λ) to λ_{\max} for UVS cones (for λ_{\max} values between 340 and 380 nm) is:

$$\lambda_{\max} = \lambda(0.8777 + 0.2309D - 0.5059D^2 + 0.9222D^3 - 0.9814D^4 + 0.5547D^5 - 0.1124D^6).$$

Visual pigment absorbance spectra were accepted if (i) the template spectrum fell within the peak-to-peak noise of the data points between 80% and 20% of normalised maximum absorbance on the shortwave and longwave limbs of the data, respectively, (ii) the absorbance spectra were flat for 100 nm beyond the wavelength at which the longwave limb first falls to an absorbance of zero, (iii) were free from obvious distortions (Levine and MacNichol, 1985), and (iv) were confirmed as photolabile by bleaching. Because of their rarity, criteria were relaxed for UVS cones, and all scans from cells which were shown to be photolabile were included. Accepted records for each photoreceptor cell type were averaged and re-analysed.

Analysis of oil droplet absorption spectra

Because of their small size, spherical shape, high refractive index and high carotenoid concentration (Goldsmith *et al.* 1984), light 'leakage' around oil droplets during MSP measurement is considerable. Consequently, recorded absorbance spectra tend to be limited to a maximum absorbance of approximately 1 and display a 'flat-topped' cut-off character (Liebman and Granda, 1975). Oil droplets are described by their 'cut-off wavelength' (λ_{cut}), defined by Lipetz (1984), from a running average fitted to offset-corrected absorbance data. For comparison with other studies (e.g. Partridge, 1989), the wavelength corresponding to 50% of maximum measured absorbance (λ_{mid}) (Lipetz, 1984) is also given (see Table 1).

Spectrophotometry of pre-retinal tissue

Absorbance measurements of the cornea, aqueous humour, lens and vitreous humour were made over the range 200–800 nm using a Shimadzu UV2101 PC UV-VIS scanning spectrophotometer fitted with a Shimadzu ISR-260 integrating sphere assembly to reduce the effects of light scattering by the tissue samples. Corneas were excised from the sclera and measured whilst sandwiched between two stainless-steel mesh inserts inside a standard (1 cm pathlength) quartz cuvette. Lenses were dissected away from the anterior segment of the eye and measured using a rectangular aluminium insert, designed to fit inside a standard cuvette, in which a 4.3 mm diameter hole (the same diameter as the lens) had been drilled to coincide with the measuring beam of the spectrophotometer and in which the lens could be positioned in its normal orientation relative to the incident light. Both corneas and lenses were bathed in 340 mosmol kg⁻¹ PBS, which was also placed in the identical inserts and cuvettes used as reference samples. Samples of aqueous humour were extracted from the anterior chambers of intact eyes, using a 10 μ l Hamilton syringe, and placed in a well created by sandwiching a sheet of aluminium (24 mm \times 40 mm \times 0.52 mm), in which a 5 mm diameter hole had been drilled to coincide with the measuring beam, between two no. 0 coverslips, using a thin ring of silicone grease as adhesive. The pathlength of this 'cuvette' was measured as 0.6 mm using precision Vernier callipers. Vitreous humour was removed from the vitreal body and measured in an identical fashion. Both the aqueous and vitreous humour were measured relative to distilled water, and the absorbance measurements obtained in this way were adjusted arithmetically to correspond to *in vivo* ocular pathlengths as measured by Martin (1986). Averaged absorbance spectra of ocular media components from single birds were fitted with an 11-point running average, to smooth random noise in the data, and averaged with similarly smoothed spectra from other individuals. The resulting mean absorbance spectra (and their variances) of each of the four ocular media components from several individuals were summed and converted to transmission for presentation.

Cone photoreceptor distribution

Counts were made from the retinæ of male and female adult starlings to determine the relative abundance and distribution of the different classes of cone photoreceptor. MSP measurements confirmed the consistent associations of different types of oil droplets with visual pigments of different λ_{\max} as reported previously (for a review, see Bowmaker *et al.* 1997). However, although the red and yellow oil droplets associated with the longwave-sensitive (LWS) and mediumwave-sensitive (MWS) visual pigments are readily distinguished using bright-field light microscopy, the oil droplets found in combination with the shortwave-sensitive (SWS) and ultraviolet-sensitive (UVS) visual pigments, and the longwave-sensitive double cones, all appear colourless to the human eye.

Because of difficulties in differentiating the three types of 'colourless' oil droplet using either autofluorescence or interference filters, a state-dependent histochemical technique was used to identify selectively bleached SWS and UVS cone photoreceptors. Increased oxidative metabolism in cone photoreceptors can be detected using a redox probe such as Nitroblue tetrazolium chloride (NBT) (Enoch, 1964*a,b*). NBT is a soluble yellow ditetrazole which can be locally reduced to an insoluble blue diformazan by increased succinic acid dehydrogenase activity in the mitochondria of photoreceptors in response to photon capture by the visual pigment. The intraretinal distributions of cone pigments in primates (Marc and Sperling, 1977) and teleost fish (Levine *et al.* 1979; Marc and Sperling, 1976) have been investigated in this way.

Subjects were dark-adapted for at least 16 h before being killed. Under infrared illumination, the eyes were removed, hemisected and placed in a dissection medium comprising 340 mosmol kg⁻¹ PBS and 11 mmol l⁻¹ D-glucose to sustain mitochondrial activity. After removing the lens and most of the vitreous humour, the posterior segments were immediately placed in an illumination medium consisting of Ham F10 nutrient mixture (Sigma) corrected to 340 mosmol kg⁻¹ by the addition of 1.75 g l⁻¹ NaCl and adjusted to pH 7.4 using 1 mol l⁻¹ NaOH. The addition of 3.4 mmol l⁻¹ NADP and 4.9 mmol l⁻¹ glucose 6-phosphate (Boehringer Mannheim) to the illumination medium, which have been shown to enhance succinate oxidation by rat liver cells (Butcher, 1972), increased the density of diformazan in stained ellipsoids. Both retinæ were then irradiated simultaneously for 10 min with either 381 nm or 434 nm monochromatic light, these wavelengths being close to the maximum spectral sensitivities of the UVS and SWS cone types, respectively. Stimulation of only the targeted cell types was optimised by the careful selection of illumination conditions (wavelength of monochromatic illumination and length of exposure), and only darkly stained cone inner segments were counted. Illumination was provided by a 150 W tungsten-halogen lamp and appropriate interference filter (Balzer B40; 10 nm FWHM bandwidth) and heat filter (Oriol 59875) combination. Typical light intensity at the point of exposure was 6×10^{18} photons s⁻¹ m⁻², measured with a portable spectrometer (SPEX 1681, Glen Spectra Ltd). After illumination, eye cups were transferred to an incubation medium consisting of the Ham F10 mixture plus 6 mmol l⁻¹ NBT (ICN) and 50 mmol l⁻¹ disodium succinate (Sigma) for 45 min at room temperature (22 °C), after which they were washed briefly in cold (3 °C) PBS and fixed for 2 min in 10% paraformaldehyde in 0.1 mol l⁻¹ sodium phosphate buffer. Each retina was then removed from the eye cup into PBS and, using the pecten and optic nerve for orientation, divided into four parts corresponding to dorsal and ventral, nasal and temporal quadrants. Two samples were taken from each quadrant, one peripheral and one central. Any remaining attached pigment epithelium was removed gently using a fine paint-brush, and the retina was mounted, receptor side up, in PBS.

Observations were made using an Olympus BHS

microscope (Olympus, Japan), fitted with a reflected light fluorescence attachment (mercury lines isolated at 334 and 365 nm) which, in preliminary trials, was used to confirm that the oil droplets associated with putative UVS cones did not autofluoresce and that those associated with the putative SWS cones did (Goldsmith *et al.* 1984*b*). The retinal photoreceptors of five birds of each sex (confirmed by laparotomy *post mortem*) were labelled separately for UVS and SWS cones. Counts were made of the blue-stained inner segments of the labelled cone type, which were distinguished from rod inner segments by size and by the presence of a colourless oil droplet, from ten fields of view for each of the samples (eight per eye) and for corresponding regions in both eyes. Counts of red, yellow and the remaining colourless oil droplets without stained inner segments were also made from the same samples. Colourless oil droplets which were not associated with a labelled inner segment were assumed to be either in the non-labelled shortwave-sensitive cone class or the principal member of the double cones. At a magnification of $\times 1250$, each field of view represented an area of 0.02 mm². Thus, only an estimated 1% of each retina was actually surveyed (approximately 26 000 cones per bird), and probably less as the retina spreads out on mounting, although this is minimised by prior fixation of the tissue. Nevertheless, having counted approximately 0.5×10^6 cones in the retinæ of 20 birds, this represents the most thorough survey of an avian retina to date.

Statistical analysis of cone distributions

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, percentages were converted to proportions and arcsine-square-root-transformed to normalise the data (Sokal and Rohlf, 1995). Significance was assessed using balanced repeated-measures multivariate analyses of variance (MANOVAs, Minitab 10.51, Minitab Inc.), with the transformed proportions of all the measured cell types as the dependent variables. Initially, three separate MANOVAs were performed: (i) using pooled data from all 20 birds to investigate the abundance and distribution of the LWS and MWS cell types; (ii) for the SWS cell types measured in 10 of those 20 birds; and (iii) for the UVS cell types, which were measured in the other 10 birds. In each case, the within-subject factors were 'quadrant', 'central/peripheral location' and 'left/right eye', with the between-subjects factor 'sex of bird'. Subsequently, two further MANOVAs were performed on the transformed proportion data from the SWS- and UVS-labelled groups, this time with the within-subjects factors being 'dorsal/ventral location' and 'nasal/temporal location'.

Results

Microspectrophotometry

Microspectrophotometric data for visual pigments and oil droplets are summarised in Table 1. The starling retina contains five different types of vitamin A₁-based visual

Table 1. *Characteristics of visual pigments and oil droplets in the European starling*

	Rod	Single cones				Double cone			
		UVS	SWS	MWS	LWS	Principal		Accessory	
Visual pigments									
λ_{\max} of mean prebleach spectrum (nm)	502.9 ± 1.7	(359)	448.7 ± 4.0	504.4 ± 3.6	563.2 ± 5.2	563.3 ± 4.1		559.5 ± 7.8	
Mean of prebleach λ_{\max} (nm)	502.8 ± 2.2	(362)	448.7 ± 2.8	503.7 ± 2.9	563.1 ± 2.8	561.3 ± 4.1		559.3 ± 2.2	
λ_{\max} of mean difference spectrum (nm)	505.6 ± 2.7	362.4	451.7 ± 7.4	505.8 ± 5.9	562.4 ± 6.9	562.9 ± 6.7		560.5 ± 14.7	
Absorbance at λ_{\max} (difference spectrum)	0.030	0.037	0.011	0.015	0.015	0.015		0.011	
Mean of difference spectrum λ_{\max} (nm)	504.7 ± 3.1	(361)	448.6 ± 2.8	503.7 ± 2.6	564.9 ± 3.8	563.0 ± 7.0		559.2 ± 7.1	
Number of cells	7	2	7	11	10	11		2	
						D	V	D	V
Oil droplets									
λ_{cut} of mean absorption spectrum (nm)	-	<350	399.3	514.1	571.7	407.4	410.5 439.8 470.9	-	478
λ_{mid} of mean absorption spectrum (nm)	-	<350	418.9	536.9	595.7	423.1	432.9 476.9 495.2	-	493
Mean λ_{cut} (nm)	-	<350	398.6 ± 3.9	515.3 ± 4.4	572.6 ± 3.2	407.3 ± 2.1	410.5 ± 2.5 438.5 ± 12.1 471.8 ± 3.0	-	479.0 ± 2.2
Mean λ_{mid} (nm)	-	<350	418.5 ± 2.0	536.2 ± 5.8	595.4 ± 3.3	423.2 ± 1.8	433.3 ± 4.8 476.3 ± 6.5 494.8 ± 3.0	-	491.0 ± 5.1
Mean diameter (μm)	-	1.6 ± 0.5	1.9 ± 0.3	2.2 ± 0.5	3.1 ± 0.4	2.5 ± 1.9	2.8 ± 0.5 2.7 ± 0.5 2.6 ± 0.5	-	1.5 ± 0.0
Number of oil droplets	-	6	20	42	27	70	75 17 7	-	5

Values in parentheses are running average values rather than those obtained from a best-fitted UVS visual pigment template and are necessary because the greater level of random noise in the individual records from UVS cones precludes accurate template fitting. Visual-pigment specific absorbances are not given owing to uncertainty regarding transverse pathlength, cone outer segments often being distorted or folded over upon themselves. Instead, absorbances measured at the λ_{\max} of the mean difference spectrum are given.

Rods do not contain oil droplets. A discrete oil droplet was not observed or measured microspectrophotometrically in the accessory member of the double cones in the dorsal retina.

Values are \pm standard deviations. 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 mean spectra. Multiple values shown for oil droplets found in the principal member of the double cones in the ventral retina correspond to the curves in Fig. 2B. They are arbitrary categorisations based on the relative absorption of the 480 nm shoulder of the spectral absorption curve relative to the main absorption peak of the spectral absorption curve. Respectively they describe oil droplets whose spectral absorption at 480 nm is (i) less than or equal to 25 % of the main absorption peak, (ii) more than 25 % but less than or equal to 50 % of the main absorption peak, and (iii) more than 50 % of the main absorption peak.

D, dorsal; V, ventral; UVS, ultraviolet-sensitive; SWS, shortwave-sensitive; MWS, mediumwave-sensitive; LWS, longwave-sensitive; λ_{\max} , wavelength of maximum absorbance; λ_{cut} , cut-off wavelength; λ_{mid} , wavelength corresponding to 50 % of maximum measured absorbance as defined by Lipetz (1984).

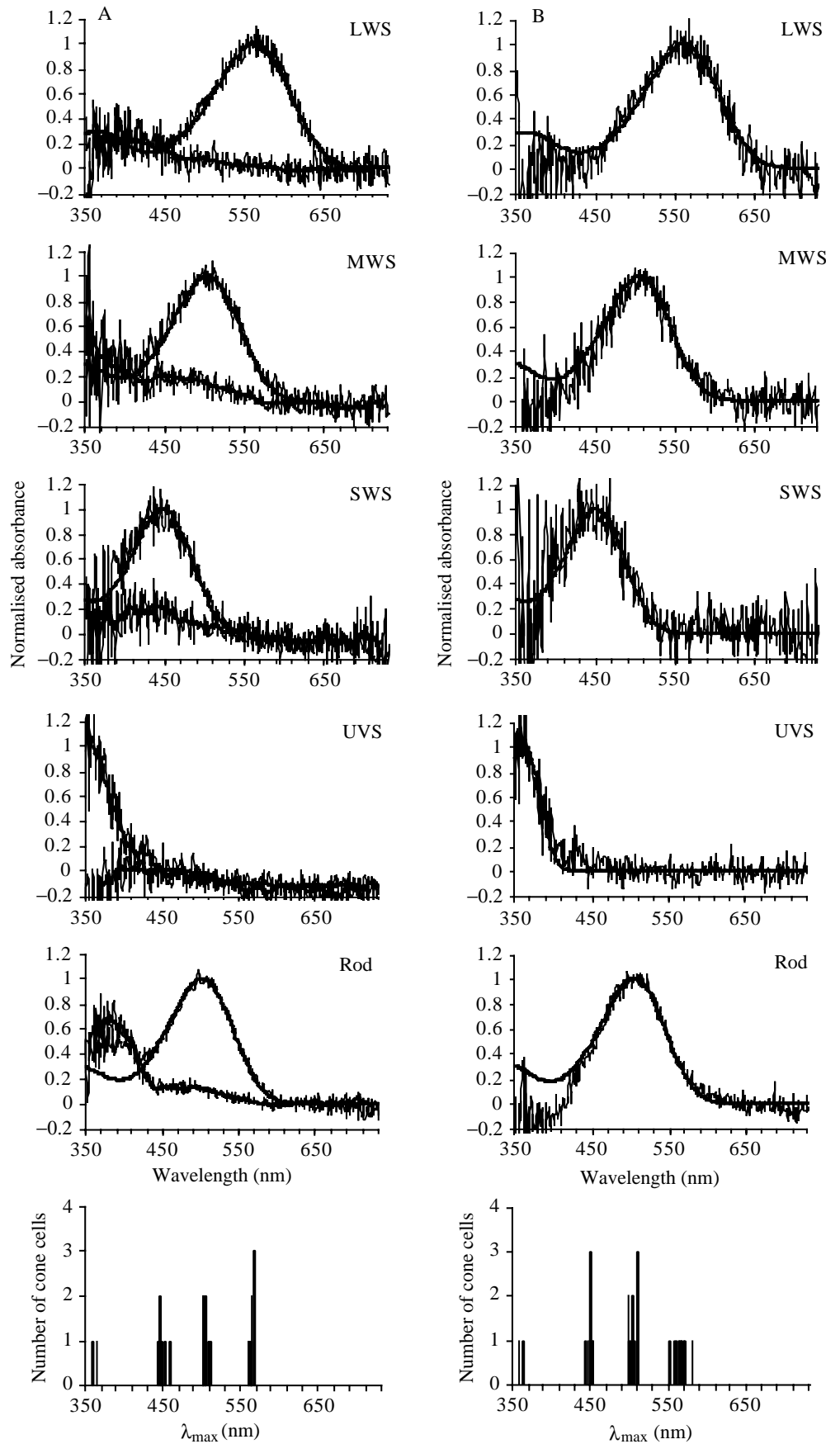


Fig. 1. Normalised absorbance spectra of visual pigments from *Sturnus vulgaris*. (A) Mean pre-bleach spectra (upper traces) with best-fitted visual pigment templates (solid lines) and mean post-bleach spectra (lower traces) with their running averages (solid lines). The mean pre-bleach spectrum of the UVS cones was not fitted well by the ultraviolet visual pigment template, so its running average absorbance is displayed instead. (B) Mean difference spectra, calculated between pre- and post-bleach absorbance spectra, and best-fitted visual pigment templates (solid lines). The histograms show the λ_{\max} values obtained for the analysis of individual photoreceptors and indicate the discrete clustering of λ_{\max} values in different cell types. UVS, ultraviolet-sensitive; SWS, shortwave-sensitive; MWS, mediumwave-sensitive; LWS, longwave-sensitive; λ_{\max} , wavelength of maximum absorbance.

pigment (i.e. rhodopsins) in six different types of photoreceptor cell (Fig. 1A,B; Table 1). A single class of rod contains a rhodopsin with a mean λ_{\max} at 503 nm ($N=7$). There are four types of single cone, each of which is associated with a different type of oil droplet. Oil droplet types are referred to using the nomenclature of Jane and Bowmaker (1988). Single cones containing red (R-type) oil droplets are paired with a longwave-sensitive (LWS) visual pigment which has a mean λ_{\max} of 563 nm ($N=10$); yellow (Y-type) oil droplets are paired with a mediumwave-sensitive (MWS) visual pigment (mean λ_{\max} 504 nm, $N=11$), spectrally very similar to that of the rod. There are two types of shortwave-sensitive single cone, one with a shortwave-sensitive (SWS) visual pigment (mean λ_{\max} 449 nm, $N=7$) which is paired with a colourless (C-type) oil

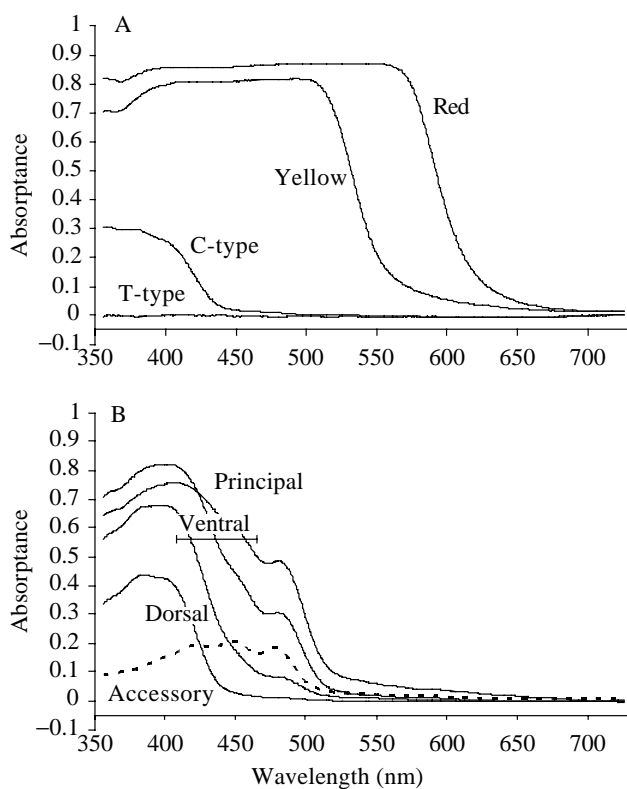


Fig. 2. Mean absorbance spectra of oil droplets from *Sturnus vulgaris*. (A) Oil droplets located in the single cones (C-type, colourless; T-type, transparent). (B) Oil droplets located in the principal (solid lines) and accessory (dashed line) members of the double cones. The three absorption spectra displayed for the Principal (P-type) oil droplets found in the principal member of the double cones in the ventral retina are arbitrary categorisations based on the relative absorption of the 480 nm shoulder of the spectral absorption curve relative to the main absorption peak of the spectral absorption curve. They describe P-type oil droplets whose spectral absorption at 480 nm is (i) less than or equal to 25% of the main absorption peak, (ii) more than 25% but less than or equal to 50% of the main absorption peak, and (iii) more than 50% of the main absorption peak (see also Table 1). In fact, as illustrated in Fig. 3B, a wide range of cut-off wavelength (λ_{cut}) is observed, the value of which, as well as the relative absorption by the 480 nm shoulder, tends to increase towards the most ventral regions of the retina.

droplet and the other an ultraviolet-sensitive (UVS) visual pigment (mean difference spectrum λ_{\max} close to 362 nm, $N=2$) which is paired with a transparent (T-type) oil droplet that has no significant absorbance at wavelengths greater than 350 nm. In addition, the retina is dominated by double cones, both members of which contain the longwave-sensitive visual pigment. The larger, principal member of the double cone pair contains a colourless (P-type) oil droplet. The accessory member displays a properly formed, spherical oil droplet (A-type; approximately 1.5 μm in diameter) only in the periphery of the ventral retina, but carotenoid is revealed in the inner segments of all accessory members by their autofluorescence when irradiated with near-ultraviolet light.

Averaged absorbance spectra for each of the oil droplet classes are shown in Fig. 2A,B. Fig. 3A,B presents histograms illustrating the variation in λ_{cut} for each of the oil droplet types. These data were obtained from two freshly caught individuals, whose oil droplets were not likely to be affected by possible

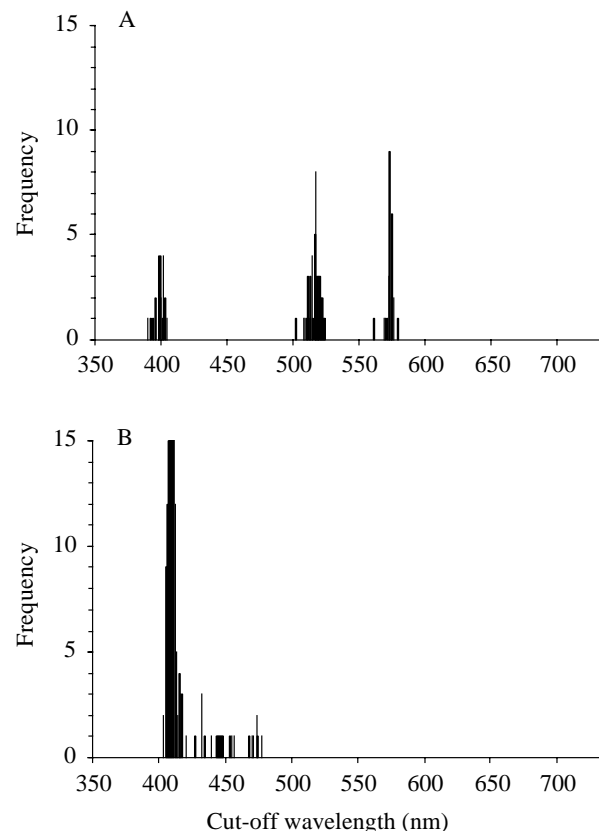


Fig. 3. Histograms showing the range of cut-off wavelength (λ_{cut}) values for oil droplets from *Sturnus vulgaris*. (A) Single cones, λ_{cut} values around 399, 514 and 572 nm describe C-type, yellow and red oil droplets respectively. (B) The principal member of the double cone clearly illustrating the range of cut-off wavelengths, which reflects a dorso-ventral gradient in relative pigmentation. λ_{cut} values are consistently more longwave towards the ventral retina as the size of the 480 nm shoulder in the absorption spectrum increases, probably due to increased ϵ -carotene concentration. Frequencies of oil droplets presented here are not directly representative of relative abundance.

dietary carotenoid deficiencies. [Scans from the oil droplets of birds which had been caught for other purposes than this study and had been kept in captivity on an artificial diet for several months displayed lower measured absorbance and, consequently, λ_{cut} values shifted to shorter wavelengths. This was particularly noticeable in the P-type oil droplet of the double cones in the ventral retina, which lacked the shoulder at approximately 480 nm in the absorbance spectra, probably reflecting a dietary deficiency of ϵ -carotene (Goldsmith *et al.* 1984.)] The red and yellow oil droplets have very high peak absorbance and act as cut-off filters (Fig. 2A), and the position of the cut-off wavelength (λ_{cut} , Table 1) is such that the peak effective spectral sensitivity of both cone types is shifted by approximately 40 nm to a longer wavelength (to approximately 605 nm and 547 nm, respectively, for the LWS and MWS single cones). C-type oil droplets generally have a lower peak absorbance (0.3 ± 0.1 , mean \pm s.d., $N=20$) and do not act as true cut-off filters, transmitting some light of wavelengths below the λ_{cut} .

The spectral absorption characteristics of the oil droplet in the principal member (P-type) of the double cone vary systematically across the retina in a dorso-ventral gradient. In the dorsal region of the retina, this droplet has a λ_{cut} at approximately 407 nm ($N=70$) and its absorbance spectrum resembles that of the C-type droplet (compare Fig. 2A,B). In the central ventral retina, the λ_{cut} of this type of droplet is shifted to slightly longer wavelengths (411 nm, $N=75$), and a shoulder in the absorbance spectrum appears at approximately 480 nm (Fig. 2B). In the most ventral regions of the retina, especially in the ventro-nasal quadrant, this shoulder is greatly increased and the λ_{cut} of the P-type oil droplet (471 nm, $N=7$) approaches that of the yellow oil droplet (514 nm, $N=42$). The peak absorbance (inferred from the λ_{cut} value) of P-type oil droplets in the ventral retina is greater than that of those found dorsally. However, even in the ventral retina, their cut-off nature probably does not affect the peak effective spectral sensitivity of the double cones because, in all cases, the λ_{cut} is shorter than the λ_{max} of the LWS visual pigment they contain. Nevertheless, shortwave sensitivity and photoreceptor bandwidth will be reduced by the presence of the oil droplets. The oil droplet in the accessory member of the double cones (A-type) was only measured in the ventral half of the retina, where it occurs as a discrete droplet approximately 1.5 μm in diameter. It has a triple-peaked absorbance spectrum (peaks at approximately 430, 450 and 480 nm), characteristic of this type of oil droplet found in other birds (Bowmaker *et al.* 1997), and a much lower peak measured absorbance (0.2 ± 0.2 , mean \pm s.d., $N=5$) than the P-type oil droplet found in the principal member (Fig. 2B). No noticeable variation in λ_{cut} was observed in the red, yellow or C-type oil droplets between the dorsal and ventral regions of the retina.

Spectrophotometry of pre-retinal tissue

The ocular media of the starling cease to transmit light below approximately 300 nm (Fig. 4A,B). At the wavelength

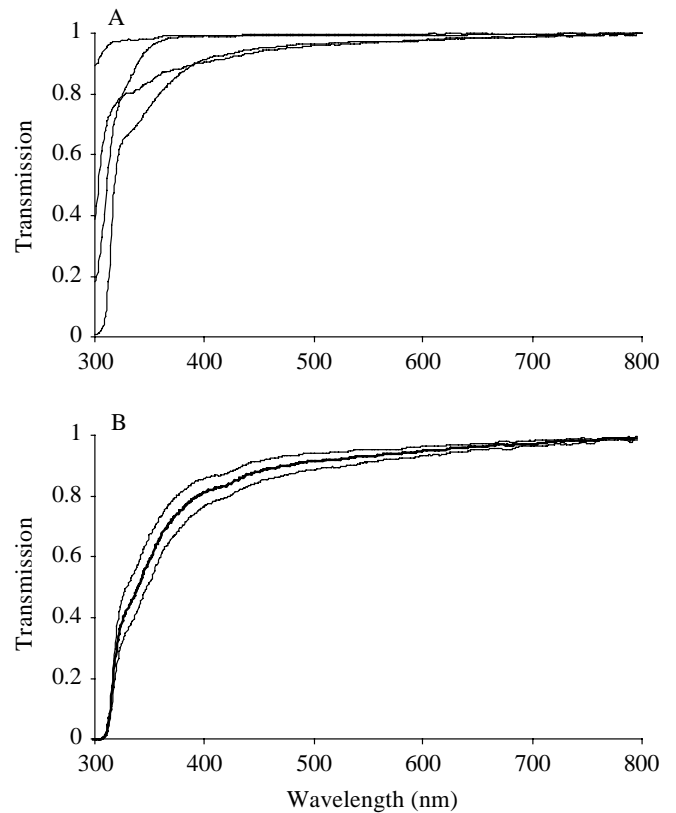


Fig. 4. Ocular media of *Sturnus vulgaris* corrected for *in vivo* pathlengths as measured by Martin (1986). (A) Mean transmission spectra (in order of increasing transmission at 300 nm) of the lens ($N=5$ individuals), aqueous humour ($N=1$), cornea ($N=4$) and vitreous humour ($N=1$). (B) Calculated transmission (thick line) \pm standard deviation (thin lines) of the combined ocular media. The wavelength of 50% transmission is 338 nm.

of 50% transmission (λ_{T50} 338 nm), corneal absorbance is 57.1% of lenticular absorbance. High transmission in the ultraviolet is essential for birds which have a dedicated ultraviolet-sensitive retinal visual pigment, and it is likely that absorbance by the lens is the limiting factor in shortwave photoreception in the starling.

Retinal mapping of cone photoreceptors

Contour plots shown in Fig. 5 summarise the distribution of cone types in the starling retina. These help visualise the distributions, but statistics are based on the eight eye regions actually sampled (four quadrants, each measured at the centre and periphery of the retina). Multivariate analysis shows that cone photoreceptor distribution varies significantly throughout the retina. Initially, when all 20 birds were pooled to compare the distribution of LWS and MWS single cones, results of the MANOVA revealed significant effects of quadrant (Wilk's $F=12.594$; d.f.=6,106; $P<0.001$) and central or peripheral location (Wilk's $F=18.820$; d.f.=2,17; $P<0.001$). Both the LWS (univariate: $F=37.98$; d.f.=1,18; $P<0.001$) and MWS (univariate: $F=4.89$; d.f.=1,18; $P=0.040$) cones are

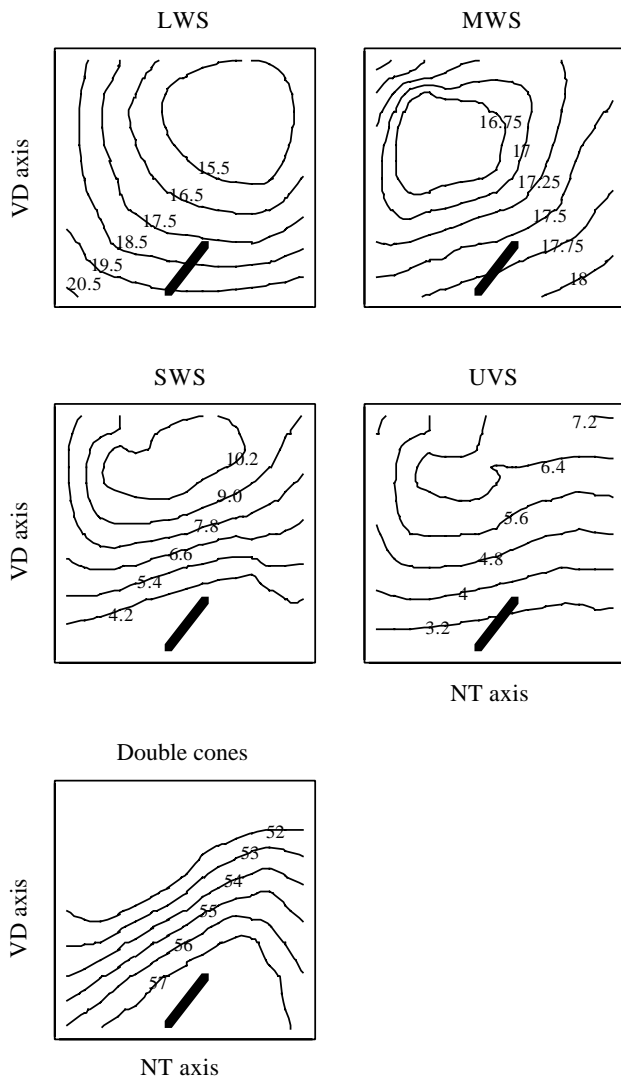


Fig. 5. Isodensity contour plots illustrating the distribution of cone types in the retina of *Sturnus vulgaris*. Values represent interpolated percentages for each cone type. Counts from both sexes and both eyes were pooled ($N=20$ birds each for LWS, MWS and double cones; $N=10$ birds each for SWS and UVS cones), as statistical tests showed that neither factor affected distribution. Values for double cones are calculated by subtraction and were not measured independently. The plots can be interpreted as if looking at the retina of the left eye from the corneal aspect. The thick diagonal line indicates the approximate position of the pecten. There are fewer LWS cones in the dorso-temporal retina, and higher densities of SWS and UVS cones dorsally. VD, ventral-dorsal axis; NT, nasotemporal axis (the bottom left hand corner of each contour plot represents the ventro-nasal quadrant); UVS, ultraviolet-sensitive; SWS, shortwave-sensitive; MWS, mediumwave-sensitive; LWS, longwave-sensitive.

significantly less abundant in the central retina than at the periphery; but the effect of quadrant was explained only by the LWS cones, which were significantly rarer in the dorso-temporal quadrant of the retina (univariate: $F=29.20$; d.f.=3,54; $P<0.001$). There was no effect of sex on the observed

distributions (multivariate: Wilk's $F=1.396$; d.f.=2,17; $P=0.275$).

SWS-labelled ($N=10$) and UVS-labelled ($N=10$) retinas were analysed separately in an identical fashion. Again, there was no significant effect of sex on SWS cone distribution (multivariate: Wilk's $F=5.072$; d.f.=4,5; $P=0.052$), but there was a clear dorso-ventral trend in the distribution of SWS cones, which are significantly more abundant in the dorsal half of the retina (multivariate: Wilk's $F=31.651$; d.f.=4,5; $P=0.001$; univariate: $F=54.71$; d.f.=1,8; $P<0.001$).

The distribution of UVS cones also varies significantly in a dorso-ventral manner (multivariate: Wilk's $F=15.501$; d.f.=4,5; $P=0.005$; univariate: $F=76.59$; d.f.=1,8; $P<0.001$) and there is a significant effect of quadrant (multivariate: Wilk's $F=5.959$; d.f.=12,55; $P<0.001$; univariate: $F=20.17$; d.f.=3,24; $P<0.001$). These effects are due to the highest proportion of UVS cones being located in the dorso-temporal region of the retina, probably replacing the LWS cones. Once again, there was no effect of sex on the observed distribution (multivariate: Wilk's $F=2.633$; d.f.=4,5; $P=0.158$).

Calculated percentages indicate that double cones tend to be more abundant in the ventral retina (Fig. 5).

Discussion

The spectral locations of the starling LWS, MWS and SWS cone visual pigment λ_{\max} values are very similar to those measured in a number of other avian species from a variety of orders. The λ_{\max} values of the rod and MWS cone pigments are almost identical to each other, which appears to be characteristic of avian retinas (Bowmaker *et al.* 1997). Although the starling is only the fourth species of bird in which a UVS visual pigment has been measured using spectrophotometric methods, the λ_{\max} value (362 nm) of these cones is similar to the values found in the UVS cones measured in the zebra finch *Taeniopygia guttata* (λ_{\max} 360–380 nm), the Pekin robin *Leothrix lutea* (λ_{\max} 355 nm) and the budgerigar *Melopsittacus undulatus* (λ_{\max} 371 nm), and it would appear from this limited data set that the spectral location of avian UVS visual pigments may also be conserved.

Ultraviolet sensitivity is now thought to be an important component of avian visual ecology. Conspecific signals in Pekin robins (Maier, 1993), zebra finches (Bennett *et al.* 1996), starlings (Bennett *et al.* 1997) and bluethroats *Luscinia s. svecica* (Andersson and Amundsen, 1997) are affected if the ultraviolet component of plumage reflectance is removed. The role of ultraviolet light in prey detection was predicted some years ago (e.g. Burkhardt, 1982) and has been recently demonstrated in kestrels *Falco tinnunculus* (Viitala *et al.* 1995). Goldsmith (1980) also produced behavioural evidence that humming birds might use ultraviolet cues in foraging for nectar.

Ultraviolet light may also be used by birds as a 'sun-compass' for orientation or as a cue to calibrate their circadian clocks (for a review, see Bennett and Cuthill, 1994). However, whether there is anything 'special' about the ultraviolet region

of the spectrum, over and above the importance of any other wavelength band, has yet to be demonstrated.

Certainly, the presence of an ultraviolet sensitive cone class (or for that matter a violet-sensitive one) in addition to the three other single cone classes suggests that birds may have the potential for tetrachromatic colour vision. Maier and Bowmaker (1993) correlated four maxima in a behaviourally measured spectral sensitivity function for the Pekin robin with peaks in the effective spectral sensitivity of the four classes of single cone predicted from MSP data. However, this does not constitute evidence of tetrachromatic colour vision, and the relevant colour-mixing behavioural experiments (e.g. Neumeier, 1992) need to be performed.

Several theories regarding the function of oil droplets in bird retinæ have been proposed (e.g. Muntz, 1972; Young and Martin, 1984). The main effect of an oil droplet cut-off filter vitread to a cone visual pigment will be to restrict the bandwidth of photoreceptor sensitivity, by reducing the amount of short-wavelength light reaching the outer segment, and to shift the wavelength of maximum sensitivity to a wavelength longer than the λ_{max} of the visual pigment (Bowmaker, 1977; Kawamuro *et al.* 1997; Maier and Bowmaker, 1993). It has been suggested that the narrowing of chromatic channels by coloured oil droplets in this manner might improve hue discrimination ability (Martin and Muntz, 1978), although at the expense of overall spectral sensitivity (Lythgoe, 1979). Indeed, Barlow (1982) proposed that the superiority of tetrachromacy over trichromatic colour vision is dependent upon such narrowing of visual pigment spectral sensitivities. The fact that C-type oil droplets appear to act as cut-off filters when the two shortwave cone pigments are spectrally close, but not when their λ_{max} values are further apart (Bowmaker *et al.* 1997), is supported by this study and adds further credence to their function in narrowing spectral sensitivity. The low density of carotenoid in the C-type oil droplets of the starling, budgerigar and zebra finch does not shift the λ_{max} of the cone and presumably serves only to prevent significant absorption of light by the β -peak of the visual pigment (Wolbarsht, 1976). Absorption of light of wavelengths below 300 nm by the ocular media precludes a visual function for the γ -peak which, in all retinal visual pigments, has a peak absorption close to 278 nm (Lythgoe, 1979) and is also thought to mediate excitation of the transduction cascade (Palacios *et al.* 1996). Oil droplets may also have an additional, dioptric function, such as light gathering (Baylor and Fettiplace, 1975; Ives *et al.* 1982; Young and Martin, 1984), which might explain the prevalence of colourless oil droplets in other vertebrate groups (Walls, 1942).

Topographic variations in oil droplet λ_{cut} will affect photopic spectral sensitivity depending on retinal area. The pigeon *Columba livia* has two distinct retinal areas: a red field in the dorso-temporal region, dominated by LWS single cones, and a yellow field, which constitutes the remaining retinal area (Muntz, 1972). The λ_{cut} of both yellow and red oil droplets is longer in the red field (Bowmaker, 1977), and the photopic spectral sensitivity of these two regions differs accordingly

(Martin and Muntz, 1978; Wortel *et al.* 1984). The red field corresponds to the binocular region of the temporal retina, which would be involved in tasks such as pecking (Clarke *et al.* 1996). This suggests that such variation may be of functional significance, most likely with regard to differences in hue discrimination ability between the two fields (Martin and Muntz, 1978).

It has been proposed that oil droplet complement is explained more by species' ecology of vision than phylogeny (Muntz, 1972; Partridge, 1989), and it is known that cones are distributed non-uniformly in the retinæ of a variety of avian species (Goldsmith *et al.* 1984; Gondo and Ando, 1995), although some maintain a largely isotropic distribution of cone types (e.g. quail *Coturnix coturnix japonica*; Budnik *et al.* 1984). Nocturnal birds have few brightly coloured oil droplets, as do swallows and swifts, whereas birds that need to see through the surface of water have a large proportion of red and yellow oil droplets (Muntz, 1972). Variations in cone distribution between dorsal and ventral regions of the retina are seen in a number of avian species (Goldsmith *et al.* 1984). Such dorso-ventral variations in cone complement in the retinæ of teleost fish have been correlated with visual environment and ethology (Levine and MacNichol, 1982).

The starling is diurnal, non-aquatic and largely ground-feeding (Feare, 1984). Interpretation of intraretinal variation therefore lies in the identification of other, perhaps quite subtle, visual tasks. The replacement of LWS cones by UVS cones in the dorso-temporal portion of the retina may be correlated with optical features of the starling eye and feeding behaviour. The dorso-temporal quadrant of the starling eye becomes increasingly myopic with greater eccentricity as the scleral surface falls behind the focal plane of the image (Martin, 1986). It is proposed that this 'ramp retina' might be used as a static accommodatory device, enabling simultaneous focus of the temporal visual field (corresponding to the ventro-nasal retina) at infinity to scan for aerial predators, as the nasal visual field (dorso-temporal retina) examines objects close to the bill while probing the ground for food. The starling is unusual in its reduction in LWS cones in the dorso-temporal retina. Most avian species studied, especially the pigeon, show the opposite trend or a more uniform distribution of red oil droplets (Bowmaker, 1977; Goldsmith *et al.* 1984; Muntz, 1972; Partridge, 1989; Waelchli, 1883). However, if the ventro-nasal region of the starling retina is indeed designed for scanning the celestial hemisphere, the increased proportion of red oil droplets and the increased pigmentation in the P-type oil droplets in this region would seem adaptive for the detection of distant objects. The visual range is greater for long-wavelength light, which is scattered less by the atmosphere than light of shorter wavelengths (Muntz, 1972). In addition, red oil droplets will reduce the degradation of the visual image by filtering short wavelengths scattered within the eye by the ocular media, thus increasing visual acuity (Lythgoe, 1979). Conversely, starlings often forage using an open-bill probing technique (Feare, 1984), which means that they locate prey visually at a very short range. The dorso-temporal region of

the retina concerned with this task would have little need for the high acuity necessary for long-distance vision, and this may explain the relative deficiency of LWS cones in the dorso-temporal retina.

Differences in the pigmentation of red and yellow oil droplets between the dorsal and ventral retina are common in many species of bird (Goldsmith *et al.* 1984; Partridge, 1989), but are not obvious in the starling. However, whereas red and yellow oil droplets in the pigeon have longer λ_{cut} values in the dorso-temporal retina (red field) (Bowmaker, 1977), the opposite is true for other terrestrial bird species (Goldsmith *et al.* 1984). C-type oil droplets are also known to have longer λ_{cut} values in the dorsal retina (Goldsmith *et al.* 1984). This too was not observed in the starling which, like the blue-throated hummingbird (*Lampornis clemenciae*) and the house finch (*Carpodacus mexicanus*) (Goldsmith *et al.* 1984), has a much higher proportion of SWS cones in the dorsal retina.

The increase in pigmentation of the P-type oil droplets in the ventral retina of the starling is intriguing. The ventral retina observes the celestial visual field and therefore receives much more light (particularly of shorter wavelengths) than the dorsal retina. Perhaps a progressive decrease in shortwave sensitivity is advantageous, either by protecting the eye from potentially damaging ultraviolet radiation (Kirschfeld, 1982) or by reducing luminance differences between the two visual hemifields to allow good vision of the ground whilst reducing excessive irradiance from the sky. In this respect, it is interesting to note that the jungle nightjar (*Caprimulgus indicus*) has been reported to possess a tapetum only in the dorsal half of the retina (Gondo and Ando, 1995). This may be an alternative or additional adaptation to increase photon capture by photoreceptors receiving light from the ventral visual hemifield.

Evidence for a centrifugally controlled circuit which might play a role in visual attention switching between the dorsal and ventral regions of the avian retina (Clarke *et al.* 1996) may also be important in deducing a functional explanation for the topographic organisation of the starling retina. Efferents from the isthmo-optic nucleus (ION) to the retina may excite ganglion cells locally in the ventral retina but inhibit those in dorsal regions, thus mediating switches in attention between the dorsal retina, involved in feeding, and the ventral retina, involved in scanning for predators. Intriguingly, ground-feeding species of bird, which have most need to switch their visual attention between food and predators and display a dorso-ventral gradation in pigment, have the largest and best developed IONs (Repérant *et al.* 1989). Swallows, which feed on the wing and probably have less need to scan for predators whilst feeding, have few ION efferents (Clarke *et al.* 1996), and it is swallows that either have no double cones (Goldsmith *et al.* 1984) or have double cones but no gradation of pigment.

The function of double cones, which occupy four times the area of a single cone (Matsusaka, 1963a cited in Meyer, 1977) and dominate the retinae of diurnal birds (Muntz, 1972), is unclear. Behavioural measures of photopic spectral sensitivity appear to show no involvement of the double cones, only peaks

in sensitivity corresponding to the corrected spectral sensitivities of the four single cone types (Maier and Bowmaker, 1993). Nevertheless, electroretinographically determined photopic spectral sensitivity functions are dominated by a broad peak at approximately 570 nm (e.g. Blough *et al.* 1972; Chen and Goldsmith, 1986), which corresponds to the peak effective spectral sensitivity of the double cones. This mismatch suggests that the neural signal from the double cones is not involved in colour vision, at least under the conditions used for the behavioural test of photopic spectral sensitivity. Double cones account for 82% of the retinal area in the great tit (*Parus major*) and form a well-developed mosaic, each single cone surrounded by four doubles (Engström, 1958). In some fish, a well-developed mosaic of single and double cones has been correlated with improved movement detection (Boehlert, 1978). The higher proportion of double cones observed in the ventral retina of the starling would be consistent with a role in enhanced detection of overhead predators through movement or achromatic contrast. It has been proposed that double cones might be involved in the detection of polarised light (Cameron and Pugh, 1991; Young and Martin, 1984), although the dedication of over 50% of the cone photoreceptors in the retina to this function alone is perhaps unlikely, and the behavioural evidence fails to support this hypothesis (Coemans *et al.* 1994; Vos Hzn *et al.* 1995).

Finally, because the UVS/violet-sensitive visual pigment dichotomy observed in the species studied using MSP so far (Bowmaker *et al.* 1997) may well reflect a phylogenetic division, and because so little variation is observed in the spectral location of avian visual pigments, it is perhaps towards oil droplet spectral absorption and the proportions and topographical distribution of retinal photoreceptors that we should turn in an attempt to identify the functional significance of species-specific variation.

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