J Comp Physiol A (2000) 186: 681–694
Digital Object Identifier (DOI) 10.1007/s003590000121

ORIGINAL PAPER

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

Visual pigments, cone oil droplets and ocular media in four species of estrildid finch

Accepted: 6 May 2000 / Published online: 10 June 2000

Abstract A microspectrophotometric study was conducted on the retinal photoreceptors of four species of bird: cut-throat finches (*Amadina fasciata*), gouldian finches (*Erythrura gouldiae*), white-headed munias (*Lonchura maja*) and plum-headed finches (*Neochmia modesta*). Spectral characteristics of the photoreceptors in all four species were very similar. Rods contained a medium-wavelength-sensitive visual pigment with a wavelength of maximum absorbance at 502–504 nm. Four spectrally distinct types of single cone contained a visual pigment with wavelength of maximum absorbance at either 370–373 nm (ultraviolet-sensitive), 440–447 nm (short-wavelength-sensitive); 500 nm (medium-wavelength-sensitive) or 562–565 nm (long-wavelength-sensitive). Oil droplets in the ultraviolet-sensitive single cones showed no detectable absorption between 330 nm and 800 nm. Oil droplets in the short-, medium-, and long-wavelength-sensitive single cones had cut-off wavelengths at 415–423 nm, 510–520 nm and 567–575 nm, respectively. Double cones contained the visual pigment with wavelength of maximum absorbance at 562–565 nm observed in long-wavelength-sensitive single cones. Only the principal member of the double cone pair contained an oil droplet (P-type, cut-off wavelength at 414–489 nm depending on species and retinal location). Spectral transmittance of the intact ocular media of each species was measured along the optic axis. Wavelengths of 0.5 transmittance for all species were very similar (316–318 nm).

Key words Colour vision · Microspectrophotometry · Photoreceptor · Retina · Bird

Abbreviations *D* dorsal · *LWS* long-wavelength-sensitive · *MSP* microspectrophotometer · *MWS* medium-wavelength-sensitive · *PBS* phosphate-buffered saline · *SWS* short-wavelength-sensitive · *UVS* ultraviolet sensitive · *V* ventral · *VS* violet-sensitive · λ_{max} wavelength of maximum absorbance · λ_{cut} cut-off wavelength · λ_{mid} wavelength of half maximum measured absorptance · $\lambda T_{0.5}$ wavelength of 0.5 transmittance

N. S. Hart · J. C. Partridge (✉) · A. T. D. Bennett · I. C. Cuthill
School of Biological Sciences, University of Bristol,
Woodland Road, Bristol BS8 1UG, UK
e-mail: j.c.partridge@bristol.ac.uk
Tel.: +44-117-928-7591; Fax: +44-117-925-7373

Introduction

The retinae of most diurnal birds studied to date contain a single class of medium-wavelength-sensitive (MWS) rod, a single class of long-wavelength-sensitive (LWS) double cone, and four classes of spectrally distinct single cone which are maximally sensitive to long, medium, short, and either violet (VS) or ultraviolet (UVS) wavelengths (Jane and Bowmaker 1988; Bowmaker et al. 1993, 1997; Maier and Bowmaker 1993; Hart et al. 1998; Das et al. 1999; Hart et al. 1999, 2000). The spectral sensitivity of a given photoreceptor cell is determined by the absorptance of the visual pigment in the outer segment and, in the case of avian or some reptilian cones, the transmittance of the oil droplet located in the ellipsoid region of the inner segment, through which some or all of the light incident upon the outer segment must have passed (Baylor and Hodgkin 1973; Bowmaker 1977; Neumeyer and Jäger 1985; Kawamuro et al. 1997).

Each type of cone visual pigment is reliably associated with a specific type of oil droplet. With the exception of the transparent, or 'T-type', oil droplets found in the VS/UVS single cones, which show no detectable absorption from at least 330 nm to 800 nm, they contain short-wavelength-absorbing pigments (carotenoids) and are generally considered to act as long-pass cut-off filters (Liebman and Granda 1975; Goldsmith et al. 1984; Lipetz 1984b). The difference in spectral transmittance between droplet types depends on the type and concentration of the carotenoids they contain.

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 1984a). This is effectively the wavelength below which light is theoretically no longer transmitted. The λ_{cut} of single cone oil droplets is usually spectrally close to the wavelength of maximum absorbance (λ_{max}) of the visual pigment with which they are associated, although the precise relationship depends on photoreceptor type: colourless ('C-type') oil droplets have their λ_{cut} at slightly shorter wavelengths than the λ_{max} of the short-wavelength-sensitive (SWS) visual pigment, whilst yellow ('Y-type') and red ('R-type') oil droplets have their λ_{cut} at slightly longer wavelengths than the λ_{max} values of the MWS and LWS visual pigments, respectively.

Intriguingly, the 'P-type' droplet found in the principal member of the double cone pair has a λ_{cut} at a considerably shorter wavelength than the λ_{max} of the LWS visual pigment it contains, and in many birds the λ_{cut} of this droplet type varies with retinal location (Goldsmith et al. 1984; Partridge 1989; Hart et al. 1998). An intact oil droplet is not always seen in the accessory member of the double cone pair, although low levels of diffuse carotenoid pigment can sometimes be detected in the ellipsoid using microspectrophotometry (Jane and Bowmaker 1988; Maier and Bowmaker 1993; Bowmaker et al. 1997; Das et al. 1999). Avian rods do not contain oil droplets.

The greatest interspecific variation in avian photoreceptor spectral sensitivities observed thus far concerns the nature of the visual pigment found in the class of single cone having a T-type oil droplet. The outer segments of these photoreceptors contain a visual pigment which has a λ_{max} value either between 403 nm and 426 nm (VS), as in the Humboldt penguin *Spheniscus humboldti* (Bowmaker and Martin 1985), chicken *Gallus gallus* (Fager and Fager 1981; Yoshizawa and Fukada 1993; Bowmaker et al. 1997), duck *Anas platyrhynchos* (Jane and Bowmaker 1988), Japanese quail *Coturnix coturnix japonica* (Bowmaker et al. 1993), peacock *Pavo cristatus* (Hart 1998), and turkey *Meleagris gallopavo* (Hart et al. 1999), or between 355 nm and 380 nm (UVS), as in the Pekin robin *Leothrix lutea* (Maier and Bowmaker 1993), zebra finch *Taeniopygia guttata* and budgerigar *Melopsittacus undulatus* (Bowmaker et al. 1997; Wilkie et al. 1998), European starling *Sturnus vulgaris* (Hart et al. 1998), canary *Serinus canaria* (Das et al. 1999), blue tit *Parus caeruleus* and blackbird *Turdus merula* (Hart et al. 2000). This visual pigment type in the pigeon (*Columba livia*) has a λ_{max} at about 409 nm when measured in situ with a microspectrophotometer (Bowmaker et al. 1997) or at 393 nm when regenerated from opsin protein expressed by cultured cells (Yokoyama et al. 1998) and as such its designation as either a VS or UVS type is debatable.

Other more subtle interspecific variations also exist. For example, λ_{max} values of the LWS visual pigment found in the LWS single cones and both members of the LWS double cone pair appear to be clustered around three spectral locations. The LWS visual pigment of the Humboldt penguin has a λ_{max} at around 543 nm (Bowmaker and Martin 1985), whilst in the tawny owl *Strix aluco* (Bowmaker and Martin 1978), great horned owl *Bubo virginianus* (Jacobs et al. 1987) and blackbird (Hart et al. 2000) the λ_{max} is at 555 nm, 555 nm, and 557 nm, respectively. The remaining birds studied have LWS visual pigments with a λ_{max} between 563 nm and 570 nm (Sillman et al. 1981; Jane and Bowmaker 1988; Bowmaker et al. 1993, 1997; Maier and Bowmaker 1993; Hart 1998; Hart et al. 1998, 1999, 2000; Das et al. 1999).

Measurements of SWS visual pigment absorbance spectra made microspectrophotometrically are generally of a lower quality than the other cone types with maximal sensitivity at longer wavelengths. Like the UVS/VS cone type, they are comparatively rare in most avian retinae and their outer segments are relatively small which makes them more difficult to measure. Nevertheless, there appears to be considerable interspecific variation in the λ_{max} of this visual pigment type: values range from 463 nm in the tawny owl (Bowmaker and Martin 1978) to as short as 430 nm in the zebra finch (Bowmaker et al. 1997), which approaches values obtained for VS visual pigments.

Evidence for spectral clustering of avian visual pigment types is conditional on obtaining further high quality data. Here, we present microspectrophotometric measurements from four species of estrildid finch: cut-throat finches (*Amadina fasciata*), gouldian finches (*Erythrura gouldiae*), white-headed munias (*Lonchura maja*) and plum-headed finches (*Neochmia modesta*). These species are considered to be closely related to the zebra finch (Sibley and Monroe 1990), whose photoreceptor spectral characteristics have already been determined (Bowmaker et al. 1997), but differ in geographical distribution and exhibit marked inter- and intra-specific variations in plumage colouration (Immelmann 1977; Goodwin 1982). Furthermore, we provide measurements of the spectral transmittance of their ocular media, which are essential in determining the short-wavelength limit of photoreception in most birds.

Materials and methods

Microspectrophotometry

Birds were obtained from breeders in southern England and kept indoors under an 18:6 light:dark cycle with ambient illumination from Truelite fluorescent tubes. Their diet consisted of foreign finch seed mix (Country Wide TM brand) and mineral grit (Prestige TM brand) ad libitum plus green food thrice weekly. At the time of measurement, most birds had been living under these conditions for at least 6 months. With the exception of plum-headed finches, where only male birds were used, photoreceptors were measured in both sexes. Three (white-headed munias, plum-headed finches and gouldian finches) or five (cut-throat finches) birds of each species were used. The gouldian finches were all black-headed morphs.

Birds were held in darkness overnight and killed by approved humane methods (cervical dislocation followed by decapitation). Retinal tissue was prepared for analysis using a microspectrophotometer (MSP) as described elsewhere (Hart et al. 1998; Hart et al. 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 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 absorptance spectra and these samples were mounted in pure glycerol.

Absorbance spectra (330–800 nm) of individual photoreceptor outer segments were measured using a computer-controlled, wavelength-scanning, single-beam MSP (Hart et al. 1998). 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.

To minimise in-scan bleaching, only one sample scan of each outer segment was made, but this was combined with two separate baseline scans. Averaging the two absorbance spectra obtained in this way improved the signal-to-noise ratio of the spectra used to determine visual pigment λ_{max} values (Bowmaker et al. 1991). Due to their smaller diameter, and the resultant low signal-to-noise ratio of the absorption 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 short wavelengths.

Following the 'pre-bleach' scans, outer segments were 'bleached' with full spectrum white light from the monochromator for 5 min and an identical number of sample and baseline scans made subsequently. The 'post-bleach' average spectrum thus created was deducted from the 'pre-bleach' average to produce a difference spectrum for each outer segment. Finally, the absorptance of the oil droplet associated with the outer segment (if present) was measured. A single sample scan was made of each 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. Higher quality oil droplet spectra, which showed less evidence of by-passing light (Lipetz 1984a), were obtained from the separate preparations mounted in glycerol, but the measurement protocol was identical.

Analysis of visual pigment absorbance spectra

Baseline and sample data were converted into absorbance[1] values at 1 nm intervals. Upward and downward scans were averaged together by fitting a weighted (delta function) three point running average to the absorbance data. Specifically, 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 normalized 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). For each pre-bleach absorbance and difference spectrum, 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 normalized 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 UVS outer segments that were shown to be photolabile were included. Acceptable spectra from each photoreceptor type were averaged and reanalysed.

Analysis of oil droplet absorptance spectra

Sample and baseline data were converted to absorptance and normalized 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 droplet absorptance spectra are described by their 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 1984a). For comparison with other studies (e.g. Partridge 1989; Hart et al. 1998, 1999), the wavelength corresponding to half maximum measured absorptance, λ_{mid} (Lipetz 1984a), is also given.

Spectrophotometry of ocular media

Absorbance measurements (200–800 nm) of the combined 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.

Each eye was placed in a rectangular aluminium insert, designed to fit inside a standard (10-mm pathlength) quartz cuvette, in which a hole corresponding to the equatorial diameter of each eye (range 6.5–7.0 mm depending on species) 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. Care was taken to avoid compression of the eyes that would alter their 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.

Results

Microspectrophotometry

Microspectrophotometric data for visual pigments (Figs. 1, 2, 3, 4) and oil droplets (Figs. 5, 6) in the four

[1] Absorbance is a logarithmic measure of light absorption and is defined as $\log_{10}$ 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 transmittance (1–absorptance). Absorbances are additive (doubling the pathlength doubles the absorbance) but when normalized, 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).

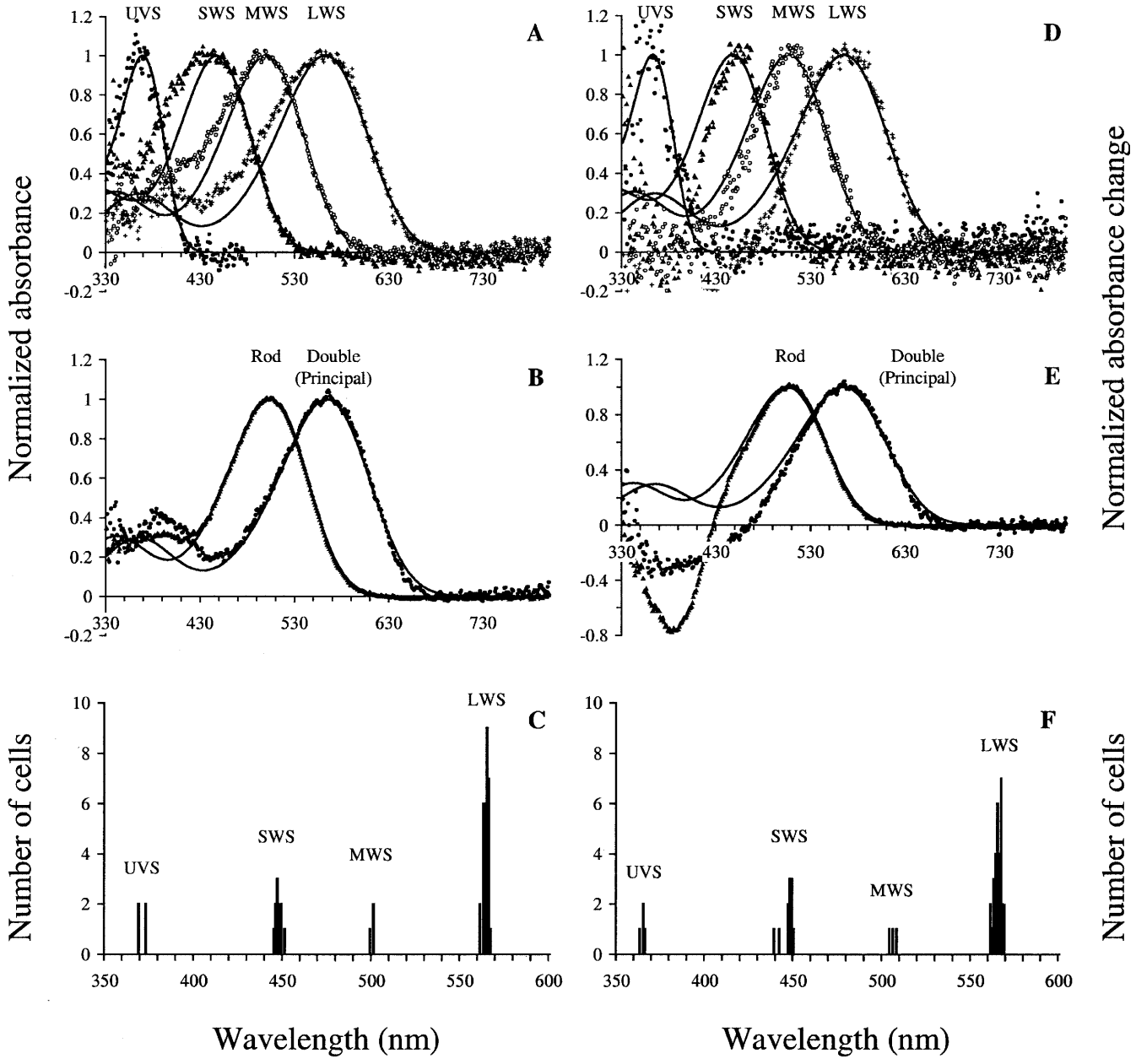


Fig. 1 Normalized mean pre-bleach absorbance (**A, B**) and difference (**D, E**) spectra of visual pigments from the cut-throat finch (*Amadina fasciata*). **A**, **D** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B**, **E** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **C, F** Histograms showing the distribution of the wavelengths of maximum sensitivity (λ_{max}) calculated for each of the cone visual pigment spectra used to create the mean spectra shown. Histogram data shown for the long-wavelength-sensitive (LWS) cone visual pigment includes spectra from LWS single cones and both members of the LWS double cones. *UVS* ultraviolet-sensitive; *SWS* short-wavelength-sensitive; *MWS* medium-wavelength-sensitive; *LWS* long-wavelength-sensitive

species of finch are summarised in Tables 1 and 2, respectively. The retinae of all species contained five different types of vitamin A_1-based visual pigment (assumed from their similarity to rhodopsin, rather than porphyropsin, visual pigment templates) in seven different types of photoreceptor cell, the spectral absorbance characteristics of which were all very similar between species.

Rods contained a MWS visual pigment with a λ_{max} at about 502–504 nm. There were four spectrally distinct types of single cone. Firstly, a UVS type with a visual pigment λ_{max} at about 370–373 nm and a transparent T-type oil droplet which showed no detectable absorptance between 330 nm and 800 nm. Secondly, a SWS type with a 440–447 nm λ_{max} visual pigment and a colourless C-type oil droplet with a λ_{cut} at 415–423 nm. Thirdly, a MWS type with a 500 nm λ_{max} visual pigment and a yellow Y-type oil droplet with a λ_{cut} at about 510–520 nm. Finally, the fourth type of single cone, a LWS type, contained a 562–565 nm λ_{max} visual pigment and a red R-type oil droplet with a λ_{cut} at 567–575 nm.

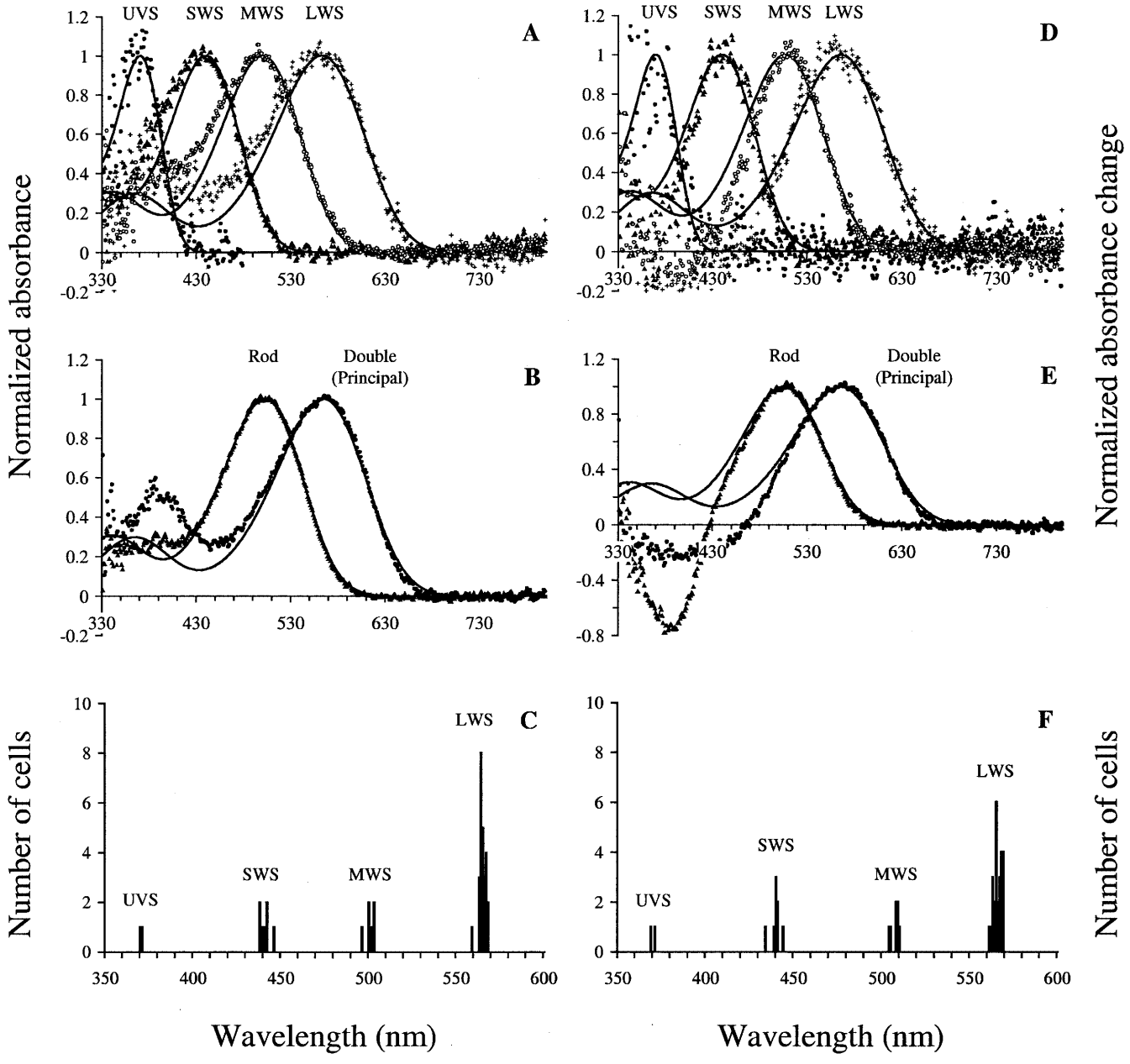


Fig. 2 Normalized mean pre-bleach absorbance (**A, B**) and difference (**D, E**) spectra of visual pigments from the gouldian finch (*Erythrura gouldiae*). **A, D** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B, E** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **C, F** Histograms showing the distribution of λ_{max} calculated for each of the cone visual pigment spectra used to create the mean spectra shown. For further details see legend to Fig. 1

Examination of Figs. 1, 2, 3, and 4 reveals that some of the pre-bleach absorbance spectra deviate from their best-fitted template spectra on the short wavelength limb, having a slightly higher absorbance at each wavelength than would be expected. This is probably due to either increased scattering of the measuring beam at shorter wavelengths or, more likely, the absorbance of stable photoproduct in the outer segments which has accumulated as a result of in-scan bleaching (Knowles and Dartnall 1977). Both of these artefacts are exacerbated by the relatively small size of the finch single-cone outer segments (compared to their rods and double cones or the single cones of other species, e.g. turkeys; Hart et al. 1999) and explain why the calculated difference spectra dip below the template on the short-wavelength limb. However, because only the long-wavelength limb is used to estimate λ_{max} (Partridge and DeGrip 1991), and because all the long-wavelength limbs fit their templates well, it is assumed that their effects on the estimates of λ_{max} are small or negligible.

Both the principal and accessory members of the double cone pair contained a LWS visual pigment that was spectrally identical to that found in the LWS single

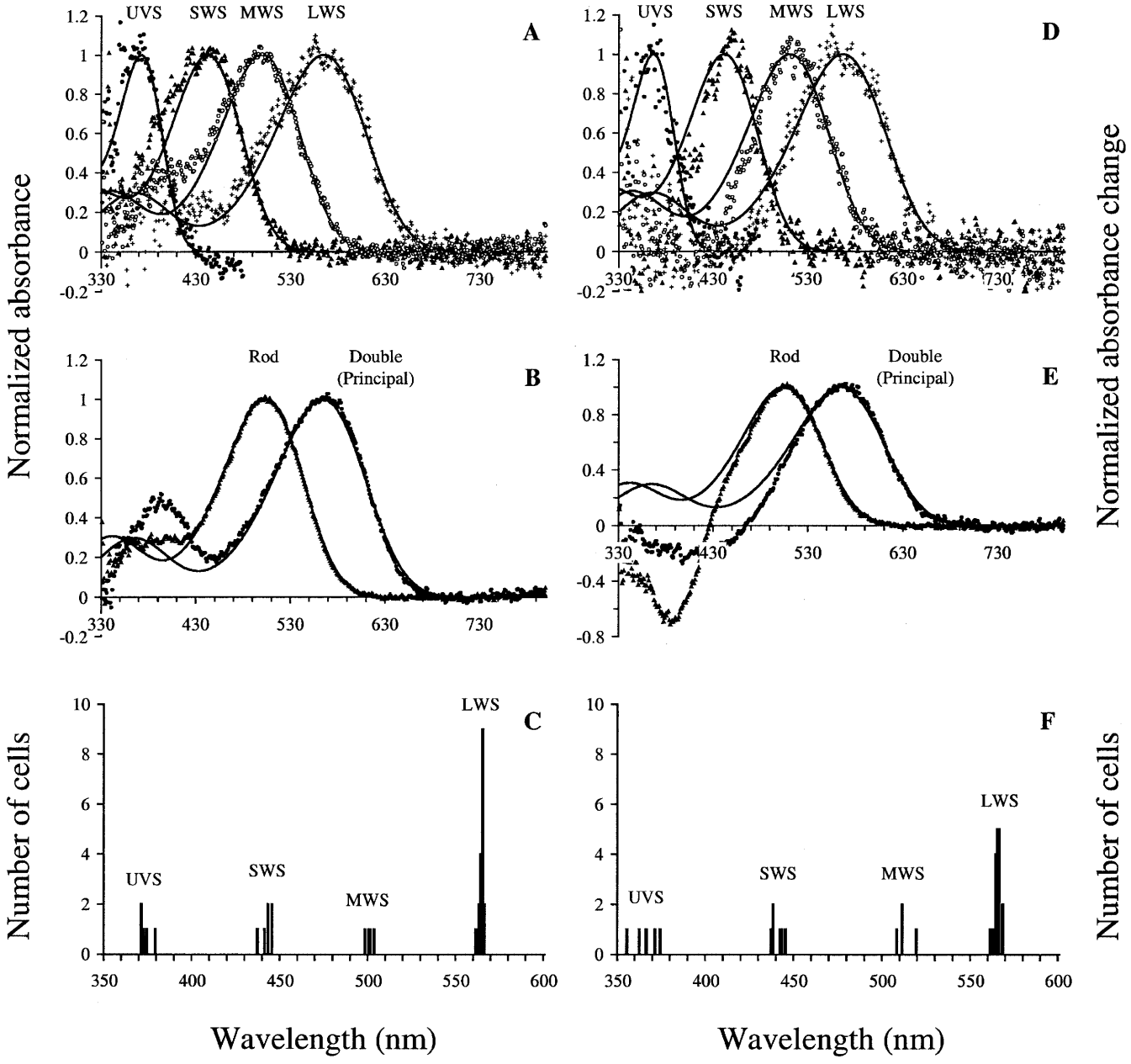


Fig. 3 Normalized mean pre-bleach absorbance (**A, B**) and difference (**D, E**) spectra of visual pigments from the plum-headed finch (*Neochmia modesta*). **A, D** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B, E** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **C, F** Histograms showing the distribution of λ_{max} calculated for each of the cone visual pigment spectra used to create the mean spectra shown. For further details see legend to Fig. 1

cones (λ_{max} 562–565 nm). An intact oil droplet (P-type) was only observed in the principal member and the spectral characteristics of this droplet type varied depending on retinal location (Table 2, Fig. 5). This was most obvious in the white-headed munias, whose P-type oil droplets had a mean λ_{cut} at 419 nm in the dorsal retina, but at 489 nm in the ventral retina. Smaller dorso-ventral differences (5–7 nm) were observed in the cut-throat and plum-headed finch mean λ_{cut} values, and in fact those of the gouldian finches were almost identical. Because P-type oil droplet absorptance spectra are not always simple step-functions (like the other oil droplet types) it is harder to describe them in terms of a single parameter (λ_{cut}). Nevertheless, in every case the λ_{mid} of P-type oil droplets located in the ventral retina also occurred at longer wavelengths (Table 2, Fig. 6E–H; two-sample *t*-tests: cut-throat finch $t = -8.25$, $P < 0.001$, $df = 28$; gouldian finch $t = -8.28$, $P < 0.001$, $df = 28$; plum-headed finch $t = -9.35$, $P < 0.001$, $df = 28$; white-headed munia $t = -45.58$, $P < 0.001$, $df = 21$), and the spectra displayed an increased absorptance at about 490 nm, compared to those found dorsally.

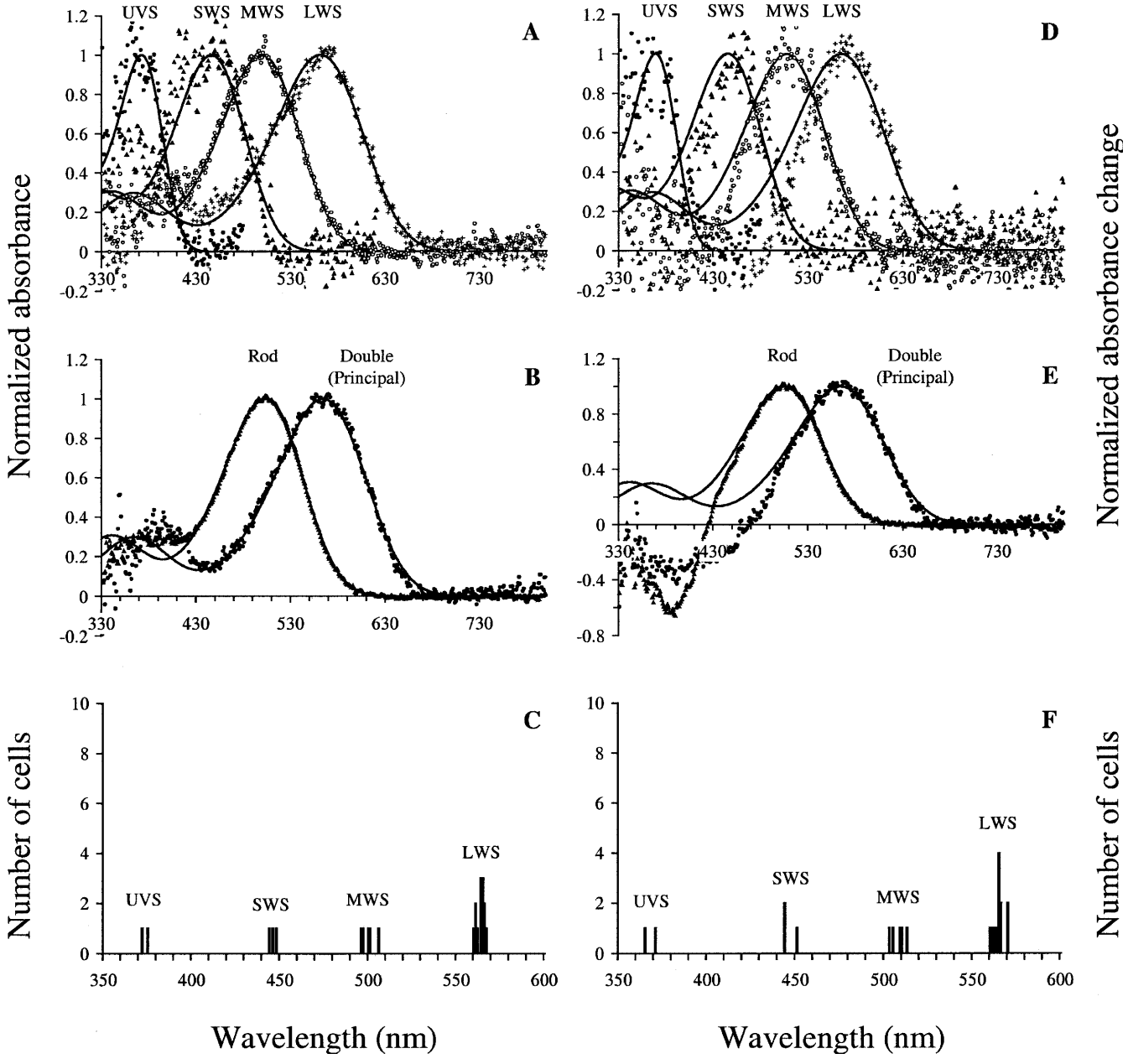


Fig. 4 Normalized mean pre-bleach absorbance (**A, B**) and difference (**D, E**) spectra of visual pigments from the white-headed munia (*Lonchura maja*). **A, D** Single cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **B, E** Rods and the principal member of the double cones (*symbols*) with best-fitted rhodopsin templates (*solid lines*). **C, F** Histograms showing the distribution of λ_{max} calculated for each of the cone visual pigment spectra used to create the mean spectra shown. For further details see legend to Fig. 1

Spectrophotometry of ocular media

Two sequential absorbance measurements of the combined ocular media of each species were averaged together, converted to transmittance and an 11-point unweighted running average fitted to the mean spectrum to smooth random noise (Fig. 7). Wavelengths of 0.5 transmittance for each species were very similar, occurring at 318 nm in the white-headed munia and cut-throat finch, 317 nm in the gouldian finch and 316 nm in the plum-headed finch. The combined ocular media of all species ceased to transmit light below approximately 300 nm.

Discussion

Comparison with other birds

In general, the spectral characteristics of the visual pigments and oil droplets of the four finch species studied are very similar, both to each other and to the only other species of finch for which microspectrophotometric data is available, the zebra finch (Bowmaker et al. 1997). They are also very similar to the other Passeriformes (the

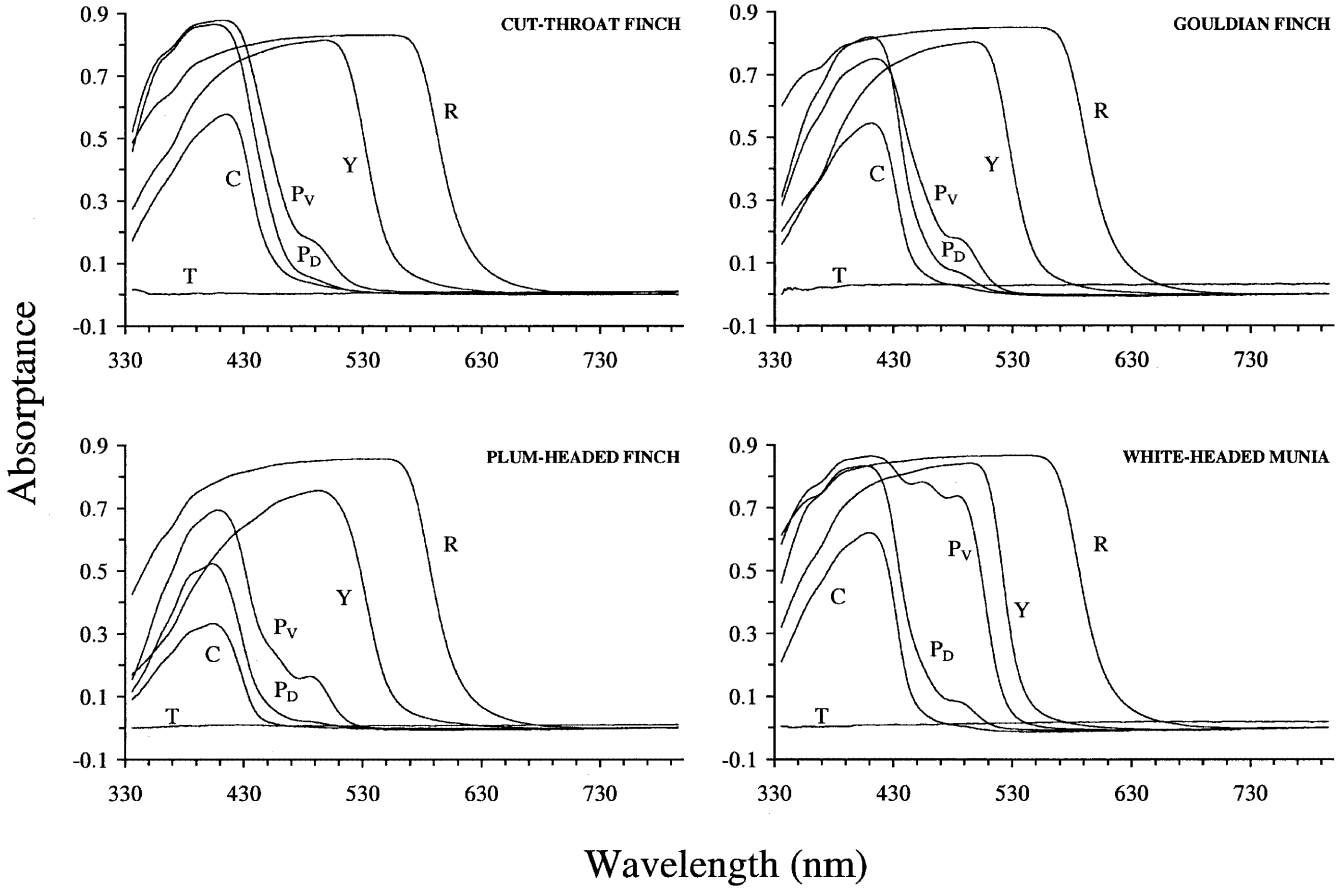
order to which finches belong) studied to date, namely the Pekin robin (Maier and Bowmaker 1993), starling (Hart et al. 1998), canary (Das et al. 1999) blue tit and blackbird (Hart et al. 2000) and the only psittaciform species investigated, the budgerigar (Bowmaker et al. 1997). The most conspicuous shared feature of these species' photoreceptors is the UVS visual pigment with a λ_{max} at around 355–380 nm that is found in single cones with transparent (T-type) oil droplets. The other species of bird for which microspectrophotometric data are available have a VS visual pigment with a λ_{max} between about 402 nm and 426 nm in this cone type (Bowmaker and Martin 1985; Jane and Bowmaker 1988; Bowmaker et al. 1993, 1997; Hart 1998; Hart et al. 1999).

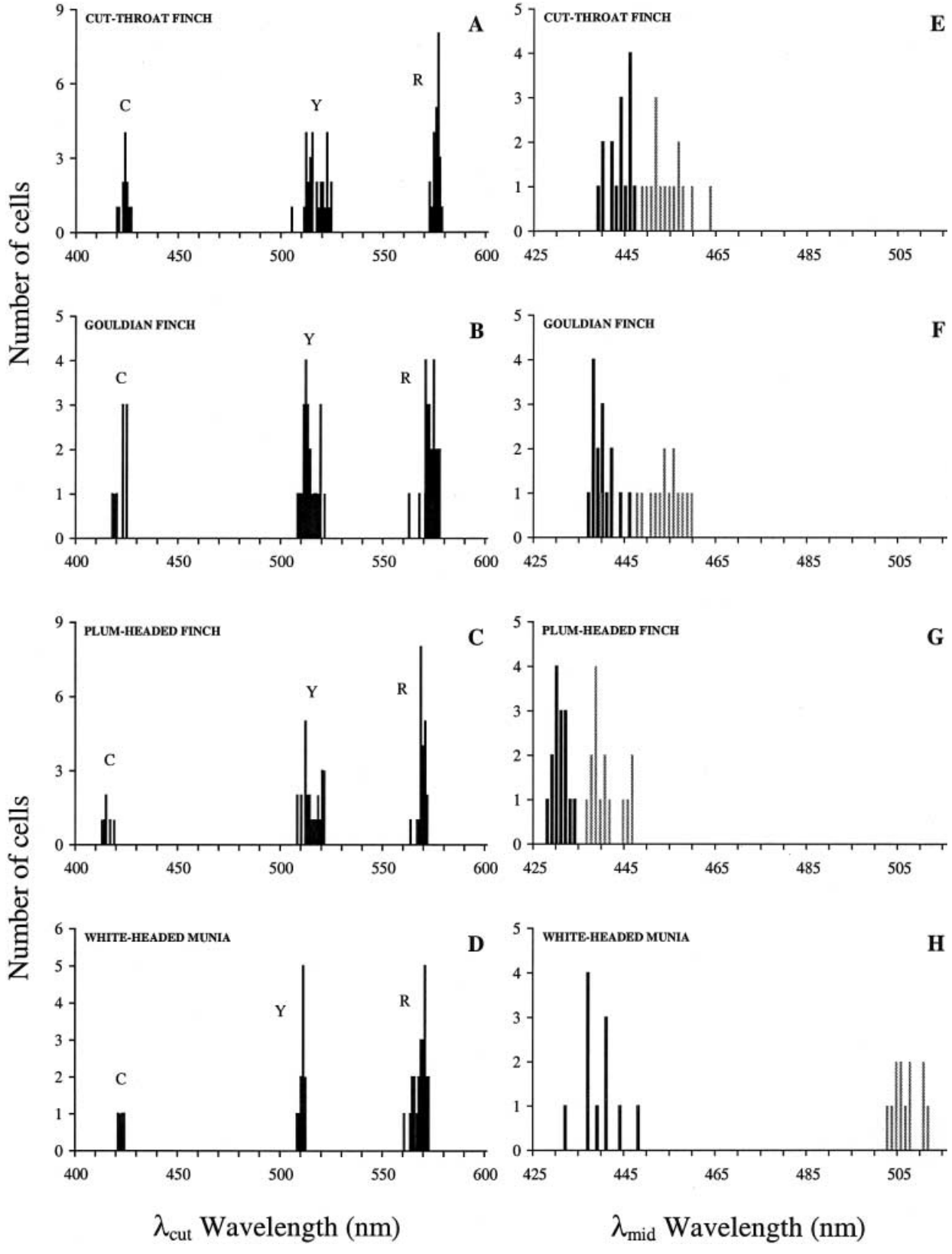
Visual ecology of SWS and UVS/VS visual pigments

Of the species which possess a dedicated UVS visual pigment, the zebra finch appears to be unusual in having a SWS visual pigment with a λ_{max} at much shorter wavelengths (about 430 nm) than the others (Bowmaker et al. 1997). The reasons for this difference are not readily apparent from comparisons of species' visual ecology. The zebra finch and the species investigated in this study all feed primarily on the seeds of grasses and occasionally supplement their diet with other seeds and small insects (Immelmann 1977; Goodwin 1982). They all tend to inhabit open grasslands interspersed with shrubs and trees and are never far from water, although their geographic distribution is varied. Of the three Australian species, zebra finches are the most widespread and occur almost everywhere except the peripheral wet sclerophyll and rain forests (Immelmann 1977). Gouldian finches are restricted to tropical northern Australia whilst the plum-headed finch is present only in the eastern hinterlands (Immelmann 1977). White-headed munias are endemic to

Fig. 5 Mean absorptance spectra of oil droplets found in the cone photoreceptors of all four species of finch investigated in this study. *T*, *C*, *Y*, *R* and *P* correspond to the T-type (transparent), C-type (colourless), Y-type (yellow), R-type (red) and P-type (pale) oil droplets found in the UVS, SWS, MWS and LWS single cones and the principal member of the double-cone pair, respectively. *Subscripts D and V* refer to whether the P-type oil droplets were measured in the dorsal or ventral retina, respectively, as noticeable differences in the spectra obtained from these two retinal regions were observed

Fig. 6 Histograms illustrating the spectral distribution of cut-off wavelength (λ_{cut}; Lipetz 1984a) values for the C-, Y- and R-type oil droplets found in the SWS, MWS and LWS single cones, respectively (**A–D**) and wavelength of half-maximum measured absorptance (λ_{mid}; Lipetz 1984a) values for the P-type oil droplets found in the principal member of the double cone pair (**E–H**) for all four species of finch investigated in the present study. P-type oil droplets measured in the dorsal retina are represented by *black bars*, whilst those measured ventrally are in *grey*. Note that P-type oil droplets measured in the ventral retina appear to have λ_{mid} values at longer wavelengths than those measured dorsally





the Malaysian peninsular and Indonesia, and the cut-throat finch inhabits the arid regions of central and eastern Africa (Goodwin 1982). Perhaps the most similar species to the zebra finch in terms of visual ecology is in fact the budgerigar, which also inhabits the arid interior of Australia and feeds largely on

Table 1 Characteristics of visual pigments measured in the four species of estrildid finch. Values are mean ± one standard deviation (SD). SDs for the wavelength of maximum absorptance (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. SDs for the mean λ_{max} values represent the variance of the individual records used to create the mean spectra. Visual pigment specific absorbances are not given owing to uncertainty regarding outer segment transverse pathlength (cone outer segments are often folded over on themselves or otherwise distorted). Instead, absorbance at the λ_{max} of the mean difference spectrum is given for each photoreceptor type (*UVS* ultraviolet-sensitive, *SWS* short-wavelength-sensitive, *MWS* medium-wavelength-sensitive, *LWS* long-wavelength-sensitive). λ_{max} values are wavelength of maximum absorbance (pre-bleach spectra) or absorbance change (difference spectra)

Species	Single cones					Double cones	
	Rods	UVS	SWS	MWS	LWS	Principal	Accessory
Cut-throat finch (*Amadina fasciata*)							
Mean λ_{max} of pre-bleach spectra (nm)	503.5 ± 0.7	370.3 ± 2.4	447.0 ± 1.6	500.0 ± 1.4	563.1 ± 1.4	564.2 ± 1.2	564.3 ± 2.1
λ_{max} of mean pre-bleach spectrum (nm)	503.4 ± 0.4	369.3 ± 1.4	447.7 ± 1.2	499.8 ± 1.2	562.5 ± 2.6	564.3 ± 1.5	563.8 ± 2.3
Mean λ_{max} of difference spectra (nm)	505.4 ± 1.5	364.3 ± 1.5	446.5 ± 3.4	505.3 ± 2.0	565.8 ± 1.8	564.8 ± 1.9	564.4 ± 2.4
λ_{max} of mean difference spectrum (nm)	505.2 ± 0.8	362.9 ± 4.7	447.6 ± 3.8	506.7 ± 2.7	565.3 ± 3.1	564.8 ± 1.8	564.8 ± 2.2
Absorbance at λ_{max} of mean difference spectrum	0.047	0.007	0.007	0.011	0.013	0.014	0.013
Number of outer segments measured	17	4	11	3	7	17	7
Gouldian finch (*Erythrura gouldiae*)							
Mean λ_{max} of pre-bleach spectra (nm)	502.4 ± 0.6	370.3 ± 0.8	440.3 ± 2.7	500.1 ± 2.5	562.3 ± 3.3	564.8 ± 1.6	564.8 ± 1.5
λ_{max} of mean pre-bleach spectrum (nm)	502.4 ± 0.5	370.5 ± 2.5	440.0 ± 1.6	500.0 ± 1.3	562.7 ± 2.9	564.3 ± 1.6	565.1 ± 1.7
Mean λ_{max} of difference spectra (nm)	504.8 ± 2.0	369.1 ± 1.6	439.4 ± 2.7	507.0 ± 2.3	565.3 ± 3.0	565.2 ± 2.4	565.7 ± 2.3
λ_{max} of mean difference spectrum (nm)	504.9 ± 1.0	369.7 ± 5.0	439.7 ± 4.1	507.0 ± 2.0	565.7 ± 4.3	565.1 ± 1.9	565.2 ± 2.3
Absorbance at λ_{max} of mean difference spectrum	0.035	0.007	0.007	0.010	0.011	0.016	0.012
Number of outer segments measured	7	2	8	7	4	17	5
Plum-headed finch (*Neochmia modesta*)							
Mean λ_{max} of pre-bleach spectra (nm)	503.0 ± 0.4	372.8 ± 3.3	442.0 ± 2.9	500.1 ± 1.9	565.0 ± 0.8	563.9 ± 0.9	562.0 ± 1.9
λ_{max} of mean pre-bleach spectrum (nm)	503.0 ± 0.4	372.4 ± 1.2	442.5 ± 2.1	499.8 ± 2.3	564.8 ± 3.5	563.9 ± 1.3	562.8 ± 2.2
Mean λ_{max} of difference spectra (nm)	504.4 ± 1.3	365.1 ± 7.3	440.1 ± 3.3	511.6 ± 4.7	566.1 ± 2.3	564.4 ± 1.5	562.5 ± 2.5
λ_{max} of mean difference spectrum (nm)	504.3 ± 1.0	366.6 ± 4.1	441.3 ± 4.3	510.7 ± 3.7	566.3 ± 4.7	564.5 ± 1.6	563.5 ± 3.2
Absorbance at λ_{max} of mean difference spectrum	0.045	0.005	0.007	0.008	0.014	0.017	0.012
Number of outer segments measured	5	5	6	4	2	15	2
White-headed munia (*Lonchura maja*)							
Mean λ_{max} of pre-bleach spectra (nm)	502.7 ± 0.8	373.2 ± 1.9	445.5 ± 1.7	499.5 ± 4.2	561.7 ± 2.7	564.3 ± 1.6	563.3 ± 2.4
λ_{max} of mean pre-bleach spectrum (nm)	502.7 ± 0.5	372.8 ± 2.3	446.3 ± 4.3	499.5 ± 1.8	561.6 ± 2.4	564.3 ± 1.5	562.5 ± 3.5
Mean λ_{max} of difference spectra (nm)	504.3 ± 1.5	367.6 ± 4.7	445.8 ± 4.1	507.3 ± 4.0	564.9 ± 5.0	564.4 ± 2.5	563.2 ± 2.0
λ_{max} of mean difference spectrum (nm)	504.3 ± 0.9	369.3 ± 6.5	445.6 ± 7.2	508.0 ± 4.0	565.3 ± 5.2	564.5 ± 2.1	563.7 ± 4.6
Absorbance at λ_{max} of mean difference spectrum	0.046	0.007	0.005	0.007	0.010	0.016	0.012
Number of outer segments measured	9	2	3	5	3	7	3

Table 2 Characteristics of cone photoreceptor oil droplets measured in the four species of estrildid finch. Values are mean ± one SD. SDs for the mean cut-off wavelength (λ_{cut}; defined as 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 1984a) and wavelength of half-maximum measured absorptance (λ_{mid}; Lipetz 1984a) values represent the variance of the individual records used to create the mean absorptance spectra. Avian rods do not contain oil droplets. No discrete droplet (usually termed A-type) was observed in the accessory member of the double cone pair. Spectra of P-type oil droplets measured in the dorsal retina differed noticeably from those located ventrally (see Results section for statistics). T-, C-, Y-, R- and P-type oil droplets are located in the UVS, SWS, MWS and LWS single cones and the principal member of the LWS double-cone pair respectively

Species	Single cones				Double cones		
	T-type (UVS)	C-type (SWS)	Y-type (MWS)	R-type (LWS)	P-type (principal)		A-type (accessory)
					Dorsal	Ventral	
Cut-throat finch (*Amadina fasciata*)							
Mean λ_{cut} of absorptance spectra (nm)	< 330	423.3 ± 2.0	516.3 ± 4.7	574.7 ± 1.4	420.3 ± 1.9	425.3 ± 5.7	–
λ_{cut} of mean absorptance spectrum (nm)	< 330	423.9	515.7	573.8	419.6	426.0	–
Mean λ_{mid} of absorptance spectra (nm)	< 330	439.3 ± 1.5	535.2 ± 4.6	597.6 ± 1.4	443.1 ± 2.6	453.3 ± 4.0	–
λ_{mid} of mean absorptance spectrum (nm)	< 330	439.0	535.3	597.8	443.1	453.8	–
Mean diameter (μm)	2.3 ± 0.2	2.3 ± 0.2	2.7 ± 0.3	2.8 ± 0.3	3.6 ± 0.2	3.5 ± 0.2	–
Mean maximum transverse absorptance	0.02 ± 0.02	0.56 ± 0.11	0.81 ± 0.05	0.83 ± 0.03	0.86 ± 0.02	0.87 ± 0.02	–
Number of oil droplets measured	7	12	30	30	15	15	–
Gouldian finch (*Erythrura gouldiae*)							
Mean λ_{cut} of absorptance spectra (nm)	< 330	421.7 ± 2.5	512.9 ± 3.3	571.9 ± 3.1	421.7 ± 1.2	419.1 ± 3.2	–
λ_{cut} of mean absorptance spectrum (nm)	< 330	422.5	511.9	571.6	420.6	418.6	–
Mean λ_{mid} of absorptance spectra (nm)	< 330	434.4 ± 2.6	530.6 ± 4.1	594.7 ± 3.0	439.8 ± 2.5	451.9 ± 5.1	–
λ_{mid} of mean absorptance spectrum (nm)	< 330	434.7	530.6	595.0	439.8	452.0	–
Mean diameter (μm)	2.3 ± 0.3	2.2 ± 0.3	2.7 ± 0.2	3.1 ± 0.2	3.0 ± 0.1	3.0 ± 0.0	–
Mean maximum transverse absorptance	0.01 ± 0.01	0.53 ± 0.12	0.80 ± 0.05	0.85 ± 0.02	0.82 ± 0.03	0.74 ± 0.09	–
Number of oil droplets measured	3	9	30	30	15	15	–
Plum-headed finch (*Neochmia modesta*)							
Mean λ_{cut} of absorptance spectra (nm)	< 330	415.2 ± 2.2	514.4 ± 3.9	568.1 ± 1.6	413.6 ± 1.1	420.6 ± 2.0	–
λ_{cut} of mean absorptance spectrum (nm)	< 330	415.9	514.2	567.8	413.9	420.4	–
Mean λ_{mid} of absorptance spectra (nm)	< 330	428.3 ± 1.5	534.2 ± 3.4	590.9 ± 1.7	430.4 ± 1.6	439.6 ± 3.5	–
λ_{mid} of mean absorptance spectrum (nm)	< 330	428.4	534.5	590.9	430.2	439.6	–
Mean diameter (μm)	2.0 ± 0.0	2.1 ± 0.2	2.3 ± 0.2	2.9 ± 0.2	3.0 ± 0.1	3.0 ± 0.1	–
Mean maximum transverse absorptance	0.01 ± 0.01	0.33 ± 0.07	0.75 ± 0.05	0.86 ± 0.02	0.52 ± 0.09	0.69 ± 0.07	–
Number of oil droplets measured	5	6	30	30	15	15	–
White-headed munia (*Lonchura maja*)							
Mean λ_{cut} of absorptance spectra (nm)	< 330	422.1 ± 1.1	510.1 ± 1.2	567.2 ± 3.2	418.6 ± 2.2	489.1 ± 4.4	–
λ_{cut} of mean absorptance spectrum (nm)	< 330	422.6	509.4	567.0	418.4	490.5	–
Mean λ_{mid} of absorptance spectra (nm)	< 330	434.0 ± 1.0	524.2 ± 1.4	589.2 ± 3.3	439.1 ± 4.2	505.8 ± 2.7	–
λ_{mid} of mean absorptance spectrum (nm)	< 330	434.1	524.2	589.4	439.1	506.0	–
Mean diameter (μm)	1.6 ± 0.4	2.3 ± 0.2	2.9 ± 0.2	3.2 ± 0.4	3.5 ± 0.4	3.7 ± 0.4	–
Mean maximum transverse absorptance	0.01 ± 0.01	0.62 ± 0.03	0.84 ± 0.03	0.87 ± 0.02	0.83 ± 0.06	0.86 ± 0.03	–
Number of oil droplets measured	3	4	14	21	11	12	–

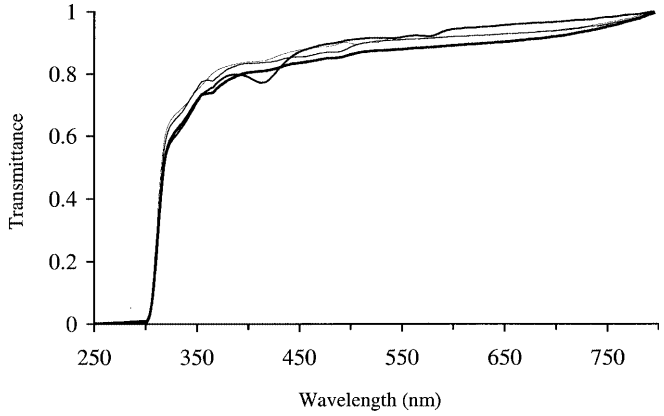


Fig. 7 Transmittance of the combined ocular media of all four species of finch. Lines, in order of increasing transmittance at 420 nm, correspond to the white-headed munia (*Lonchura maja*), the cut-throat finch (*Amadina fasciata*), the gouldian finch (*Erythrura gouldiae*), and the plum-headed finch (*Neochmia modesta*). Wavelengths of 0.5 transmittance occurred at 318 nm in the white-headed munia and cut-throat finch, 317 nm in the gouldian finch and 316 nm in the plum-headed finch

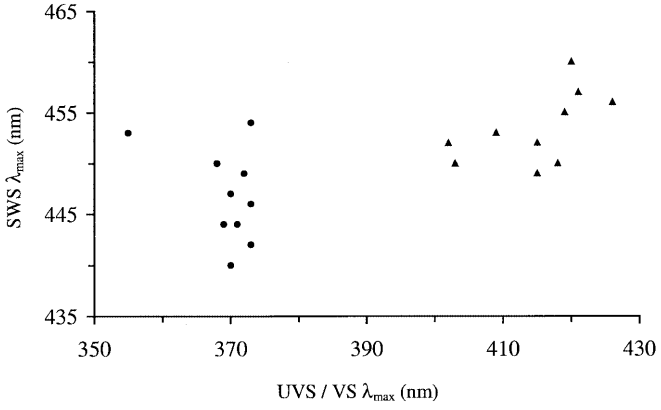


Fig. 8 Scatter plot showing UVS or VS visual pigment λ_{max} value versus SWS visual pigment λ_{max} value in each bird species for which microspectrophotometric data is available. The apparent relationship between these values was investigated by calculating the Spearman rank correlation coefficient (Minitab 12, Minitab, USA) for the whole data set (UVS and VS species combined; Spearman's $\rho = 0.632$, $P = 0.003$, $n = 20$) and both the VS species (*triangles*; Spearman's $\rho = 0.670$, $P = 0.034$, $n = 10$) and UVS species (*circles*; Spearman's $\rho = -0.154$, $P = 0.670$, $n = 10$) separately. Visual pigment data used were from the finches in this study, the starling and peacock (Hart 1998; Hart et al. 1998), blue tit and blackbird (Hart et al. 2000), canary (Das et al. 1999), budgerigar, pigeon, chicken and Manx shearwater *Puffinus puffinus* (Bowmaker et al. 1997), turkey (Hart et al. 1999), Humboldt penguin (Bowmaker and Martin 1985), Pekin robin (Maier and Bowmaker 1993), Japanese quail (Bowmaker et al. 1993) and mallard, Aylesbury and Khaki Campbell ducks (Jane and Bowmaker 1988)

grass seeds (Crome and Shields 1992). However, the budgerigar more closely resembles the four species of finch described herein, having a SWS visual pigment λ_{max} at 444 nm (Bowmaker et al. 1997), and so the λ_{max} 430 nm SWS visual pigment of the zebra finch probably cannot simply be explained as an adaptation to the light environment of central Australia.

Whilst the functional significance of a short-wavelength-shifted SWS cone visual pigment in the zebra finch retina is unclear, there is an interesting relationship between the λ_{max} value of the SWS cone visual pigment associated with the C-type oil droplet and the λ_{max} value of the cone visual pigment (either VS or UVS) associated with the T-type oil droplet (Fig. 8). It appears that birds have a SWS visual pigment λ_{max} at slightly longer wavelengths when the single cone containing a T-type oil droplets has a VS visual pigment ('VS species') than when it has a UVS one ('UVS species'). This relationship was investigated by calculating the Spearman rank correlation coefficient (Minitab 12, Minitab, USA) for all avian species in which both types of visual pigment have been measured microspectrophotometrically, with the exception of the zebra finch for which the estimate of UVS cone visual pigment λ_{max} is insufficiently accurate (Bowmaker et al. 1997). The correlation was significant when considering both the whole data set (UVS and VS species combined; Spearman's $\rho = 0.632$, $P = 0.003$, $n = 20$) and the VS species in isolation (Spearman's $\rho = 0.670$, $P = 0.034$, $n = 10$), but not when considering just the UVS species (Spearman's $\rho = -0.154$, $P = 0.670$, $n = 10$; the variation in UVS λ_{max} seen amongst the UVS species is so low that a covariation between UVS λ_{max} and SWS λ_{max} would not be expected). These results suggest that, in addition to UVS species generally having a SWS visual pigment λ_{max} at shorter wavelengths compared to VS species, among VS species the λ_{max} of the VS visual pigment is positively correlated with SWS visual pigment λ_{max} value.

Possible reasons for this correlation may be the need to minimise potentially disadvantageous excessive overlap between the adjacent UVS/VS and SWS chromatic channels (Barlow 1982) or to space receptor spectral sensitivities evenly across the visible spectrum (Vorobyev and Menzel 1999). It has also been noted previously that C-type oil droplets in VS species have their λ_{cut} at longer wavelengths than in UVS species (Bowmaker et al. 1997; Hart et al. 1998). This is presumably due to the relative concentration of carotenoid in the oil droplets as those with a λ_{cut} at longer wavelengths have a higher measured transverse absorptance (Bowmaker et al. 1997). C-type oil droplets with their λ_{cut} at longer wavelengths and long-wavelength-shifted SWS visual pigment λ_{max} values will both serve to reduce the degree to which the short-wavelength limb of the SWS cone spectral sensitivity function overlaps the long-wavelength limb of the VS cone spectral sensitivity function, increasing chromatic contrast (Vorobyev et al. 1998) and also perhaps colour constancy (Osorio et al. 1997; Dyer 1999) in this spectral region.

Why some birds use a VS visual pigment instead of a UVS one is unclear, especially when the opsin proteins responsible for both visual pigment types are thought to be derived from the same ancestral visual pigment gene (Okano et al. 1992; Wilkie et al. 1998; Yokoyama et al. 1998; Das et al. 1999). The broad similarity in avian

photoreceptor spectral sensitivities observed suggests that the visual system of diurnal birds is a generalised one that is designed to cope with a wide range of visual tasks. Nevertheless, the large variation in λ_{max} of visual pigments in the UVS/VS single-cone type suggests there is considerable interspecific variation in the utility of ultraviolet wavelengths in avian visual ecology. The two groupings tend to reflect the degree of phylogenetic relatedness between the different species indicated by Sibley and Ahlquist (1990) and Sibley and Monroe (1990). Specifically, the majority of species that possess a UVS cone visual pigment are passeriforms. The budgerigar, which also has a UVS cone visual pigment, is a member of the Psittaciformes, which are more closely related to the Passeriformes than the Galliformes (chicken, quail, peacock and turkey) and Anseriformes (duck), which possess a VS cone visual pigment instead of the UVS type. These data are most parsimoniously explained by a single evolutionary split at the divergence of the passeriform and psittaciform lineages from the galliform and anseriform lineages.

However, the Manx shearwater, Humboldt penguin (both Ciconiformes) and pigeon (Columbiformes), which have nominal VS cone visual pigments but with λ_{max} values at slightly shorter wavelengths than those found in the galliform and anseriform species studied (402 nm, 403 nm and 393 nm or 409 nm, respectively; Bowmaker and Martin 1984; Bowmaker et al. 1997; Yokoyama et al. 1998), are more closely related to the Passeriformes and Psittaciformes than the Galliformes and Anseriformes. Therefore, it is possible the lineages that gave rise to the Passeriformes and Psittaciformes underwent a further division with regard to the spectral location of the λ_{max} of this cone visual pigment type. Phylogenetic relatedness, however, is often inextricably linked to ecology, and a great deal more comparative data on the absorption properties of avian retinal photoreceptors, and the characteristics of the visual environment which have driven the spectral tuning of the ancestral opsin genes, are required before further conclusions can be made.

Ocular media

The ocular media of species which have a UVS visual pigment generally transmit more short wavelengths [wavelength of 0.5 transmittance, $\lambda T_{0.5}$ 316–318 nm for the finches in this study, 320 nm in the Pekin robin (Maier 1994), 317 nm and 343 nm in the blue tit and blackbird, respectively (Hart et al. 2000), and 338 nm in the starling (Hart et al. 1998)] than those with a VS visual pigment [$\lambda T_{0.5}$ 358 nm in the turkey (Hart et al. 1999) and 370–380 nm in ducks (Jane and Bowmaker 1988)]. Transmittance by the ocular media certainly determines the short-wavelength limit of photoreception, but it is too early to say whether the spectral characteristics of the ocular media have driven the selection of the UVS/VS visual pigment λ_{max} value or vice versa. Either way, the visual systems of species with a VS visual pigment appear to operate over a more restricted range of short wavelengths than those with a UVS one.

Double-cone oil droplets

Another similarity displayed by the four species of finch in this study is the differing spectral characteristics of P-type oil droplets, located in the principal member of the double cones, depending on whether the cells are located in the dorsal or ventral retina. This increased pigmentation of P-type oil droplets in the ventral retina has been observed in a number of other species (Goldsmith et al. 1984; Partridge 1989; Hart et al. 1998, 2000) and is probably a strategy to offset the increased short-wavelength radiance from the sky (impinging on the ventral retina) relative to that received by the dorsal retina from the ground, possibly as either some sort of brightness matching function or as a protective mechanism against potentially damaging ultraviolet wavelengths (Kirschfeld 1982). The spectral characteristics of avian cone oil droplets (but not visual pigments) are known to be affected by deficiencies in dietary carotenoid (Wallman 1979; Bowmaker et al. 1993) and lengthy captivity (Hart et al. 1998). As the severity of these effects might be species specific, further investigation will be required to assess whether the marked difference between dorsal and ventral P-type spectra seen in the white-headed munias relative to the other finches is of functional significance.

In conclusion, although the photoreceptor spectral characteristics of the relatively few birds studied to date fall in to broadly similar categories, we only have a superficial picture and there is obviously much more to be discovered about the subtle inter-specific adaptations displayed by species with different visual requirements.

Acknowledgements This research was supported by the BBSRC (Research Grant Number 7/S05042 to ICC and JCP). The authors would like to thank Sadie Iles for assistance with bird care. 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

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

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

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 (1984) Colour vision in the penguin, *Spheniscus humboldti*: a microspectrophotometric study. Vision Res 24: 1702

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

Bowmaker JK, Astell S, Hunt DM, Mollon JD (1991) Photosensitive and photostable pigments in the retinae of old world monkeys. J Exp Biol 156: 1–19

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

Crome F, Shields J (1992) Parrots and pigeons of Australia. Collins, Angus and Robertson, London

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

Dyer AG (1999) Broad spectral sensitivities in the honeybee's photoreceptors limit colour constancy. J Comp Physiol A 185: 445–453

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

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

Goodwin D (1982) Estrildid finches of the world. Oxford University Press, Oxford

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 (*Sturnus 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, Bennett ATD (2000) Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). J Comp Physiol A 186: 375–387

Immelmann K (1977) Australian finches in bush and aviary. Angus and Robertson, London

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

Kirschfeld K (1982) Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. Proc R Soc Lond Ser B 216: 71–85

Knowles A, Dartnall HJA (1977) The photobiology of vision. Academic Press, New York

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

Liebman PA, Granda AM (1975) Super dense carotenoid spectra resolved in single cone oil droplets. Nature (Lond) 253: 370–372

Lipetz LE (1984a) 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

Lipetz LE (1984b) Pigment types, densities and concentrations in cone oil droplets of *Emydoidea blandingii*. Vision Res 24: 605–612

Maier EJ (1994) Ultraviolet vision in a passeriform bird: from receptor spectral sensitivity to overall spectral sensitivity in *Leothrix lutea*. Vision Res 34: 1415–1418

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

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

Osorio D, Marshall NJ, Cronin TW (1997) Stomatopod photoreceptor spectral tuning as an adaptation for colour constancy in water. Vision Res 37: 3299–3309

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

Sibley CG, Ahlquist JE (1990) Phylogeny and classification of birds: a study in molecular evolution. Yale University Press, New Haven

Sibley CG, Monroe BL Jr (1990) Distribution and taxonomy of birds of the world. Yale University Press, New Haven

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

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

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, Bennett ATD, Marshall NJ, Cuthill IC (1998) Tetrachromacy, oil droplets and bird plumage colours. J Comp Physiol A 183: 621–633

Wallman J (1979) Role of the retinal oil droplets in the colour vision of japanese quail. In: Granda AM, Maxwell JH (eds) Neural mechanisms of behaviour in the pigeon. Plenum Press, New York, pp 327–351

Wilkie SE, Vissers PMAM, Das D, DeGrip WJ, Bowmaker JK, Hunt DM (1998) The molecular basis for UV vision in birds: spectral characteristics, cDNA sequence and retinal localization of the UV-sensitive visual pigment of the budgerigar (*Melopsittacus undulatus*). Biochem J 330: 541–547

Yokoyama S, Radlwimmer FB, Kawamura S (1998) Regeneration of ultraviolet pigments of vertebrates. FEBS Lett 423: 155–158

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