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Visual Pigments and Oil Droplets from Six Classes of Photoreceptor in the Retinas of Birds

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Microspectrophotometric examination of the retinal photoreceptors of the budgerigar (shell parakeet), *Melopsittacus undulatus* (Psittaciformes) and the zebra finch, *Taeniopygia guttata* (Passeriformes), demonstrate the presence of four, spectrally distinct classes of single cone that contain visual pigments absorbing maximally at about 565, 507, 430–445 and 360–380 nm. The three longer-wave cone classes contain coloured oil droplets acting as long pass filters with cut-offs at about 570, 500–520 and 445 nm, respectively, whereas the ultraviolet-sensitive cones contain a transparent droplet. The two species possess double cones in which both members contain the long-wave-sensitive visual pigment, but only the principal member contains an oil droplet, with cut-off at about 420 nm. A survey of the cones of the pigeon, *Columba livia* (Columbiformes), confirms the presence of the three longer-wave classes of single cone, but also reveals the presence of a fourth class containing a visual pigment with maximum absorbance at about 409 nm, combined with a transparent droplet. No evidence was found for a fifth, ultraviolet-sensitive receptor. In the chicken, *Gallus gallus* (Galliformes), the cone class with a transparent droplet contains "chicken violet" with maximum absorbance at about 418 nm. The rods of all four species contain visual pigments that are spectrally similar, with maximum absorbance between about 506 and 509 nm. Noticeably, in any given species, the maximum absorbance of the rods is spectrally very similar to the maximum absorbance of the middle-wavelength-sensitive cone pigments. © 1997 Elsevier Science Ltd.

Visual pigment Cone Oil droplet Bird Colour vision

INTRODUCTION

Diurnal neognathus birds probably have, at least at the retinal level, one of the most elaborate mechanisms for colour vision within the vertebrates. The retinas of these avian species contain a complex complement of photoreceptors, rods, double cones and at least four classes of single cone. The cones are characterised by brightly coloured oil droplets, a feature restricted to birds and some reptiles (for a review, see Bowmaker, 1991). The droplets are located in the distal ellipsoid region of the inner segment and act as selective cut-off (long pass) filters interposed between the incident light and the visual pigment (Bowmaker & Knowles, 1977; Goldsmith *et al.*, 1984; Partridge, 1989). The four classes of single cone are spectrally distinct with maximum sensitivities extending from the near ultraviolet, close to 360 nm, to the red, close to 600 nm and are thought to subserve tetrachromatic colour vision (Goldsmith, 1991; Maier & Bowmaker, 1993).

Although such general statements can be made concerning avian colour vision, in fact few species have been studied in any detail. In the chicken, *Gallus gallus*, four cone visual pigments with wavelengths of maximum absorbance (λ_{max}) at about 415, 460, 505 and 562 nm, have been identified by visual pigment extraction techniques (Fager & Fager, 1981; Yen & Fager, 1984; Yoshizawa & Fukada, 1993) and electroretinography (Govardovskii & Zueva, 1977) and the two long-wave pigments located to specific cone classes by microspectrophotometry (Bowmaker & Knowles, 1977). Recently, the genes encoding these four cone visual pigments and the rod pigment have been isolated, sequenced and expressed (Takao *et al.*, 1988; Kuwata *et al.*, 1990; Okano *et al.*, 1992; Wang *et al.*, 1992), confirming their presence in the retina and suggesting a close evolutionary relationship between the spectrally similar rod pigment and the middle-wave-sensitive (MWS) cone pigment.

More detailed microspectrophotometric analysis of the photoreceptors of the closely related Japanese quail, *Coturnix coturnix japonica*, another member of the *Galliformes* (Bowmaker *et al.*, 1993), has established

CLASSIFICATION:

Aves:	Psittaciformes:	*Melopsittacus undulatus*
	Passeriformes:	*Taeniopygia guttata*
	Columbiformes:	*Columba livia*
	Galliformes:	*Gallus gallus*

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the location of the four cone pigments within distinct cone classes. The pattern identified in the quail appears to be common amongst diurnal avian species and probably can be used as a model for other species. The long-wave-sensitive (LWS) pigment, with λ_{max} 567 nm (P567), dominates the retina and is found in both members of the double cones and in a class of single cone containing a red (R-type) oil droplet. The filtering effect of the R-type droplet, with a cut-off at about 570 nm, narrows the spectral sensitivity of the single cone class by removing short wavelengths, and displaces the maximum sensitivity of the cell to longer wavelengths above 600 nm. In contrast, the oil droplet (P-type) of the principal member of the double cones has a cut-off at much shorter wavelengths so that the spectral sensitivity of the double cones is broad with a maximum close to 570 nm. The three remaining classes of single cone are identified as green-sensitive with a P505 associated with a yellow (Y-type) droplet with a cut-off at about 510 nm, blue-sensitive with a P460 associated with a clear or colourless (C-type) droplet cutting off at about 450 nm, and violet-sensitive with a P420 associated with a transparent (T-type) droplet that exhibits no significant absorbance throughout the spectrum.

The role of the four classes of single cone in colour vision is supported by the behaviourally determined increment spectral sensitivity function of the passeriform bird, the Pekin robin or red-billed Leiothrix, *Leiothrix lutea* (Maier, 1992). *Leiothrix* shows high sensitivity in the near ultraviolet with the sensitivity function exhibiting four marked peaks with maxima at about 370, 460, 520 and 620 nm. These sensitivity maxima closely match spectral sensitivity functions for the four classes of single cone derived from microspectrophotometric analysis of their visual pigments and oil droplets (Maier & Bowmaker, 1993). As was inferred from the behavioural data and confirmed by microspectrophotometry, the fourth class of single cone in *Leiothrix* contains an ultraviolet-sensitive visual pigment with λ_{max} at about 355 nm and not at about 420 nm, as in the two galliform species. In addition to species possessing a fourth cone visual pigment with λ_{max} at either 420 or 360 nm, other species such as the Humboldt penguin, *Spheniscus humboldti* (Bowmaker & Martin, 1985) and Manx shearwater, *Puffinus puffinus* (Bowmaker, unpublished) have their shortest-wave pigment with λ_{max} close to 400 nm.

Ultraviolet sensitivity is well established in avian species and has been most fully demonstrated in the pigeon, *Columba livia*, both behaviourally (Wright, 1972; Romeskie & Yager, 1976; Kreithen & Eisner, 1978; Emmerton & Delius, 1980; Emmerton, 1983; Emmerton & Remy, 1983; Remy & Emmerton, 1989) and electrophysiologically (Blough, 1957; Graf & Norren, 1974; Norren, 1975; Chen *et al.*, 1984; Wortel *et al.*, 1984; Chen & Goldsmith, 1986; Vos Hzn *et al.*, 1994), but has also been shown in three species of hummingbird (Goldsmith, 1980; Goldsmith *et al.*, 1981), 13 species of passerines (Chen *et al.*, 1984; Chen & Goldsmith, 1986), the Pekin robin (Maier, 1992), the kestrel, *Falco tinnunculus* (Viitala *et al.*, 1995) and the zebra finch, *Taeniopygia guttata* (Bennett *et al.*, 1996).

Although four spectrally distinct classes of single cone have been demonstrated in a number of birds, with ultraviolet sensitivity subserved by a class of cones with λ_{max} at either 360–380 nm or 400–420 nm, the possibility that the pigeon has five classes of single cone, with two classes maximally sensitive in the violet-ultraviolet range, cannot be ruled out. Early microspectrophotometric analysis of pigeon photoreceptors (Bowmaker, 1977) identified only three cone pigments with λ_{max} at about 460, 515 and 567 nm, but accumulating evidence from behaviourally determined wavelength discrimination functions (Wright, 1979; Emmerton & Delius, 1980), and from electrophysiological studies (Graf & Norren, 1974; Norren, 1975; Romeskie & Yager, 1976; Wortel *et al.*, 1984; Vos Hzn *et al.*, 1994) indicate that two additional spectrally distinct cone mechanisms may be present with maxima at about 410 and 365 nm. If this is the case, then the pigeon has the potential for pentachromatic colour vision and it would be difficult to conceive the pigeon as the only avian species so endowed.

The complement of cones within avian retinas raises a number of questions: (i) Is the presence of four spectrally distinct cone pigments common to other avian groups? (ii) What are the spectral locations utilized by avian cone pigments at short wavelengths between 350 and 450 nm? (iii) Since most birds appear to possess only two classes of short-wave-sensitive cones, are only specific pairs found? (iv) Does the pigeon possess three short-wave cone mechanisms and, if so, is it unique in this feature? (v) Since the MWS cone pigment and the rod pigment are evolutionarily and spectrally close, how similar are the λ_{max} of the two pigments in any given species.

In this paper we have addressed these questions with a detailed microspectrophotometric analysis of two additional avian species, the zebra finch, *Taeniopygia guttata*, a passeriform, and the budgerigar or shell parakeet, *Melopsittacus undulatus*, a member of the *Psittaciformes* for which no previous data are available. In addition, we have re-examined the retinal photoreceptors of both pigeon and chicken, since it is from these two species that most of the published behavioural, physiological and molecular data have been derived.

METHODS

The absorbance spectra of individual photoreceptors were measured with a modified dual-beam Liebman microspectrophotometer under computer control (Liebman & Entine, 1964; Knowles & Dartnall, 1977; Mollon *et al.*, 1984; Bowmaker *et al.*, 1991a). With the help of an infrared converter, the measuring beam (normally 2 μm square cross-section) was aligned to pass transversely through a given structure, either an outer segment or an oil droplet, while the reference beam passed through a clear space adjacent to the photoreceptor. In all cases, measurements were made only from morphologically distinct intact cones where both beams could be positioned so that neither beam overlapped with any

TABLE 1. λ_{max} of visual pigments from four avian species

	Melopsittacus undulattus	*Taeniopygia guttata*	*Columba livia*	*Gallus gallus*
LWS (red) cones				
λ_{max} of mean spectrum, nm	564.0 ± 2.6	567.2 ± 2.7	567.3 ± 2.9	569.8 ± 2.7
λ_{max} of difference spectrum, nm	563.2 ± 3.3	569.0 ± 3.6	569.6 ± 4.5	568.9 ± 4.1
mean of λ_{max}, nm	565.9 ± 6.5	566.3 ± 4.5	566.3 ± 5.2	570.9 ± 5.0
maximum absorbance	0.010/0.007*	0.013/0.009	0.012/0.009	0.014/0.011
number of cells	19	24	14	6
MWS (green) cones				
λ_{max} of mean spectrum, nm	509.3 ± 4.9	502.8 ± 2.8	507.1 ± 1.5	507.8 ± 1.5
λ_{max} of difference spectrum, nm	506.9 ± 4.7	508.5 ± 4.3	506.7 ± 4.0	507.2 ± 2.7
mean of λ_{max}, nm	499.1 ± 12.0	504.7 ± 9.1	506.0 ± 2.5	507.1 ± 5.3
maximum absorbance	0.012/0.010	0.010/0.006	0.023/0.016	0.018/0.012
number of cells	19	17	7	11
SWS (blue) cones				
λ_{max} of mean spectrum, nm	444.3 ± 4.0	429.5 ± 5.2	452.7 ± 4.6	455.2 ± 3.1
λ_{max} of difference spectrum, nm	443.0 ± 6.7	429.8 ± 6.0	463.5 ± 6.7	455.2 ± 4.8
mean of λ_{max}, nm	439.5 ± 8.0	427.0 ± 6.5	452.2 ± 5.6	452.6 ± 3.8
maximum absorbance	0.008/0.006	0.008/0.007	0.014/0.007	0.012/0.009
number of cells	16	13	7	4
UVS (uv or violet) cones				
λ_{max} of mean spectrum, nm	370.9 ± 5.2	*ca 360–380*†	408.8 ± 6.7	418.6 ± 2.5
λ_{max} of difference spectrum, nm	—	—	—	418.0 ± 9.3
mean of λ_{max}, nm	370.7 ± 3.1		403.8 ± 10.2	417.5 ± 5.1
maximum absorbance	<0.008	0.009/0.004	0.006/0.003	0.009/0.005
number of cells	5	8	6	4
Rods				
λ_{max} of mean spectrum, nm	508.7 ± 0.6	507.0 ± 0.5	505.5 ± 0.6	505.5 ± 1.2
λ_{max} of difference spectrum, nm				507.5 ± 1.7
mean of λ_{max}, nm	508.6 ± 1.4	506.7 ± 5.3	505.3 ± 1.3	505.7 ± 2.1
maximum absorbance	0.045	0.033	0.040	0.029/0.028
number of cells	16	24	16	14

*The two values represent the maximum absorbance of the absorbance spectrum and that derived from the difference spectrum.
†The UV data for *Taeniopygia* are limited and can only be used as indicative of a pigment with λ_{max} in the region of 360–380 nm.
—, difference spectrum has too low density to determine λ_{max}.

other object. The dichroism of visual pigment molecules was exploited by polarizing the beams so that the e-vector of the beams was perpendicular to the long axis of the outer segment. Spectra were scanned from 750 to 350 nm in 2-nm steps and back from 351 to 749 nm at the interleaved wavelengths. To minimise the effects of bleaching, only one absorbance spectrum was usually obtained from a given outer segment, but two independent estimates of the baseline absorbance spectrum were obtained by arranging both beams to pass outside the cell. In the case of violet- and ultraviolet-sensitive cones, where bleaching is less of a problem, up to three pairs of records were obtained for each outer segment and the mean of these used for analysis.

Analysis of visual pigment spectra

A standardised computer programme was employed to estimate the wavelength of maximum absorbance (λ_{max}) of each outer segment. First the two spectra from a cell were averaged and then the absorbance values at pairs of adjacent wavelengths were averaged to obtain a mean curve from the outward and return records. Each of the 20 absorbance values on the long wavelength limb of the curve (corresponding to a 40-nm segment and to absorbances in the range of approximately 45–90% of the maximum of the cell) was then referred to a standard template curve in order to obtain an estimate of λ_{max}. This operation amounts to finding the spectral location of a standard curve that gives the per cent absorbance value under consideration. A second estimate of λ_{max} was obtained from the top of the absorbance curve by fitting each of 50 consecutive absorbance points, centred on the highest point, to the template curve and averaging the resulting estimates. The template curve used in the analysis was the Dartnall standard curve for rhodopsin (Knowles & Dartnall, 1977; p. 76) placed with its λ_{max} at 502 nm and expressed on an abscissal scale of log frequency, since absorbance curves of visual pigments have almost the same shape when expressed on such an abscissa (Mansfield, 1985; Bowmaker *et al.*, 1991a). This relationship breaks down at short wavelengths (Hárosi, 1994) and the template used for the UVS pigments is based on that of Hárosi (1994) with a half-bandwidth of about 5000 cm^{-1}. Absorbance spectra were obtained both before and after exposure to white light from all putative outer segments in order to confirm the presence of a visual (photosensitive) pigment. Bleaching was achieved by passing white light from the monochromator

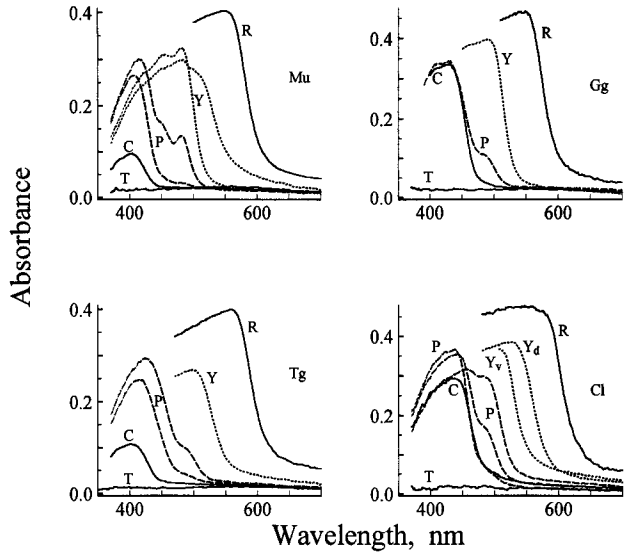

FIGURE 1. Mean absorbance spectra of cone oil droplets from four avian species. Mu, *Melopsittacus undulatus*; Tg, *Taeniopygia guttata*; Gg, *Gallus gallus*; Cl, *Columba livia*. Letters indicate droplet type. **R**ed, **Y**ellow, **C**lear and **T**ransparent are all found in single cones, whereas the **P**ale droplets are located in the Principal member of double cones. Note the variability of the P-type droplets. In *Columba livia*, the Y-type droplets differ between the dorsal (Y_d) and ventral (Y_v) retinas.

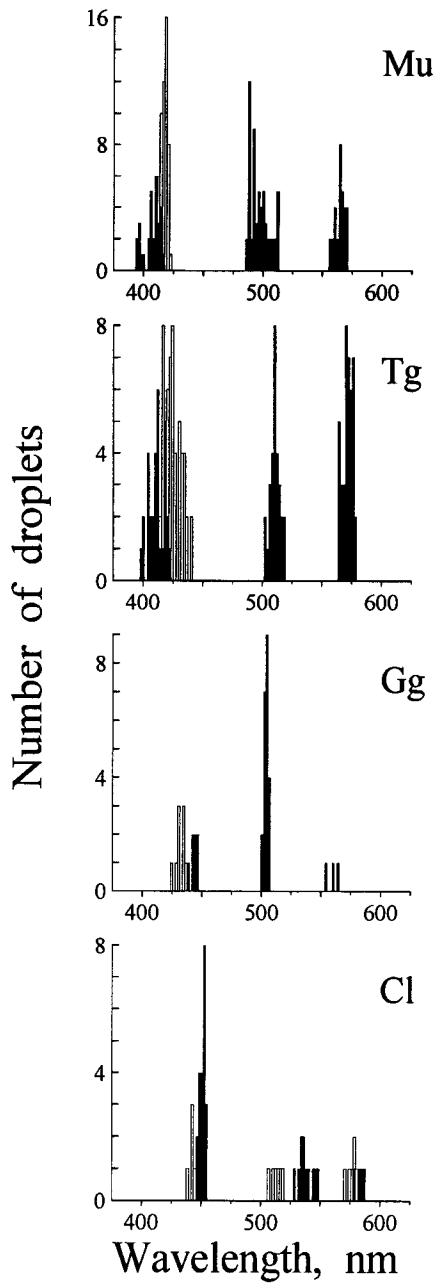

FIGURE 2. Distribution histograms of the cut-off wavelengths (λ_{cut}) of individual oil droplets from four avian species. Mu, *Melopsittacus undulatus*; Tg, *Taeniopygia guttata*; Gg, *Gallus gallus*; Cl, *Columba livia*. The solid histograms are for R-type (λ_{cut} around 570 nm), Y-type (λ_{cut} around 510 nm) and C-type (λ_{cut} around 400-450 nm). The open histograms with λ_{cut} below 450 nm are for P-type droplets. The open histograms with λ_{cut} above 500 nm in *Columba livia* (bottom panel) are for Y-type and R-type droplets from the ventral retina, whereas the solid histograms are for the equivalent droplets in the dorsal retina.

through the measuring beams of the microspectrophotometer for about 2 min in the case of LWS pigments, but up to 10 min for UVS pigments.

Normally only records that passed rigid selection criteria were used for detailed analysis, the criteria for LWS and MWS cones being (i) a transverse density at the λ_{max} greater than 0.01; (ii) a standard deviation from the right-hand limb estimate of λ_{max} of less than 12 nm; and (iii) the difference between the two estimates of λ_{max} less than 6 nm. For SWS and UVS cones, because of their smaller size and rarity, the criteria had to be relaxed. In the case of SWS cones, all records from putative outer segments that showed convincing evidence of photosensitivity (bleaching) were included, whereas all records from putative UVS cone outer segments that were clearly attached to inner segments containing a transparent droplet were included in the analysis, whether or not unequivocal evidence of photosensitivity was obtained. In contrast, for rods, because of their relatively large diameter, more stringent criteria could be applied: a standard deviation from the right-hand limb estimate of λ_{max} of less than 2 nm and the difference between the two estimates of λ_{max} of less than 2 nm.

The absorbance spectra from all cells of a given class that passed the criteria were averaged together to produce the mean spectra shown in Figs 4–6, and it was from these averaged data that the "λ_{max} of the mean spectrum" (Table 1) was obtained. The "mean of the λ_{max}" (Table 1) is the mean of the λ_{max} determined from the records of individual cells.

Analysis of oil droplet spectra

The oil droplets in avian and reptilian cones have diameters of approx. 2–4 μm and may contain high concentrations of carotenoids (Liebman & Granda, 1975; Goldsmith *et al.*, 1984). As a consequence, light leakage around the droplet becomes significant during spectral measurements and accounts for the "flat-topped" spectra, especially of the R and Y types shown in Fig. 1. Essentially, the droplets act as cut-off filters and were classified according to their "cut-off" wavelength (λ_{cut}) as calculated by the method suggested by Lipetz (1984).

RESULTS

Photoreceptor classes and oil droplets

The different classes of cones can be most easily distinguished by their respective oil droplets which have, in most cases, distinct absorbance spectra (Fig. 1). The R-type droplets of LWS single cones have λ_{cut} at about 560–580 nm, whereas the Y-type droplets of MWS single cones have λ_{cut} between about 500 and 540 nm (Fig. 2). In the pigeon two distinct forms of Y-type droplet could be identified: the dorsal retina contains droplets with λ_{cut} at about 539 nm, whereas the ventral retina contains droplets with λ_{cut} at about 513 nm (Fig. 1). There is also an indication that the R-type droplets have a cut-off at longer wavelengths in the dorsal retina than in the ventral retina (Fig. 2). Two varieties of Y-type droplet were also identified in the budgerigar (Fig. 1), but there was no clear distinction between dorsal and ventral retina. T-type droplets were present in all species, but they are rare and although no systematic count of the different types of droplet was attempted, they probably account for less than 10% of the total oil droplet complement.

Although the R-, Y- and T-type droplets are easily distinguished microspectrophotometrically, some problems arise in separating the C-type droplets of single cones from the P-type droplets of the double cones. In the budgerigar and zebra finch the C-type droplets appear to have relatively low densities (about 0.1), but it is not clear whether these values represent true low concentrations of carotenoid or are simply the result of excessive light scattering around the droplets. The values for λ_{cut} ranging from about 410–450 nm (Fig. 2) assume that the droplets

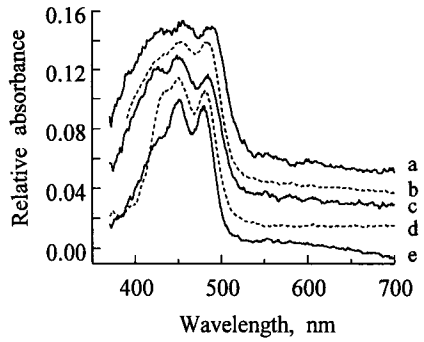

FIGURE 3. Mean absorbance spectra of carotenoids from the accessory member of double cones. The spectra have been arbitrarily shifted in the vertical axis for clarity, but all have a maximum absorbance of about 0.1. a, *Columba livia*; b, *Gallus gallus*; c, *Melopsittacus undulatus*; d, difference spectrum between the two forms of the P-type droplets (Fig. 1) in *Melopsittacus undulatus*; e, carotenoid spectrum from droplets in small unidentified photoreceptors in *Melopsittacus undulatus*.

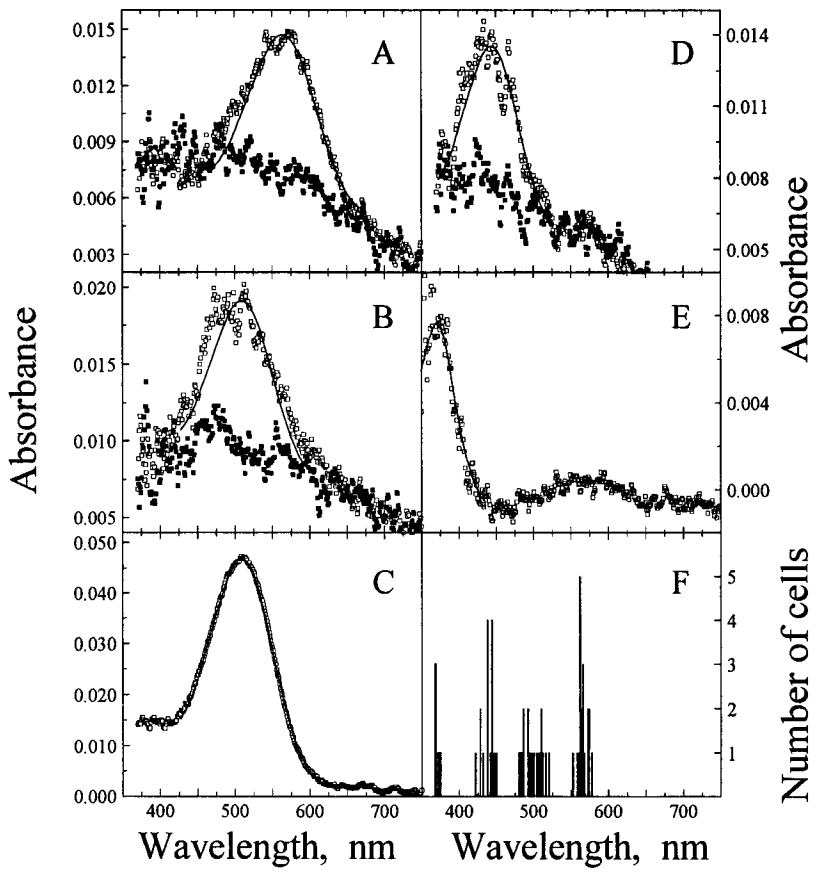

FIGURE 4. Mean absorbance spectra of visual pigments from *Melopsittacus undulatus*. Open symbols, before bleaching; filled symbols, after exposure to white light. (A) LWS cone (from both LWS single cones and from double cones); (B) MWS single cones; (C) rods; (D) SWS single cones; (E) UVS single cones; (F) distribution histograms of the individual λ_{max} from all cones. The solid lines in (A)–(E) are visual pigment template curves with λ_{max} at 564, 509, 509, 444 and 371 nm, respectively (see Methods).

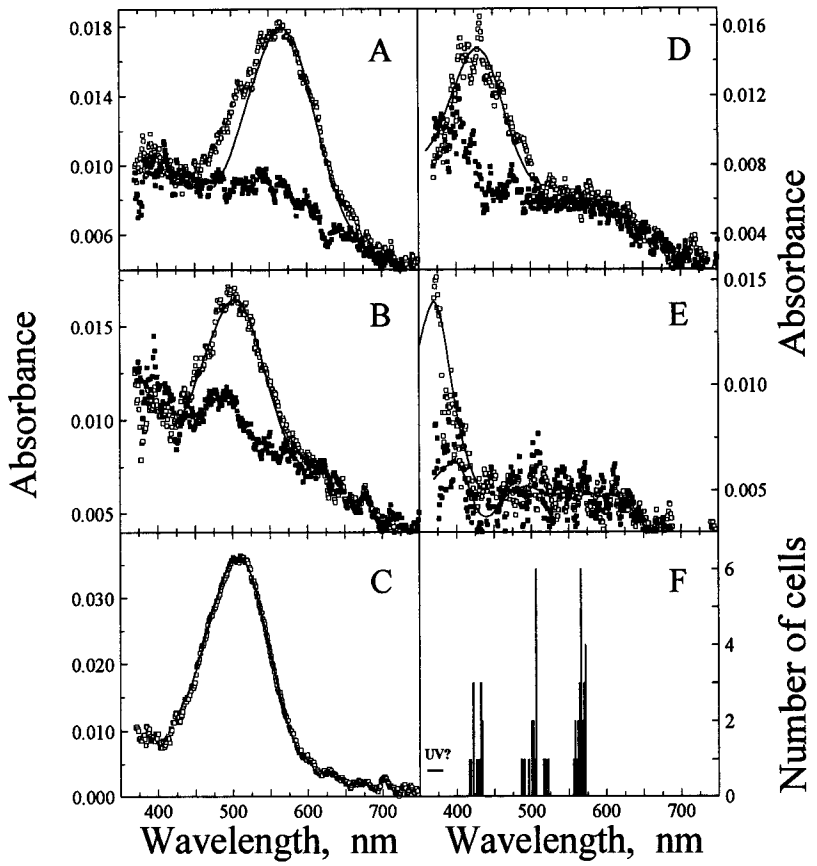

FIGURE 5. Mean absorbance spectra of visual pigments from *Taeniopygia guttata*. Open symbols, before bleaching; filled symbols, after exposure to white light. (A) LWS cone (from both LWS single cones and from double cones); (B) MWS single cones; (C) rods; (D) SWS single cones; (E) UVS single cones; (F) distribution histograms of the individual λ_{max} from all cones. The solid lines in (A)–(E) are visual pigment template curves with λ_{max} at 567, 504, 507, 430 and 370 nm, respectively (see Methods). The bar in (F) at wavelengths less than 380 nm indicates that UVS cones with λ_{max} below 380 nm were present: the data are limited and can only be viewed as indicative of a pigment with λ_{max} in the region of 360–380 nm.

do indeed act as cut-off filters, but if this is not the case, their spectral locations give a comparative indication of the λ_{max} of the droplets. P-type droplets also have λ_{cut} at similar wavelengths (Fig. 2), but, in general, differ from the C-type in having a higher absorbance and often an additional "shoulder" in their absorbance spectra at about 480 nm (Fig. 1). The height of the 480-nm shoulder varies from cone to cone across the retina and between individual birds and cannot easily be related to retinal location. The P-type droplets are located in the principal member of the double cones, but in many instances the two members of the double cone become separated in the samples prepared for microspectrophotometry. The identification of a droplet with relatively low absorbance and no 480-nm shoulder as a P-type as distinct from a C-type can then become problematic.

The accessory member of the double cones rarely displays an oil droplet, though in the chicken a minute droplet can sometimes be distinguished. However, the distal region (ellipsoid) of the inner segment where oil droplets are normally located contains a carotenoid with a characteristic triple peaked absorbance spectrum (Fig. 3). In the P-type droplets of the principal member of the double cones, the presence of the 480-nm absorbance is most marked in the budgerigar (Fig. 1) where in some droplets it forms a distinct absorbance peak. The variation in the absorbance spectra of the P-type droplets clearly indicates that these droplets contain mixtures of carotenoids and, in the budgerigar, the difference spectrum obtained by subtracting the two P-type spectra shown in Fig. 1 reveals a typical triple peaked carotenoid spectrum (Fig. 3, curve d) similar to that of the carotenoid in the accessory member of the double cones (Fig. 3, curve c). A further type of small droplet was also distinguished in the budgerigar, whose spectrum appears distinct from that of the accessory droplet (Fig. 3, curve e). The spectrum had two distinct "twin" peaks at about 480 and 450 nm with only a small short-wave shoulder at about 430 nm. The outer segments of these cones were never identified and it is not clear whether they simply constitute a variety of accessory droplet or form an additional class of single cone.

Visual pigments

In all the four species, a rod and four spectrally distinct classes of cone visual pigment were identified. The mean absorbance spectra of the visual pigments from *Melopsittacus undulatus*, *Taeniopygia guttata* and *Columba livia* are shown in Figs 4–6. Similar data were obtained for the chicken. The distributions of the λ_{max} of the

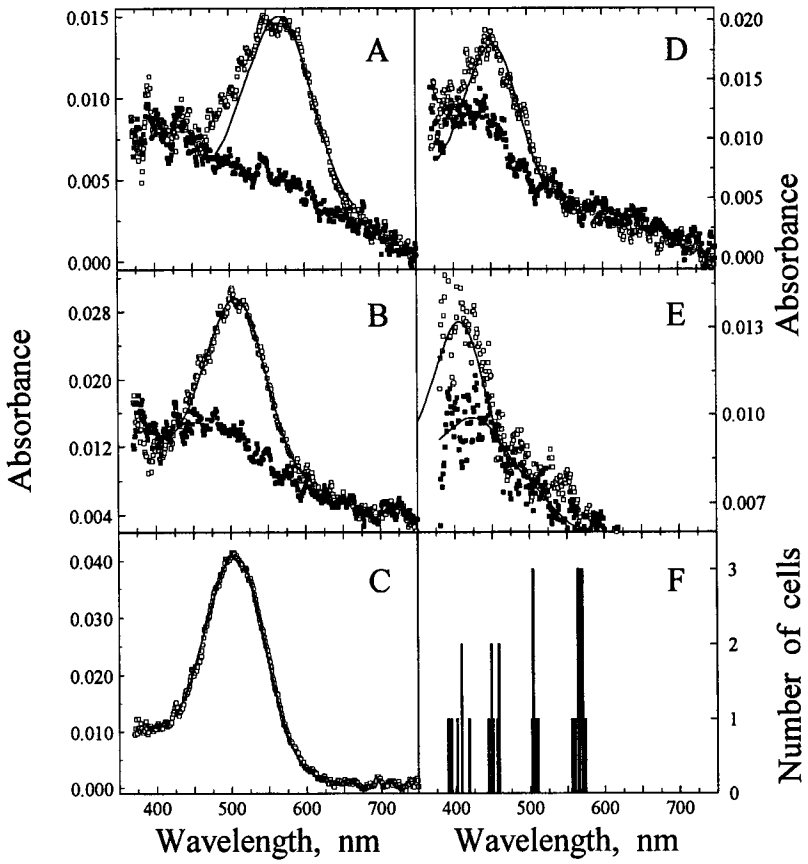

FIGURE 6. Mean absorbance spectra of visual pigments from *Columba livia*. Open symbols, before bleaching; filled symbols, after exposure to white light. (A) LWS cone (from both LWS single cones and from double cones); (B) MWS single cones; (C) rods; (D) SWS single cones; (E) violet-sensitive single cones; (F) distribution histograms of the individual λ_{max} from all cones. The solid lines in (A)–(E) are visual pigment template curves with λ_{max} at 567, 507, 506, 453 and 409 nm, respectively.

individual cones used in the analysis from the three species are shown in the bottom right-hand panels of Figs 4–6. Full details of the λ_{max} and transverse densities of the outer segments are given in Table 1. The LWS and MWS cone pigments have similar λ_{max} across the four species at about 565–570 nm and 503–509 nm, respectively. The SWS cones again have similar λ_{max}: at 430 nm in *Taeniopygia guttata*, 444 nm in *Melopsittacus undulatus* and about 455 nm in *Columba livia* and *Gallus gallus*. The fourth cone pigment has λ_{max} below 420 nm, at about 409 and 418 nm in *Columba livia* and *Gallus gallus*, respectively, but at shorter wavelengths in the near ultraviolet in *Melopsittacus undulatus* and *Taeniopygia guttata* (Table 1). It must be emphasised that in these two species, the data obtained from the outer segments of clearly identified intact cones with T-type droplets can only indicate the presence of a visual pigment absorbing maximally between about 360 and 380 nm, since spectra are limited to 350 nm. In *Melopscittacus undulatus* a clear peak could be distinguished in the data from each of five cells so that the λ_{max} of each cell could be calculated from the right-hand limb, giving a mean λ_{max} of approx. 371 nm (Table 1 and Fig. 4). However, in *Taeniopygia guttata*, the data are insufficient to determine the λ_{max} precisely within the range of 360–380 nm.

The four classes of single cone follow a logical pattern in their combinations of oil droplets and visual pigments; the LWS pigment combines with the R-type droplet, the MWS pigment with the Y-type droplet, the SWS pigment with the C-type droplet and the UVS pigment with the T-type droplet. The double cones contain the LWS visual pigment in both members, with the principal member containing the P-type droplet and the accessory member lacking a clearly defined droplet.

The rods contain visual pigments that are spectrally similar, with λ_{max} between approx. 505 and 509 nm. Noticeably, in any given species, the λ_{max} of the rods are spectrally very similar to the λ_{max} of the MWS cone pigments (Table 1).

DISCUSSION

The complement of photoreceptor classes within the retina of diurnal birds appears to be highly consistent across species. The retinas are cone dominated, with the majority of cells consisting of double cones (about 50–60% in *Galliformes* and *Columbiformes*, e.g., Bowmaker, 1977; Bowmaker & Knowles, 1977; Oishi *et al.*, 1990; Bowmaker *et al.*, 1993) in which the two members are morphologically distinct, but nevertheless contain the same LWS visual pigment with λ_{max} close to 565–

TABLE 2. Summary of avian visual pigments, λ_{max} (nm) determined from microspectrophotometry

	UV	Violet	Blue	Green	Red	Rod
Sphenisciformes:						
Humboldt penguin (*Spheniscus humboldti*)	—	403	450	543		504
Procellariformes:						
Manx shearwater (*Puffinus puffinus*)	—	402	452	P	P	505
Anseriformes:						
Mallard duck (*Anas platyrhynchos*)	—	420	452	502	570	505
Psittaciformes:						
budgerigar (*Melopsittacus undulatus*)	371	—	444	508	564	509
Galliformes:						
domestic chicken (*Gallus gallus*)	—	418	455	507	569	506
Japanese quail (*Coturnix japonica*)	—	419	456	505	569	505
Columbiformes:						
pigeon (*Columba livia*)	(366)	409 (410)	453	507	568	506
Strigiformes:						
tawny owl (*Strix aluco*)	—	—	463	503	555	503
Passeriformes:						
Pekin robin (*Leiothrix lutea*)	355	—	453	501	567	500
zebra finch (*Taeniopygia guttata*)	*ca 360–380*	—	430	506	568	507

P = known to be present, but λ_{max} not determined. () = ERG measurements from Vos Hzn *et al.* (1994). Ultraviolet data for *Taeniopygia* are limited and can only be used as indicative of a pigment with λ_{max} in the region of 360–380 nm. Data for *Spheniscus humboldti* (Bowmaker & Martin, 1985); *Puffinus puffinus* (Bowmaker, unpublished); *Anas platyrhynchos* (Jane & Bowmaker, 1988); *Coturnix japonica* (Bowmaker *et al.*, 1993); *Strix aluco* (Bowmaker & Martin, 1978); *Leiothrix lutea* (Maier & Bowmaker, 1993).

570 nm (Table 2). In addition, there are at least four spectrally distinct classes of single cones. Three classes appear to be highly conserved containing visual pigments with λ_{max} close to 565–570, 500–510 and 430–460 nm, but the fourth class may contain either a violet- or ultraviolet-sensitive pigment with λ_{max} ranging from about 420 to 360 nm (Table 2). The LWS and MWS cones occur in about equal numbers comprising a further 30–40% of the cones, whereas the SWS and UVS cones are rare and together comprise the remaining 10% of cones.

The budgerigar, *Melopsittacus undulatus*, and the zebra finch, *Taeniopygia guttata*, whose visual pigments have not previously been reported, are members of two widely different avian families, Psittaciformes and Passeriformes, respectively. Nevertheless, they both exhibit similar patterns of photoreceptors, though the budgerigar retina contains a higher number of rods than the zebra finch. Although no systematic count of the ratio of rods to cones was made, it was clear from observation of numerous retinal preparations for microspectrophotometry that the rod outer segments in the budgerigar were both larger and more common than in the zebra finch. This higher proportion of rods may reflect the more crepuscular behaviour of budgerigars which tend to be active in the cooler dawn and dusk rather than in the heat of the day.

Oil droplets

Avian oil droplets fall into highly conserved spectral classes matched to the four cone visual pigments and to the type of cone, single or double. The R-type droplets in all four species have a λ_{cut} at about 570 nm, similar to that found in a number of other species (e.g., Partridge, 1989) and is close to the λ_{max} of the LWS visual pigment. Since the droplet filters the incident light, it effectively limits the visually active spectral region of the cone to wavelengths longer than the visual pigment's λ_{max}, with a maximum sensitivity displaced to around 600–620 nm (e.g., Jane & Bowmaker, 1988; Maier & Bowmaker, 1993). Similarly, the λ_{cut} at about 500–520 nm of the Y-type droplets is close to the λ_{max} of the MWS visual pigment and will displace the maximum sensitivity of these cones to longer wavelengths, in the region of 530–550 nm. In the pigeon, there is a clear difference between the λ_{cut} of Y-type droplets (and probably R-type droplets) from the dorsal "red" sector of the retina and those in the ventral "yellow" sector and these differences clearly have functional significance (Bowmaker, 1977; Martin & Muntz, 1978; Wortel *et al.*, 1984). In the budgerigar, although two forms of Y-type droplet were identified, there appeared to be no extreme segregation of the two forms across the retina and it is unclear whether the variation has any functional significance.

Although the R- and Y-type droplets function as cut-off filters, the role of C- and T-type droplets is not so apparent. The maximum absorbance recorded from C-type droplets varied from approx. 0.35 in the chicken to only about 0.1 in the budgerigar and zebra finch. Given the optical problems of recording from these droplets, it appears likely that in the chicken and pigeon, the C-type droplets may well act as cut-off filters with a λ_{cut} at about 445–450 nm, close to the λ_{max} of the SWS visual pigment, P455. However, in the budgerigar and zebra finch this is probably not the case. The low densities of about 0.1 in the C-type droplets may reflect a genuine low concentration of carotenoids, since absorbances of about 0.3 were recorded in these species from P-type droplets

which have similar spectral maxima to the C-type and do not appear to differ in diameter.

The functional significance of the low optical density of the C-type droplets will be that the bandwidth of the spectral sensitivity of these single cones is not reduced by the oil droplet, thereby increasing their shortwave sensitivity. It is interesting that in the chicken and pigeon where the SWS and UVS cones are spectrally close (within 30–40 nm), the C-type droplets act as cut-off filters so that the SWS cones will have a narrow spectral sensitivity, with a peak sensitivity displaced to longer wavelengths than the λ_{max} of the visual pigment. In contrast, in the zebra finch and budgerigar, where the two cone classes have λ_{max} separated by about 70 nm, the C-type droplets have low density and so the SWS cones will have a much broader sensitivity function. This correlation also holds for the Mallard duck (Jane & Bowmaker, 1988) and quail (Bowmaker *et al.*, 1993) that are related to the chicken, and for the Pekin robin (Maier & Bowmaker, 1993) which is closely related to the zebra finch. Such differences in the overlap of the spectral sensitivities of these two classes of short-wave cone may be reflected in aspects of visual performance, such as wavelength discrimination at short wavelengths.

Visual pigments

Although the LWS cone pigment is highly conserved across avian species, with λ_{max} around 565–570 nm, there are notable exceptions: the penguin, *Spheniscus humboldti*, and tawny owl, *Strix aluco*, whose LWS pigments have λ_{max} at about 543 and 555 nm, respectively (Bowmaker & Martin, 1985, 1978). A λ_{max} of 555 nm may be more general amongst the *Strigiformes*, since the Great Horned Owl, *Bubo virginianus* also has a cone mechanism maximal at about 555 nm (Jacobs *et al.*, 1987). The three spectral locations, 565–570, 555 and 543 nm, are similar to the spectral positions of LWS cone pigments in some primates (Nathans *et al.*, 1986; Neitz *et al.*, 1991; Williams *et al.*, 1992) and teleosts (Yokoyama & Yokoyama, 1990) and it is possible that the amino acid substitutions in opsin that are responsible for spectral tuning in these diverse groups are also responsible for spectral tuning in the avian system.

A striking feature of the MWS cone pigments in avian species is that their spectral absorbance is very similar to that of the rods. In the eight species where microspectrophotometric data are available (Table 2), the rods all have λ_{max} within the narrow range of 500–509 nm and in each species, the λ_{max} of the MWS cone pigment is within 1 or 2 nm of that of the rods. This is markedly different from the situation in many teleosts and mammals, where there is little correlation between the spectral absorbance of the rods and MWS cones (Jacobs, 1993; Bowmaker, 1995). Earlier microspectrophotometric measurements from chicken and pigeon suggested small spectral differences of about 10 nm (Bowmaker & Knowles, 1977; Bowmaker, 1977) between rods and MWS cones, but the λ_{max} obtained for MWS cone pigments were based on only a few records of inferior quality in comparison with those presented here. Other values for the λ_{max} of the chicken MWS cone pigment have been published. Difference spectra derived from hydroxylamine bleaching of retinal extracts gave a λ_{max} around 500 nm (Yen & Fager, 1984) and 508 nm from expressed pigment (Okano *et al.*, 1989), whereas Wang *et al.* (1992) obtained a value of 495 nm from photobleaching of the expressed pigment. Again, slightly different λ_{max} values have been reported for the rod pigment from chicken, 500 nm from retinal extracts (Wald *et al.*, 1955), around 500 nm from early microspectrophotometry (Liebman, 1972) and 503 nm from the expressed pigment (Okano *et al.*, 1989). Presumably, these small variations are the result of the very different methods used to determine the λ_{max}, but taken together, they demonstrate the close similarity in the spectral absorbance of the two pigments. This is supported by the high degree of homology between the amino acid sequences of the two opsins (Okano *et al.*, 1992), especially at amino acid sites known to be involved in spectral tuning.

Ultraviolet sensitivity

Both the zebra finch and the budgerigar possess a class of single cones maximally sensitive in the near ultraviolet, with λ_{max} below 380 nm. A similar class of cone has previously been reported in another member of the *Passeriformes*, the Pekin robin, *Leiothrix lutea* (Maier & Bowmaker, 1993). Behaviourally determined increment thresholds for the Pekin robin clearly demonstrate its sensitivity to wavelengths below 400 nm (Maier, 1992) and similarly, behavioural experiments looking at mate choice in zebra finches also demonstrate the importance of ultraviolet vision to this species (Bennett *et al.*, 1996). Unfortunately, there appears to be little behavioural data on the colour vision of budgerigars (Baltz & Clark, 1996) or indeed parrots in general, and certainly no indication of their performance in the ultraviolet.

In contrast to the zebra finch and budgerigar, the chicken and quail, both members of the Galliformes have their shortest wavelength cone at significantly longer wavelengths, close to 420 nm (Govardovskii & Zueva, 1977; Fager & Fager, 1981; Okano *et al.*, 1992; Bowmaker *et al.*, 1993). Similarly, the Mallard duck, *Anas platyrhynchos*, a member of the Anseriformes, a group closely related to the Galliformes, also possesses a P420 (Jane & Bowmaker, 1988). In the duck the lens is far less transparent to the near ultraviolet than the pigeon lens (Jane & Bowmaker, 1988), which appears to be similar to that of the chicken (Govardovskii & Zueva, 1977). Even though, behaviourally, the duck would appear to be sensitive to the near ultraviolet (Parrish *et al.*, 1981), it must be assumed that the Passeriformes (as represented by the zebra finch and Pekin robin) and the Psittaciformes (as represented by the budgerigar) make greater use of the near ultraviolet spectrum below about 400 nm than do the Galliformes and Anseriformes. Ultraviolet sensitivity may well be common within the Passeriformes since a peak sensitivity close to 370 nm has been isolated from the electroretinogram in 13 other

passerine species (Chen *et al.*, 1984; Chen & Goldsmith, 1986).

The violet-sensitive pigment of the chicken (P418) has a λ_{max} similar to that of the SWS "blue" cone of humans and other primates with λ_{max} at about 420 nm (Dartnall *et al.*, 1983; Oprian *et al.*, 1991; Merbs & Nathans, 1992). At the level of amino acids, the opsins of these avian and primate visual pigments are about 80% identical and are classified as members of the same evolutionary family of SWS pigments (Nathans *et al.*, 1986; Okano *et al.*, 1992; Hunt *et al.*, 1995). This family of opsins also includes the short-wave cone pigments of some rodents including the mouse, *Mus musculus*, that have λ_{max} close to 365 nm (Jacobs *et al.*, 1991; Chiu *et al.*, 1994), as well as the ultraviolet-sensitive cone pigment of the goldfish, *Carassius auratus* (Bowmaker *et al.*, 1991b; Hisatomi *et al.*, 1996). It would seem most likely that the UVS cone pigments of avian species also belong to the same family of opsins.

Ultraviolet sensitivity is well established in the pigeon from both behavioural and electrophysiological experiments (see Introduction) and the pigeon would appear to be absolutely more sensitive than the chicken at wavelengths below 420 nm (Wortel *et al.*, 1987). However, the underlying cone mechanisms have not been directly determined, though there is strong evidence for sensitivity peaks at about 410 and 370 nm, in addition to the three known single cone classes at longer wavelengths. These data suggest that the pigeon, in contrast to the Passeriformes, may have an additional SWS cone population with the potential for a pentachromatic colour vision system. The present microspectrophotometric measurements confirm the presence of the longer-wave cone pigments with λ_{max} at about 453, 507 and 568 nm (Table 2), but also demonstrate directly the existence of a fourth, violet-sensitive cone pigment with λ_{max} close to 409 nm. This rare class of single cone contains a transparent droplet and is spectrally similar to the 420-nm pigments of the chicken, quail and duck (Fager & Fager, 1981; Bowmaker *et al.*, 1993; Jane & Bowmaker, 1988, respectively) and the 403-nm cone class found in the Humboldt penguin, *Spheniscus humboldti* (Bowmaker & Martin, 1985). However, no evidence was found in the present microspectrophotometric study of the pigeon retina for a cone population containing a visual pigment with λ_{max} below 380 nm. Similarly, only four classes of cone opsin have been demonstrated in the pigeon retina by opsin antibody labelling (Cserháti *et al.*, 1989). Nevertheless, neither of these findings can confirm the absence of a fifth cone class in pigeons. Microspectrophotometry can only randomly survey the photoreceptors in a given retina and can easily miss a small population of cones, a problem that may be enhanced if the photoreceptors are not uniformly distributed across the retina, so that the present survey may simply have failed to detect a rare population of UVS cones. If such a population does exist, it is probable that its opsin belongs to the same evolutionary family as the violet-sensitive P409 and the two opsins would, therefore, have been indistinguishable from each other in the antibody studies of Cserháti *et al.* (1989). The presence in the retina of two spectrally distinct cone populations originating by gene duplication from the same evolutionary group of opsins is found in both teleosts (Yokoyama & Yokoyama, 1990) and primates including man (Nathans *et al.*, 1986; Deeb *et al.*, 1994; Dulai *et al.*, 1994) and a similar occurrence would account for the two postulated violet/ultraviolet-sensitive cones in the pigeon.

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