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Cone photoreceptor oil droplet pigmentation is affected by ambient light intensity

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Summary

The cone photoreceptors of many vertebrates contain spherical organelles called oil droplets. In birds, turtles, lizards and some lungfish the oil droplets are heavily pigmented and function to filter the spectrum of light incident upon the visual pigment within the outer segment. Pigmented oil droplets are beneficial for colour discrimination in bright light, but at lower light levels the reduction in sensitivity caused by the pigmentation increasingly outweighs the benefits generated by spectral tuning. Consequently, it is expected that species with pigmented oil droplets should modulate the density of pigment in response to ambient light intensity and thereby regulate the amount of light transmitted to the outer segment. In this study, microspectrophotometry was used to measure the absorption spectra of cone oil droplets in chickens (*Gallus gallus domesticus*) reared under bright (unfiltered) or dim (filtered) sunlight. Oil droplet pigmentation was found to be dependent on the intensity of the ambient light and the duration of exposure to the different lighting treatments. In adult chickens reared in bright light, the oil droplets of all cone types (except the violet-sensitive single cones, whose oil droplet is always non-pigmented) were more densely pigmented than those in chickens reared in dim light. Calculations show that the reduced levels of oil droplet pigmentation in chickens reared in dim light would increase the sensitivity and spectral bandwidth of the outer segment significantly. The density of pigmentation in the oil droplets presumably represents a trade-off between the need for good colour discrimination and absolute sensitivity. This might also explain why nocturnal animals, or those that underwent a nocturnal phase during their evolution, have evolved oil droplets with low pigment densities or no pigmentation or have lost their oil droplets altogether.

Key words: microspectrophotometry, avian colour vision, carotenoid, photon catch, spectral tuning.

Introduction

A feature of the retinal photoreceptors of many vertebrates is a large spherical organelle, commonly referred to as an ‘oil droplet’ because of its high lipid content (Johnston and Hudson, 1976), located in the ellipsoid at the distal end of the inner segment. Usually, the oil droplet completely covers the entrance aperture of the outer segment, and most of the incident light passes through it before reaching the visual pigment (Wortel and Nuboer, 1986). The oil droplets found in the photoreceptors of some species of chondrostean fishes (Walls and Judd, 1933), anuran amphibians (Hailman, 1976), geckos (Ellingson et al., 1995), monotremes (Walls, 1942) and marsupials (O’Day, 1935; Arrese et al., 2002; Arrese et al., 2005) do not have obvious pigmentation. The cone oil droplets of birds, turtles, lizards and the Australian lungfish, however, have a pale green, greenish yellow, golden yellow or ruby red colouration, depending on the spectral identity of the cone and the species (Walls and Judd, 1933; Robinson, 1994; Bailes et al., 2006).

Avian oil droplets are the best studied, with all but one spectral type containing high concentrations of diet-derived, short-wavelength-absorbing carotenoid pigments (Goldsmith et al., 1984; Davies, 1985). Birds possess a single type of medium-wavelength-sensitive (MWS) rod, four spectrally distinct types of single cone and a single type of double cone (for reviews, see Hart, 2001; Hart and Hunt, in press). Single cones containing an ultraviolet- (UVS) or violet-sensitive (VS) visual pigment [SWS1 opsin; for terminology see (Yokoyama, 2000)] have a non-pigmented ‘T-type’ oil droplet, with no significant absorption of wavelengths between at least 330 and 800 nm. Single cones expressing a short-wavelength-sensitive (SWS) visual pigment (SWS2 opsin) in their outer segment have a ‘colourless’ or pale green ‘C-type’ oil droplet. The cut-off wavelength, or λ_{cut} (Lipetz, 1984), of the C-type oil droplet in different bird species varies from 392 to 444 nm. Single cones containing a MWS visual pigment (RH2 opsin) have a golden yellow Y-type oil droplet (λ_{cut} = 505–516 nm), and those containing a long-wavelength-sensitive (LWS) visual

pigment (M/LWS opsin) have a red R-type oil droplet (λ_{cut} = 552–586 nm). The outer segments of both the principal and accessory members of the double cone pair contain the same LWS visual pigment found in the LWS single cones. Usually, only the principal member of the double cone contains an oil droplet (P-type), but a smaller droplet (A-type) might occasionally be seen in the accessory member (Bowmaker et al., 1997; Hart et al., 1998). The P-type oil droplet might appear colourless, pale green, greenish yellow or yellow depending on the spectral location of the λ_{cut} (range = 407–489 nm). Avian rods do not contain oil droplets.

The incorporation of pigmented compounds into the oil droplet creates an intracellular spectral filter that has a marked effect on the spectral sensitivity of the cone (Neumeyer and Jäger, 1985; Wortel and Nuboer, 1986). For example, in the case of avian MWS and LWS single cones, calculations show that absorption of short wavelengths by the Y- and R-type oil droplets will shift the wavelength of peak sensitivity of the cones approximately 40 nm towards longer wavelengths (540 nm and 605 nm, respectively) and reduce the sensitivity of the outer segment by 50% or more (Bowmaker and Knowles, 1977; Hart and Vorobyev, 2005). For all cone types, the absorption of short wavelengths by the pigmented oil droplets narrows the spectral sensitivity function of the photoreceptor and reduces the overlap between adjacent spectral classes, potentially improving the discrimination of broadband (i.e. natural) reflectance spectra (Govardovskii, 1983; Vorobyev, 1997; Vorobyev et al., 1998; Vorobyev, 2003) and enhancing colour constancy (Dyer, 2001).

Nocturnal birds also have coloured cone oil droplets, but they are less densely pigmented than those of diurnal species. The tawny owl (*Strix aluco*) has dark yellow, pale yellow and pale red oil droplets (Bowmaker and Martin, 1978), whereas the tawny frogmouth (*Podargus strigoides*) lacks red oil droplets altogether and has only yellow, pale green and transparent oil droplets (N.S.H., unpublished observations). The reduced pigmentation of oil droplets in nocturnal species suggests that, at low light levels, heavily pigmented oil droplets either are of no use or reduce photon capture sufficiently to be a hindrance to vision.

While the reduction in oil droplet pigmentation over evolutionary time might have occurred as a result of genetic selection for individuals that were better able to see and thus survive under nocturnal or crepuscular lighting conditions, short-term phenotypic changes in oil droplet pigmentation might also be adaptive for optimising visual performance under different environmental conditions. To investigate this possibility, we have used microspectrophotometry to measure objectively the spectral absorptance characteristics of the cone oil droplets of chickens reared under either bright or dim light.

Materials and methods

Experimental animals and light treatments

All procedures were approved by the University of Queensland Animal Ethics Committee and were pursuant to the ethical guidelines of the National Health and Medical Research Council of Australia. Day-old Rhode Island Red/White cockerels (*Gallus gallus domesticus*) were obtained from a local hatchery (Bond Nelbex, Brisbane, Australia) in February. Initially, chicks (*N*=18) were kept in heated (30°C) indoor cages with a 12 h:12 h L:D cycle. Illumination was provided by overhead fluorescent lights (Sylvania 'Cool White' 13 W and 18 W; Sylvania Lighting Australasia Pty. Ltd, Lisarow, Australia), which gave a downwelling illuminance of 1000 lx at the level of the food troughs. After 2 weeks, the chicks were transferred to a larger unheated indoor cage (2.7×2.4×2.2 m, length × width × height, respectively), also with a 12 h:12 h L:D cycle. Illumination was provided by two overhead fluorescent strip lights (Phillips TL-D 36 W; Phillips Australia, North Ryde, Australia), which gave a downwelling illuminance of 92 lx at a height of 50 cm from the floor.

After a further 2 weeks (i.e. at 4 weeks of age) the chicks were divided randomly into two treatment groups, designated as 'bright' light (*N*=9) and 'dim' light (*N*=9), for the remainder of the experiment. Each group was placed into one of two large outdoor mesh cages (4×3×2 m, length × width × height, respectively) with bare earth floors. The cages were adjacent to each other and partially shaded by nearby eucalyptus trees. Both cages had a 1.5×1.5 m waterproof (corrugated steel) shade roof in the north-east corner. Over the experimental period, both cages had the same minimum and maximum recorded ambient temperatures of 12°C and 36°C, respectively. The only difference between the two treatments was that the cage housing the dim light group of chicks was covered on all sides and over the roof by a single layer of closely woven black plastic weed matting (Mitre 10, Brisbane, Australia) secured to the cage mesh and further shaded by an opaque plastic tarpaulin (6×4 m) suspended 1 m above the cage. The characterisation of the intensity and spectral distribution of the ambient light in each of the treatment cages is described in the next section.

Both groups had access to a covered wooden chicken coop and were provided with straw for bedding. Food and water were provided *ad libitum* throughout the experiment. Up to 4 weeks of age, the feedstock was Riverina Chick Starter Crumbles (Riverina Australia Pty. Ltd, Brisbane, Australia), which is derived predominantly from wheat grains. Thereafter, chicks were fed SupaStok Coarse Grain Mix (Ridley AgriProducts Pty. Ltd, Pakenham, Australia), which on the basis of mass consists of 26% sorghum, 26% wheat, 26% corn, 8% barley, 3% sunflower seeds and 11% other ingredients. No artificial carotenoid supplements were provided.

Spectroradiometry and photometry

Spectral irradiance in each of the treatment cages was recorded using a calibrated, computer-controlled Ocean Optics S2000 charge-coupled device (CCD) spectroradiometer (Ocean Optics Inc., Dunedin, FL, USA) connected to an Ocean Optics CC3-UV cosine-corrected irradiance probe by a 12 m long, 1 mm diameter, UV-visible-transmitting fibre optic. The probe was positioned 50 cm above the ground (approximately the head height of an adult chicken) in the centre of each cage and

measurements were made (N=5) with the probe pointing both directly upwards and directly downwards to record the downwelling and upwelling radiation impinging upon the ventral and dorsal retinal surfaces of the chickens' eyes, respectively.

Measurements of illuminance (lux) were also made using a calibrated, hand-held light-meter (Lutron LX-107HA; Lutron Electronic Enterprise Co. Ltd, Taipei, Taiwan). Unlike the spectral irradiance measurements, lux measurements are based on human photopic spectral sensitivity functions, but they are useful for comparison with previous studies in which light intensities have been measured in lux (lx). The recording probe was positioned 50 cm off the ground and measurements were made from five different locations within the cage (once in each corner and in the centre) with the probe pointing both directly upwards and directly downwards. All measurements were made at the end of the experimental period and were taken under full sunlight at approximately midday.

Microspectrophotometry

Chickens were euthanised with an overdose of sodium pentobarbitone (Lethabarb, Virbac Australia Pty. Ltd, Peakhurst, Australia), followed by cervical dislocation and weighed using an electronic balance (accuracy ±1 g). The left eye from each chicken was removed and bisected at the equator, immediately anterior to the *ora terminalis*. Only the left eye was used so as to standardise the sampling procedure and avoid introducing errors caused by differences in the way in which the left and right eyes might be dissected. This is important because oil droplet spectra vary subtly depending on retinal location and there is also some evidence to suggest that the proportions of cone photoreceptors vary between the left and right retinae of the same bird (Hart et al., 2000). The posterior segment of the globe containing the retina was immersed in cold (4°C) phosphate-buffered saline (167 mmol l^{-1} NaCl, 3 mmol l^{-1} KCl, 10 mmol l^{-1} Na_2HPO_4, 2 mmol l^{-1} KH_2PO_4; osmolality 340 mosmol kg^{-1}; pH 7.3; Oxoid Ltd, Basingstoke, UK) and the vitreous dissected away. Two samples of neural retina approximately 2×2 mm were cut from the fundus, both with their peripheral edge a distance of 1 mm from the *ora terminalis*. The first piece was taken from the ventral peripheral retina, 2 mm nasal to the base of the pecten; the second piece was removed from the dorsal peripheral retina exactly opposite the site of the ventral sample. Each piece of retinal tissue was transferred to a drop of glycerol (APS Finechem, Seven Hills, Australia) placed in the centre of a 24×60 mm No. 1 glass coverslip and the retina oriented with the photoreceptor layer uppermost. The preparation was then covered with a 22×22 mm No. 0 glass coverslip, blotted gently with filter paper to remove excess glycerol and the edges of the top coverslip sealed with nail varnish to prevent movement of the retina. Preparations were stored in a refrigerator at 4°C for up to 6 h before use.

Transverse absorptance spectra (330–800 nm) of cone photoreceptor oil droplets were made using a computer-controlled, single-beam, wavelength-scanning microspectrophotometer (MSP) described in detail elsewhere (Hart, 2004). A sample scan was made by aligning the measuring beam (dimensions 1×1 μm) within an oil droplet and recording the amount of light transmitted at each wavelength across the spectrum. A baseline scan was made in an identical fashion from a cell-free area of the preparation adjacent to the measured cell. Baseline transmittance was subtracted from that of the sample at each corresponding wavelength to create a single baseline-corrected scan that was subsequently converted to absorptance. Absorptance spectra were obtained from at least 10 different oil droplets for each cone class that contains a pigmented oil droplet in both the dorsal and ventral retinal samples. The so-called 'transparent' T-type oil droplets found in the VS single cones were not measured. The diameter of each droplet measured was recorded (to an accuracy of ±0.25 μm) with the use of a calibrated acetate sheet placed over an image of the retina, supplied by a CCD camera attached to the MSP, projected onto a television screen. One chicken from each treatment group was sampled at intervals from 10 to 33 weeks of age (i.e. from 6 to 29 weeks of treatment under the different lighting regimes). In a given sampling week, chickens from different light-treatment groups were measured on different days but within 5 days of each other.

Analysis of oil droplet absorptance spectra

Oil droplet absorptance spectra were normalized to the maximum, and long-wavelength offset absorbances obtained by fitting an 11-point unweighted running average to the data. Spectra were then described by their cut-off wavelength, λ_{cut}, as defined by Lipetz (Lipetz, 1984). Briefly, a tangent line was fitted (see below) to the long-wavelength limb of the normalized absorptance spectrum and the λ_{cut} calculated as the wavelength at which the tangent line had a value of 100% normalized absorptance. The λ_{cut} is particularly useful as an objective measure of oil droplet pigmentation because it is directly related to carotenoid concentration (Lipetz, 1984; Hart and Vorobyev, 2005). For the C-, Y- and R-type single cone oil droplets, the tangent line was fitted using absorptance values on the long-wavelength limb between 70% and 30% of the normalized maximum. For double cone P-type oil droplets displaying a secondary peak in the long-wavelength limb of their absorptance spectrum, the tangent line was fitted using different absorptance value ranges, as follows: where the secondary peak was less than 50% of the normalized maximum, the tangent line was fitted using absorptance values between 70% and 60% of the maximum; where the secondary peak was more than or equal to 50% of the normalized maximum, the tangent line was fitted using absorptance values between 40% and 30%.

Modelling the spectral sensitivity of cone outer segments

The relative quantal spectral sensitivity of the outer segments of the SWS, MWS and LWS single cones and the principal member of the LWS double cones in the ventral retina of the bright-light and dim-light groups was modelled

as described previously (Hart, 2002; Hart, 2004; Hart and Vorobyev, 2005). Cone outer segment absorptance was modelled using the rhodopsin (vitamin A_1-based) visual pigment templates of Govardovskii et al. (Govardovskii et al., 2000), a specific (decadic) absorbance of $0.014\,\mu m^{-1}$ (Bowmaker and Knowles, 1977) and a cone outer segment length of 16 μm (Morris and Shorey, 1967). Microspectrophotometrically measured oil droplet absorptance spectra recorded from the last chicken sampled (after 29 weeks of light treatment, i.e. 33 weeks of age) in each treatment group were fitted with an 11-point running average, corrected for any long-wavelength absorptance offset, converted to transmittance and normalized. Spectral sensitivity was defined as the product of outer segment axial absorptance and oil droplet transmittance.

Statistical analysis

Pearson product-moment correlation coefficients were calculated to determine whether mean λ_{cut} or mean diameter was significantly correlated with time (weeks of light treatment) for each of the different pigmented oil droplet types, in both the dorsal and ventral retina of the dim-light and bright-light groups. Subsequently, the data were split into juvenile (≤18 weeks of age) and adult (≥24 weeks of age) sets (Limburg, 1975). Trends in mean λ_{cut} value and mean diameter for the different oil droplet types across treatment groups and between dorsal and ventral retinal regions in both juveniles and adults were analysed using a General Linear Model (GLM), with body mass as a covariate. The effect of light treatment on body mass was analysed using a two-sample *t*-test. Statistical analyses were performed with the aid of Minitab 14.20 (Minitab Inc., State College, PA, USA).

Results

Spectroradiometry and photometry

Spectral irradiance measurements made in both treatment cages are summarised in Fig. 1. The spectral composition of the illumination in both the bright-light and dim-light cages was similar, with the shading applied to the dim-light cage affecting predominantly the intensity of the incident sunlight (Fig. 1A). There were some differences in the spectral distribution of habitat light between the bright-light and dim-light cages, with the treatment groups differing less in intensity in the UV (<400 nm) and far-red regions (>700 nm) of the spectrum than at medium wavelengths (Fig. 1B). However, over the range of wavelengths to which the chickens would be most sensitive (see below), the ratio of spectral irradiances between the treatment groups was almost uniform. Measurements of mean downwelling and upwelling illuminance in the treatment cages, made with the probe pointing both up towards the sky and down towards the floor, respectively, were 70 250 lx and 7173 lx for the bright-light cage and 14 lx and 2 lx for the dim-light cage. These values are a guide only, as absolute intensities will vary with time of day, degree of cloud cover and seasonal factors.

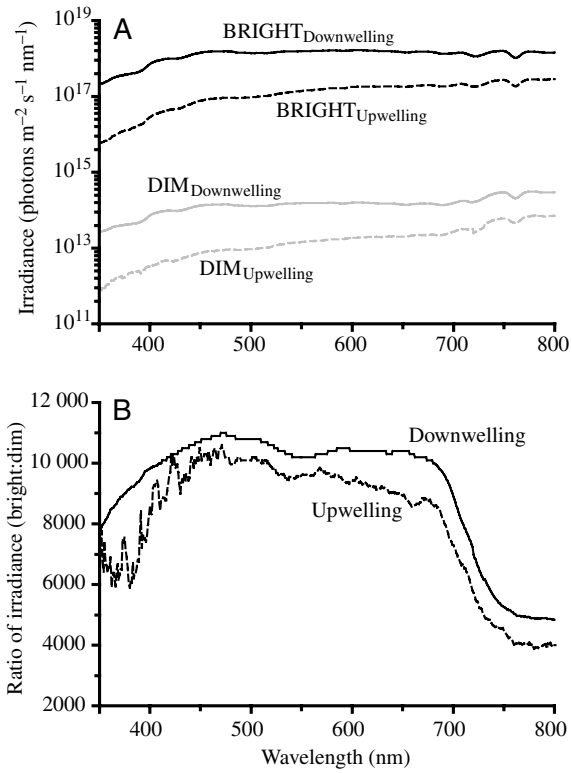

Fig. 1. (A) Semi-logarithmic plot of habitat spectral irradiance (350–800 nm) for the different light treatments employed in the study. 'BRIGHT' refers to the unshaded cage ('bright-light') treatment, 'DIM' refers to the shaded cage ('dim-light') treatment. The subscripts 'Downwelling' and 'Upwelling' refer to irradiance measurements made with the recording probe pointing either directly upwards towards the sky or directly downwards towards the substrate, respectively. (B) Ratios of downwelling (solid line) and upwelling (broken line) spectral irradiance between the bright-light and dim-light treatment groups.

Microspectrophotometry

Absorptance spectra of the different types of pigmented oil droplets found in the retinal cone photoreceptors of 33-week-old chickens are shown in Fig. 2. These spectra are similar to those obtained for the chicken by Bowmaker and Knowles (Bowmaker and Knowles, 1977) and Bowmaker et al. (Bowmaker et al., 1997), although there are distinct differences in the absorption spectra of specific droplet types depending on retinal location and light-treatment group. These differences are also evident in Fig. 3, in which mean oil droplet cut-off wavelength (λ_{cut}) is plotted against weeks of treatment for both bright-light and dim-light groups.

Calculations of the Pearson product-moment correlation coefficient for each oil droplet type in both the dorsal and ventral retina and in the different light-treatment groups show that in several instances the λ_{cut} values and/or the diameters of the oil droplets were significantly correlated with time (Table 1). For all significant relationships found, the correlation was positive, i.e. oil droplet diameter and λ_{cut} tended to increase with age. In view

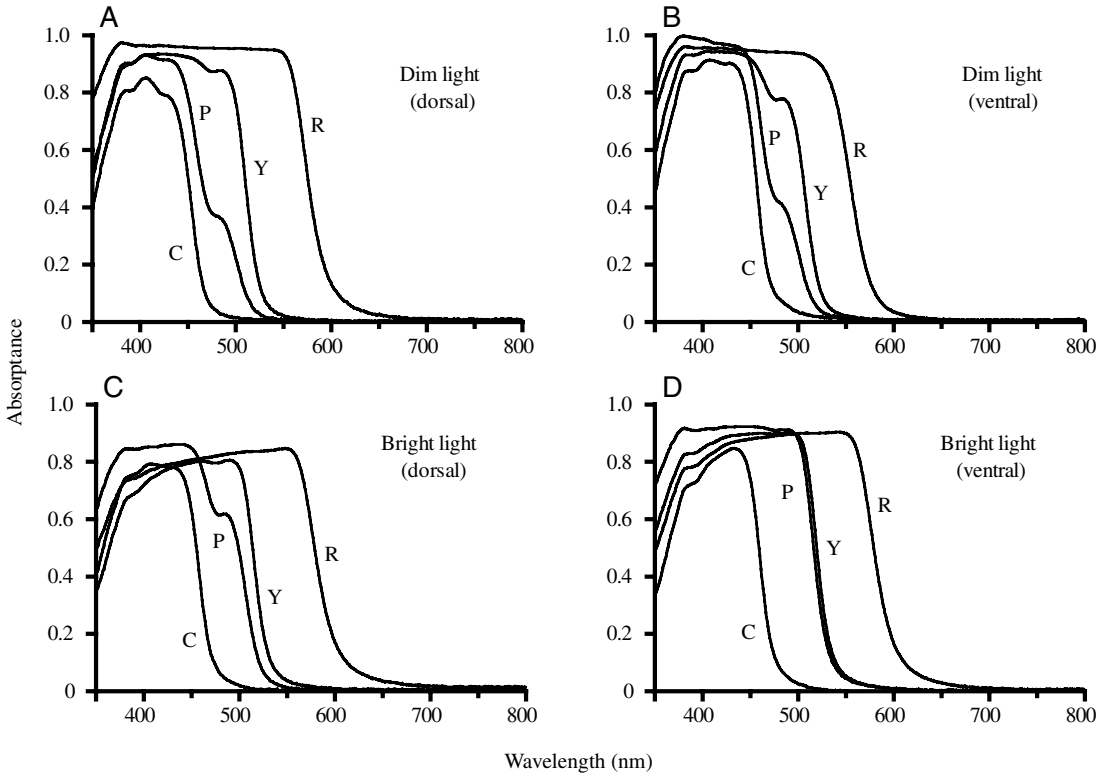

Fig. 2. Mean absorptance spectra of pigmented cone oil droplets in 33-week-old chickens measured using microspectrophotometry. Panels correspond to oil droplets measured in the dorsal (A) and ventral (B) retina of a chicken reared in dim light and the dorsal (C) and ventral (D) retina of a chicken reared in bright light. C, Y, R and P refer to the oil droplets found in the short- (SWS), medium- (MWS) and long-wavelength-sensitive (LWS) single cones and the principal member of the LWS double cones, respectively. Note the difference in spectra between light-treatment groups, especially with respect to the Y-, R- and P-type oil droplets, and also between dorsal and ventral retina locations within the same bird. Each spectrum is the average of the individual spectra from 10 different oil droplets of a given oil-droplet type.

of these correlations, and because other retinal characteristics – such as the relative proportions of the different oil droplet types [Pézard, cited in Meyer (Meyer, 1977)] – are reported to be affected by the state of maturity, the data were split into juvenile and adult sets for subsequent analyses.

In juvenile chickens, the λ_{cut} values of the Y-, R- and P-type oil droplets in the bright-light group were at longer wavelengths than those in the dim-light group, although the difference was significant only for the P-type droplets (Tables 2, 3; Fig. 3). In adult chickens, however, all pigmented oil droplet types, regardless of retinal location, had λ_{cut} values shifted significantly towards longer wavelengths and were therefore more densely pigmented in the group reared in bright light compared with the group reared in dim light (Tables 2, 4; Fig. 3). The absolute shift in λ_{cut} for the C-type droplets was smaller than for the other droplet types (Table 2), which might reflect the fact that the light intensity in light-treatment groups differed less at short wavelengths (<400 nm) than at other regions of the spectrum (Fig. 1B) to which the chickens would be sensitive (Fig. 4). These results suggest that the effect of the different light treatments on oil droplet pigmentation is progressive and occurs over several weeks. There was no significant effect of light treatment on body mass (two-sample t-test; juvenile t=–1.88, d.f.=8, P=0.097; adult t=–0.75, d.f.=6, P=0.480) or of body mass on oil droplet λ_{cut} in either juvenile or adult chickens (GLM: juvenile – C-type $F_{1,7}$=0.04, P=0.841; Y-type $F_{1,7}$=0.24, P=0.640; R-type $F_{1,7}$=0.31, P=0.593; P-type $F_{1,7}$=0.70, P=0.430; adult – C-type $F_{1,5}$=0.22, P=0.661; Y-type $F_{1,5}$=4.87, P=0.078; R-type $F_{1,5}$=0.03, P=0.875; P-type $F_{1,5}$=0.34, P=0.585).

With regard to retinal location, the mean λ_{cut} values of C- and P-type oil droplets in the ventral retina of adult chickens were shifted significantly towards longer wavelengths compared with those in the dorsal retina in both light-treatment groups (Tables 1, 4; Figs 2, 3). This was particularly obvious in the P-type oil droplets, in which the increased contribution of a secondary absorption peak at approximately 485 nm increased the absorption of short-medium wavelengths in ventrally located cones (Fig. 2); in the case of the bright-light group, the absorptance spectrum of the ventral P-type oil droplet in the double cones closely resembles that of the Y-type oil droplet in the MWS single cones. Conversely, R-type oil droplets in the dorsal retina of both adult and juvenile chickens were more densely pigmented and had mean λ_{cut} values shifted significantly towards longer wavelengths than those in the ventral retina (Tables 2–4; Figs 2, 3).

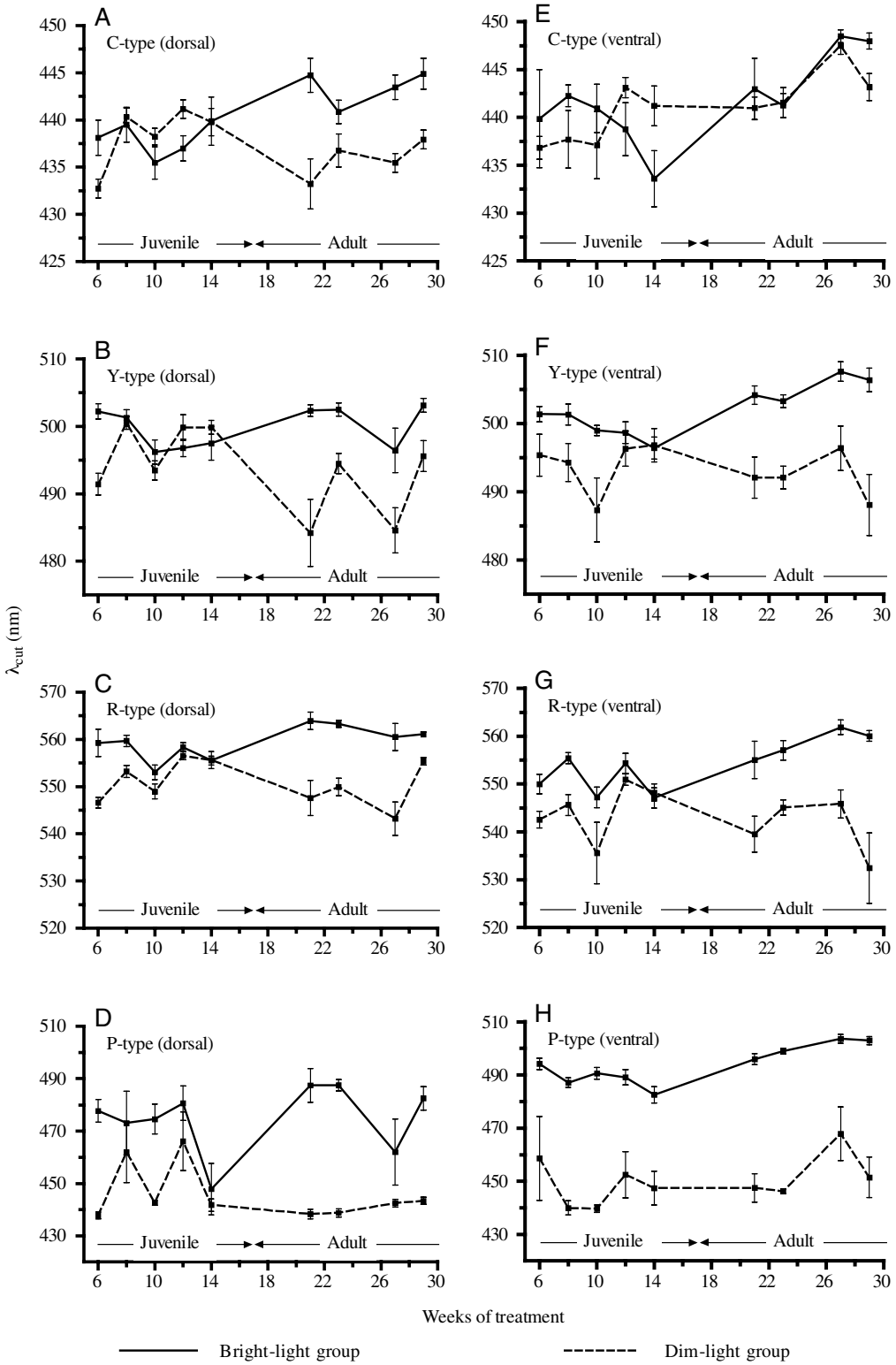

Fig. 3. Variations in cut-off wavelength (λ_{cut}) of the different pigmented cone oil droplets in the dorsal and ventral retina as a function of the type and duration of light treatment. Solid line, 'bright-light' group; broken line, 'dim-light' group. Bars around data points represent ±1 s.d. for the mean value (N=10). Panels A–D show data for C-, Y-, R- and P-type oil droplets located in the dorsal retina and panels E–H show data for the same droplet types located in the ventral retina. The earliest microspectrophotometric data were obtained after 6 weeks of exposure to the different lighting conditions, i.e. when the chickens were 10 weeks old. Chickens reach sexual maturity between 18 and 24 weeks of age and the state of maturity is indicated on each graph, with the border between juvenile and adult status taken as the midpoint of this range (i.e. 21 weeks of age), which occurred 17 weeks after the onset of the different light treatments.

Table 1. *Summary of calculated Pearson product–moment correlation coefficients (r) showing the relationships between the mean cut-off wavelength (λ_{cut}) values and the mean diameters for the different pigmented cone oil droplets (in the dorsal and ventral retina and both dim-light- and bright-light-treatment groups) as a function of time (weeks of treatment)*

			Dim-light		Bright-light	
Type	Parameter	Location	*r*	*P*	*r*	*P*
C-type	λ_{cut}	D	–0.124	ns	0.813	0.008
		V	0.796	0.010	0.642	ns
	Diameter	D	0.617	ns	0.418	ns
		V	0.977	<0.001	0.222	ns
Y-type	λ_{cut}	D	–0.399	ns	0.148	ns
		V	–0.256	ns	0.728	0.026
	Diameter	D	0.691	0.039	0.820	0.007
		V	0.921	<0.001	0.858	0.003
R-type	λ_{cut}	D	–0.097	ns	0.533	ns
		V	–0.280	ns	0.752	0.019
	Diameter	D	0.798	0.010	0.719	0.029
		V	0.840	0.005	0.931	<0.001
P-type	λ_{cut}	D	–0.308	ns	0.129	ns
		V	0.314	ns	0.736	0.024
	Diameter	D	0.626	ns	0.919	<0.001
		V	0.963	<0.001	0.856	0.003

C-, Y-, R- and P-type oil droplets are found in the short- (SWS), medium- (MWS) and long-wavelength-sensitive (LWS) single cones and the principal member of the LWS double cones, respectively. D, dorsal; ns, not significant; V, ventral.

There was a significant effect of light treatment on the diameters of the C- and P-type oil droplets in the juvenile chickens (Table 3) and of the C-type oil droplets in the adult chickens (Table 4), and in each case the diameter of the oil droplets was larger in the dim-light group than in the bright-light group (Table 2). Moreover, the diameter of the C-type oil droplets in the juvenile chickens, and the C- and R-type oil droplets in the adults, was significantly larger in the dorsal retina than the ventral retina.

Effects on spectral sensitivity

The absorptance spectra of all pigmented oil droplet types from adult chickens reared in dim light had λ_{cut} values at shorter wavelengths, and were therefore less densely pigmented than the corresponding oil droplet types in the adult chickens reared in bright light. These differences in spectral absorptance characteristics markedly affected the modelled spectral sensitivity of the corresponding cone photoreceptor outer segments (Fig. 4). In the case of the MWS and LWS single cones, the reduction in pigmentation of the Y- and R-type oil droplets in the chickens reared in dim light resulted in the wavelength of peak sensitivity of their outer segments being shifted towards shorter wavelengths, from 535 to 526 nm and from 600 to 585 nm, respectively, and increased the overall sensitivity of the outer segments, by 52% and 58%, respectively, compared with those in the chickens reared in bright light.

The effects of the dim-light treatment on the spectral sensitivities of the outer segments of SWS single cones containing a C-type oil droplet and of the LWS principal members of the double cones containing a P-type oil droplet were smaller in terms of spectral shift (475 to 472 nm and 573 to 572 nm, respectively) compared with the MWS and LWS single cones but were substantial in terms of overall sensitivity (increases of 11% and 18%, respectively). If the significant difference in C-type oil droplet diameter between bright- and dim-light-treatment groups (see above) is taken into consideration, the increase in spectral sensitivity of the SWS cones in the dim-light group would be considerably greater (62% more than SWS cones in the bright-light group).

Discussion

The density of carotenoid pigments in the cone oil droplets of the chicken retina has been shown to be affected by the intensity of light experienced during development. Chickens reared in bright light developed more densely pigmented oil droplets in both the single and double cones than chickens raised in dim light. This effect appears to be progressive and was more pronounced in adult chickens that had experienced the altered lighting conditions for several months. It is possible, however, that significant changes in pigment density can occur over much shorter timescales than those considered in the present study. Bowmaker et al. showed that when newly hatched, carotenoid-deprived quail chicks possessing oil droplets with no detectable levels of pigment absorptance were

Table 2. *Summary of mean (n=10 measurements for each parameter at each location from the left eye of each bird) cut-off wavelength (λ_{cut}) values and diameters for pigmented cone oil droplets in juvenile and sexually mature chickens as a function of light treatment and retinal location*

			Juvenile[a]		Adult[b]	
Type	Parameter	Location	Dark	Light	Dark	Light
C-type	λ_{cut} (nm)	D	438.5±3.4	438.0±1.8	435.9±2.0	443.5±1.9
		V	439.2±2.8	439.1±3.3	443.3±3.0	445.2±3.6
	Diameter (μm)	D	3.7±0.1	3.5±0.3	4.2±0.4	3.7±0.2
		V	3.4±0.2	3.2±0.2	3.9±0.1	3.2±0.1
Y-type	λ_{cut} (nm)	D	497.0±4.3	498.8±2.8	489.7±6.2	501.1±3.1
		V	494.0±3.9	499.4±2.1	492.2±3.4	505.4±2.0
	Diameter (μm)	D	3.4±0.2	3.2±0.2	4.1±0.3	3.8±0.2
		V	3.5±0.1	3.3±0.2	4.0±0.3	3.8±0.3
R-type	λ_{cut} (nm)	D	552.2±4.3	557.2±2.8	549.0±5.1	562.2±1.7
		V	544.6±5.9	550.8±3.9	540.8±6.2	558.5±3.0
	Diameter (μm)	D	3.8±0.1	3.7±0.2	4.2±0.2	4.2±0.2
		V	3.6±0.3	3.5±0.1	4.0±0.2	4.0±0.2
P-type	λ_{cut} (nm)	D	450.1±13.0	470.8±13.1	440.7±2.6	479.9±12.2
		V	447.6±8.2	488.7±4.3	453.3±10.0	500.4±3.6
	Diameter (μm)	D	3.9±0.1	3.7±0.3	4.6±0.6	4.4±0.4
		V	4.0±0.2	4.0±0.5	4.7±0.3	4.7±0.4

Birds were [a]10–18 weeks old; *N*=5 birds per light-treatment group; [b]25–33 weeks old; *N*=4 birds per light-treatment group.
Values are means ± 1 s.d. See Table 1 for more details.

returned to a carotenoid-rich diet, they could accumulate sufficient carotenoids over the course of one week to generate oil droplets almost identical in spectral absorptance to those of normal adult birds (Bowmaker et al., 1993).

Although the absorptance spectra and λ_{cut} values of the oil droplets of the red jungle fowl (*Gallus gallus*), from which the domestic chicken is derived (Fumihito et al., 1994), are unknown, those of the chickens reared in bright light are most similar to those of other wild-caught, wild-type diurnal bird species (e.g. Hart et al., 1998) and may well represent the 'natural' condition for the chicken. Moreover, the absorptance spectra of oil droplets in chickens reared in dim light resemble those of carotenoid-deprived quails (*Coturnix coturnix japonica*), the oil droplets of which are much less densely pigmented than those of quail fed a carotenoid-rich 'natural' diet (Bowmaker et al., 1993). Light intensities measured in rainforest habitats similar to those of the jungle fowl vary from approximately 50–1000 lx in the shade to 10 000–18 000 lx in areas illuminated *via* small gaps in the canopy (Endler, 1993) (J. A. Endler, personal communication). These values are similar to the illuminance levels that would have been experienced by the bright-light-treatment group in the present study.

Presumably, the chickens reared in dim light accumulated or maintained less carotenoid pigment in the oil droplets to compensate for the reduced levels of light available for vision. A reduction in pigmentation would allow more of the incident photons to pass through the oil droplet and be absorbed by the visual pigment within the outer segment. On the basis of electroretinographic measurements of the spectral sensitivity of the SWS single cone in the pigeon, Wortel and Nuboer calculated that less than 10% of the light reaching the outer segment bypasses the oil droplet (Wortel and Nuboer, 1986). Consequently, while scattered light and any wave-guiding behaviour of oil droplets might reduce slightly the effects of a decrease in oil droplet pigmentation (or an increase in oil droplet diameter), the predicted increases in spectral sensitivity of the cone outer segments in the chickens reared in dim light are likely to be significant for enhancing visual sensitivity.

The modulation of oil droplet pigment density in response to changing light intensity is perhaps analogous and/or additional to the adaptive anatomical and physiological changes observed in the photoreceptors of other vertebrate groups. For example, Penn and Williams showed that the rod photoreceptors of laboratory rats raised in dim light (3 lx) had longer outer segments and a higher density of visual pigment molecules than those raised under brighter light (400 lx) and that the number of photons absorbed by the retina over time was approximately constant regardless of incident light levels, a process they called photostasis (Penn and Williams, 1986). Similarly, the outer segments of double cone photoreceptors in the retina of the blue acara (*Aequidens pulcher*) are significantly longer in animals raised in dim (<1 lx) 'white' light than those raised in bright (33–700 lx) 'white' light (Kröger et al., 1999).

Changes in oil droplet pigmentation at the rate observed in

Table 3. *Summary of statistical analyses (GLM ANOVA) of mean cut-off wavelength (λ_{cut}) values and mean diameters for pigmented cone oil droplets in juvenile chickens (10–18 weeks old) as a function of light treatment (dim light versus bright light) and retinal location*

		Light treatment		Dorsal *versus* ventral	
Type	Parameter	$F_{1,7}$	P	$F_{1,8}$	P
C-type	λ_{cut}	0.001	ns	0.648	ns
	Diameter	11.76	0.011	17.13	0.003
Y-type	λ_{cut}	3.154	ns	1.497	ns
	Diameter	5.350	ns	0.920	ns
R-type	λ_{cut}	1.993	ns	53.39	<0.001
	Diameter	3.216	ns	3.091	ns
P-type	λ_{cut}	31.84	<0.001	3.189	ns
	Diameter	14.55	0.007	4.862	ns

ns, not significant. See Table 1 for more details.

Table 4. *Summary of statistical analyses (GLM ANOVA) of mean cut-off wavelength (λ_{cut}) values and mean diameters for pigmented cone oil droplets in sexually mature chickens (25–33 weeks old) as a function of light treatment (dim light versus bright light) and retinal location*

		Light treatment		Dorsal *versus* ventral	
		$F_{1,5}$	P	$F_{1,6}$	P
C-type	λ_{cut}	6.688	0.049	16.73	0.006
	Diameter	33.68	0.002	18.41	0.005
Y-type	λ_{cut}	165.0	<0.001	1.748	ns
	Diameter	6.53	ns	0.246	ns
R-type	λ_{cut}	196.1	<0.001	6.810	0.040
	Diameter	1.2×10^{-5}	ns	17.10	0.006
P-type	λ_{cut}	106.0	<0.001	14.61	0.009
	Diameter	1.163	ns	1.010	ns

ns, not significant. See Table 1 for more details.

the present study (i.e. over the course of several weeks) might be adaptive for optimising visual performance under the varying environmental conditions experienced by a bird throughout its life, such as those arising from seasonal variations in ambient light intensity or a shift between habitats during development or migration. Similar intra-specific changes in spectral filtering have been observed in mantis shrimps living in either deep- or shallow-water habitats, in which both the intensity and spectral distribution of the ambient light differ markedly (Cronin et al., 2001; Cronin and Caldwell, 2002). Alternatively, the modulation of oil droplet pigmentation might represent another form of retinal photostasis that regulates photon capture across the retina, perhaps in response to local differences in intensity caused by the physiological optics of the eye (Penn and Williams, 1986). The phenomenon of photostasis might also explain some of the intra-individual variations in oil droplet λ_{cut} between dorsal and ventral retinal locations. In particular, the P-type oil droplets of double cones located in the ventral retina are more densely pigmented than those located in the dorsal retina. The ventral retina (which views the sky) will receive more light, especially of shorter wavelengths (Fig. 1), than the dorsal retina (which views the ground), and the extra filtering of wavelengths below 500 nm might compensate for these intensity differences.

An alternative role for the increase in oil droplet carotenoid pigment density in chickens reared in bright light compared with those reared in dim light might be in the reduction of photo-oxidative damage in the retina. Carotenoids are capable of quenching reactive oxygen species and organic free radicals, such as those created as a result of intense irradiation of biological tissue (Kirschfeld, 1982; Miki, 1991). They might also reduce photo-oxidative damage indirectly by blocking the

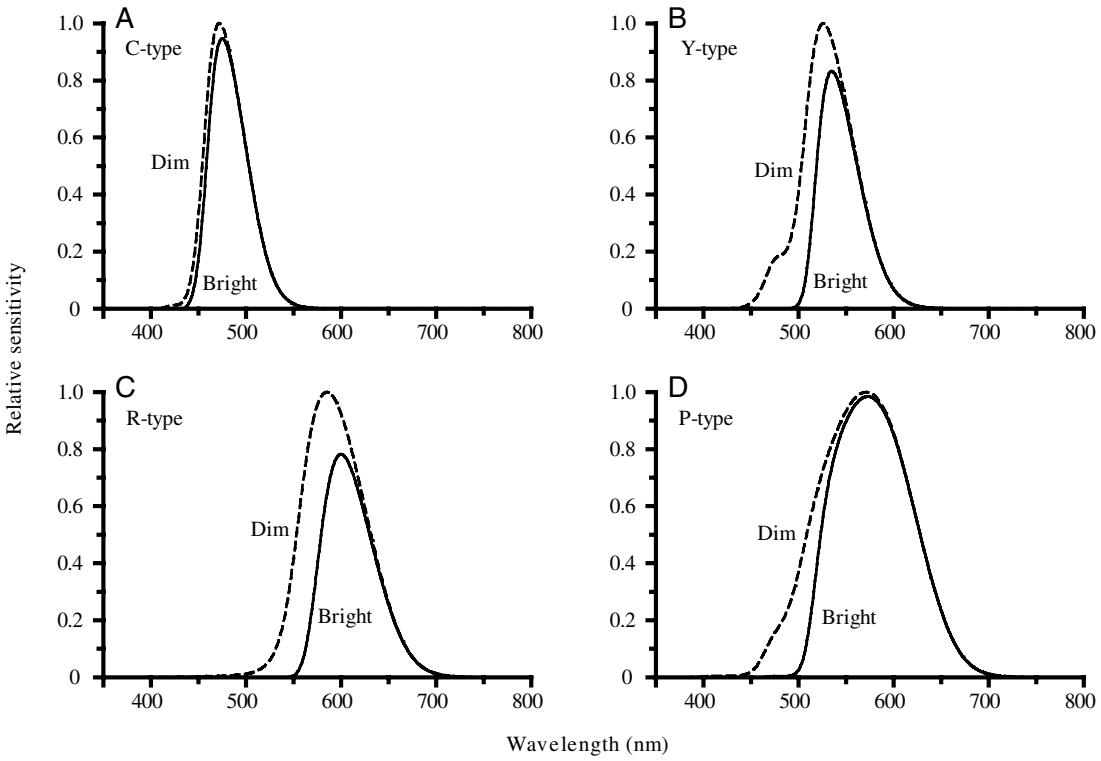

Fig. 4. Calculated relative quantal spectral sensitivity functions for the outer segment of cones containing pigmented oil droplets in 33-week-old adult chickens reared in bright (solid line) or dim (broken line) light. (A) C-type oil droplets in the short-wavelength-sensitive single cones; (B) Y-type oil droplets in the medium-wavelength-sensitive single cones; (C) R-type oil droplets in the long-wavelength-sensitive (LWS) single cones and (D) P-type oil droplets in the principal member of the LWS double cones. Note the increased sensitivity and spectral bandwidth of all cone types in the dim-light group, owing to the reduced density of carotenoid pigments in their respective oil droplet types, compared with that of the bright-light group (see Figs 2, 3).

transmission of high-energy UV radiation to the outer segment. However, the variation in oil droplet colouration with cone type, the predictable relationship between oil droplet λ_{cut} and visual pigment λ_{max} in some cone types across species (Hart and Vorobyev, 2005) and the position of the oil droplet in the photoreceptor imply a significant role in visual function. Moreover, it is not readily apparent how carotenoids sequestered within the oil droplet would be able to quench photo-excited molecules throughout the photoreceptor, let alone the rest of the retina, through which the light has already passed before reaching the oil droplets.

Carotenoids are mobilised from the skin, liver and fat reserves of birds exposed to oxidative stress (Costantini and Dell'omo, 2006) or in response to activation of the immune system (Faivre et al., 2003). Whether carotenoids are mobilised from the retinal cone oil droplets in response to such physiological insults, and if so whether this might affect visual sensitivity, is unknown, but it is a possibility that should be considered when studying the effects of stress on visually guided behaviours, such as mate choice.

Variations in the intensity and photoperiod of the ambient light experienced during development are known to have other effects on the visual system. For example, constant darkness ($\leq$1 lx) or continuous light causes enlargement of the eye and a reduction in corneal curvature in both turkeys (Ashton et al., 1973; Siopes et al., 1984; Davis et al., 1986) and chickens (Jenkins et al., 1979; Oishi and Murakami, 1985; Li et al., 2000; Liu et al., 2004) that results in hyperopia. Such effects on the developing avian eye have received considerable attention, partly because of the use of the chicken as a model for growth-related ocular diseases in humans, but also because of the importance of lighting conditions in poultry farming. Light levels in the cages of intensively farmed chickens and turkeys might be as low as 1 lx, either to save fuel costs, improve feed-conversion efficiency by discouraging activity or reduce injurious pecking (Manser, 1996; Moinard and Sherwin, 1999; Prescott and Wathes, 1999). It is obvious from the foregoing that birds reared under such low light conditions will exhibit morphological changes in their eyes that might be detrimental to their welfare, and alternatives such as environmental enrichment should be encouraged [e.g. as proposed by Sherwin et al. (Sherwin et al., 1999)]. These issues also highlight the need to maintain animals in conditions that are as close to their natural habitat as possible prior to conducting any spectral or anatomical studies of the retina.

Yew et al. reared chickens under different coloured lights and showed that those raised under blue light had a higher proportion of R-type (LWS single cone) oil droplets than those

raised under white, red or yellow light (Yew et al., 1978). However, both wavelength discrimination ability and threshold spectral sensitivity in chickens and pigeons are reportedly unaffected by early spectral deprivation (Rudolph and Honig, 1972; Brenner et al., 1983), suggesting that any variations in visual performance due to changes in cone proportions are compensated for at higher stages of the visual system. Nevertheless, there is evidence from electrophysiological studies that differences in oil droplet absorptance spectra might affect the spectral sensitivity of the visual system, which suggests that the changes in oil droplet pigmentation and λ_{cut} observed in the present study could have significant effects on vision. Brenner et al. showed that the dorso-temporal retina or 'red field' of the pigeon has a peak spectral sensitivity at longer wavelengths than the ventro-nasal retina or 'yellow field' (Brenner et al., 1983). Both the Y- and R-type oil droplets of the MWS and LWS single cones, respectively, in the red field of the pigeon retina are more densely pigmented and have λ_{cut} values at longer wavelengths than those same cone types in the yellow field (Bowmaker, 1977). Together with a higher proportion of MWS and LWS single cones in the red field compared with the rest of the retina [(Waelchli, 1883), cited in Bowmaker (Bowmaker, 1979)], these differences in oil droplet spectra might account for the observed differences in spectral sensitivity. Similarly, the dorsal retina of the chicken has a higher sensitivity to wavelengths between approximately 350 and 450 nm than the ventral retina (Wortel et al., 1987). This difference in relative sensitivity at short wavelengths might be because of the reduced filtering of UV radiation by the P-type oil droplets in the dorsal retina compared with those in the ventral retina (Fig. 2).

Short-term, phenotypic changes in ocular anatomy or physiology, such as the density of oil droplet pigmentation, reflect not only the susceptibility of visual systems to altered lighting conditions, but also their functional plasticity. Using a noise-limited model of spectral thresholds, Vorobyev showed that the benefit to colour discrimination of pigmented oil droplets in the avian retina was dependent on the intensity of the ambient light (Vorobyev, 2003). Spectral tuning by pigmented oil droplets allowed birds to discriminate more colours in bright light. However, the reduction in photon capture caused by the pigmented oil droplets, and the accompanying decrease in signal-to-noise ratio of the cone responses, meant that the benefit of coloured oil droplets to vision was marginal at light levels approximating those around twilight (~1–10 lx). The reduction in levels of oil droplet pigmentation in chickens reared in dim light in the present study suggests that absolute sensitivity is maintained at the expense of spectral tuning (colour vision) under these conditions. Speculating further, it is possible that, over evolutionary time, this would create a selection pressure of sufficient strength to cause the reduction or loss of oil droplet colouration in animals, including birds, that become more nocturnal in habit. Indeed, the adoption of nocturnality has been proposed as the reason for the loss of pigmentation in the cone oil droplets of marsupial (metatherian) mammals and the loss of oil droplets altogether in placental (eutherian) mammals (Walls, 1942).

In conclusion, although the cellular mechanisms responsible for modulating oil droplet pigment density are unknown, and are clearly a subject for further investigation, it is evident that the eyes of both vertebrates and invertebrates have several short- and long-term mechanisms to adapt to variations in the intensity and/or spectral distribution of the ambient illumination. Moreover, it is possible that many of these photostatic mechanisms offer a means for adaptive changes in visual system design over an evolutionary timescale.

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