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

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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

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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.