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Morphology and spectral absorption characteristics of retinal photoreceptors in the southern hemisphere lamprey (*Geotria australis*)

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Abstract

The morphology and spectral absorption characteristics of the retinal photoreceptors in the southern hemisphere lamprey *Geotria australis* (Agnatha) were studied using light and electron microscopy and microspectrophotometry. The retinae of both downstream and upstream migrants of *Geotria* contained two types of cone photoreceptor and one type of rod photoreceptor. Visual pigments contained in the outer segments of these three photoreceptor types had absorbance spectra typical of porphyropsins and with wavelengths of maximum absorbance (downstream/upstream) at 610/616 nm (long-wavelength-sensitive cone, LWS), 515/515 nm (medium-wavelength-sensitive cone, MWS), and 506/500 nm (medium-wavelength-sensitive rod). A "yellow" photostable pigment was present in the myoid region of all three types of photoreceptor in the downstream migrant. The same short-wavelength-absorbing pigment, which prevents photostimulation of the beta band of the visual pigment in the outer segment, was present in the rods and LWS cones of the upstream migrant, but was replaced by a large transparent ellipsosome in the MWS cones. Using microspectrophotometric and anatomical data, the quantal spectral sensitivity of each photoreceptor type was calculated. Our results provide the first evidence of a jawless vertebrate, represented today solely by the lampreys and hagfishes, with two morphologically and physiologically distinct types of cone photoreceptors, in addition to a rod-like photoreceptor containing a colored filter (a cone-like characteristic). In contrast, all other lampreys studied thus far have either (1) one type of cone and one type of rod, or (2) a single type of rod-like photoreceptor. The evolution or retention of a second type of cone in adult *Geotria* is presumably an adaptation to life in the brightly lit surface waters of the Southern Ocean, where this species lives during the marine phase of its life cycle. The functional significance of the unique visual system of *Geotria* is discussed in relation to its life cycle and the potential for color vision.

Keywords: Lamprey, Photoreceptor, Color vision, Spectral sensitivity, Filter

Introduction

Lampreys and hagfishes are the sole survivors of the very early agnathan (jawless) stage in vertebrate evolution (Hardisty, 1982). Recent studies have shown that lampreys, or their very close relatives, had already evolved by the lower Cambrian period [*ca*. 540 million years ago (Shu et al., 1999)]. Although lamprey eyes possess several primitive characteristics not found in jawed (gnathostomatous) fishes, their structure still conforms to the basic vertebrate plan (Duke-Elder, 1958; Land & Nilsson, 2002). While there has been some disagreement as to the precise identity of the complement of photoreceptors within the lamprey retina, it is now generally accepted that the retina of the northern hemisphere lampreys contains one type of rod and one type of cone photoreceptor. This conclusion is based on morphological (Walls, 1928; Yamada & Ishikawa, 1967; Öhman, 1971, 1976; Stell, 1972; Holmberg & Öhman, 1976; Holmberg, 1977; Dickson & Graves, 1979, 1982; Tonosaki et al., 1989), immunohistochemical (Negishi et al., 1987; Ishikawa et al., 1987), cytochemical (Ishikawa et al., 1989), spectral (Crescitelli, 1956, 1972; Govardovskii & Lychakov, 1984; Harosi & Kleinschmidt, 1993), biochemical (Wald, 1942, 1957), and electrophysiological (Holmberg et al., 1977; Govardovskii & Lychakov, 1984) studies of two genera of the northern hemisphere lampreys (*Petromyzon* and *Lampetra*). Interestingly, however, the rod photoreceptors appear to be capable of operating under light levels similar to that of cones (Govardovskii & Lychakov, 1984).

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The wavelength of maximum absorbance (λ_{max}) of the single type of cone photoreceptor in upstream migrants of *Lampetra fluviatilis* (Govardovskii & Lychakov, 1984) and *Petromyzon marinus* (Harosi & Kleinschmidt, 1993) lies at 555 nm and 600 nm, respectively. The λ_{max} for the rod photoreceptor lies at 517 nm in *L. fluviatilis* and 525 nm in *P. marinus*.

The southern hemisphere lamprey *G. australis*, the sole representative of the southern hemisphere family Geotriidae (Potter, 1980), is anadromous. Fully metamorphosed young adults of *G. australis* migrate downstream to the sea (Potter et al., 1980), where they feed parasitically by attaching themselves to fish and extracting blood and/or muscle tissue from their hosts (Potter & Hilliard 1987). During the parasitic phase, *G. australis* feeds in the brightly lit surface waters and increases in length from about 75–640 mm, after which it reenters rivers and takes about 15–16 months to migrate to its upstream spawning grounds (Hardisty & Potter 1971; Potter & Hilliard 1987).

Recent ultrastructural studies have demonstrated that downstream migrants of the southern hemisphere lamprey *G. australis* possess a retina that contains two distinct types of cone and a single type of rod (Collin et al., 1999), a finding consistent with that of Walls (1942) rather than Meyer-Rochow and Stewart (1996). The two cone types in *G. australis* (designated C1 and C2) are morphologically very similar and, due to the presence of a pyramid-shaped pedicle at their terminals (in contrast to the rod spherules), the sclerad location of their nuclei within the outer nuclear layer (ONL) and their tapered outer segments are considered to be similar to gnathostomatous cones (Collin et al., 1999). These two cone-like photoreceptors share many features with the cone photoreceptors of holarctic lampreys (Collin et al., 1999). However, the size, shape, and staining characteristics of the mitochondria within their inner segment and the presence of an accumulation of spherical to ovoid-shaped deposits of secretory material bound within the endoplasmic reticulum (refractile bodies) of the myoid distinguish both types of cone in *G. australis* from the cone of the northern hemisphere lampreys. Without further morphological and spectral evidence, it is impossible to draw any direct homology between either of the two cone-like photoreceptors in *G. australis* with the cone photoreceptors of the northern hemisphere (holarctic) lampreys.

The rod-like photoreceptors in downstream *G. australis* are characterized by a long, cylindrical outer segment, a nucleus that lies within the vitread region of the ONL and a spherical terminal containing up to three synaptic ribbons (Collin et al., 1999). These morphological features are essentially identical to those of the rod-like photoreceptors found in the eyes of the holarctic lampreys *Ichthyomyzon unicuspis* (S. P. Collin, unpublished data), *Petromyzon marinus* (Dickson & Graves, 1979; 1982), *Lampetra fluviatilis* (Öhman, 1971, 1976; Holmberg & Öhman, 1976; Holmberg, 1977), *Lampetra tridentata* (Stell, 1972; S. P. Collin & I. C. Potter, unpublished data), *Lampetra lamottenii* (Walls, 1928), and *Lampetra japonica* (Yamada & Ishikawa, 1967; Tonosaki et al., 1989). Therefore, the rod receptors in *G. australis* may be homologous to the rod receptors described for holarctic lampreys but further evidence is required to substantiate this conclusion.

The aim of this study was to (1) determine the spectral absorption characteristics of the visual pigments and intracellular spectral filters present in the photoreceptors of *G. australis* during both their downstream and upstream migration, (2) identify any morphological or physiological homologies between these receptors and those of holarctic lampreys, and (3) discuss the possible functional significance of the development of a second type of cone photoreceptor in the context of the visual ecology of *G. australis* and the potential for color vision.

Methods

Nine downstream migrating (75–110 mm in total length) and nine upstream migrating (560–640 mm in total length, Figs. 1A & 1B) adults of *G. australis* (Geotriidae, Agnatha) were collected from streams and rivers in south-western Australia using an electric fish shocker. All individuals were maintained in laboratories in either Perth or Brisbane, where temperature and light/dark regimes paralleled those in the field. The animals were kept at 17° C under a 12-h light/12-h dark cycle, mimicking, as much as possible, the environmental conditions in which the animals were captured, for example, providing a suitable substrate for the burrowing downstream migrants. Both downstream and upstream migrants were examined as soon as possible after capture (less than 8 weeks).

Microscopy

Following an overdose of methane tricaine sulfonate salt (MS 222, 1:2000) under the ethical guidelines of the National Health and Medical Research Council of Australia, ten individuals (5 downstream and 5 upstream) were sacrificed for light microscopical and ultrastructural characterization of the photoreceptor types. The technique closely follows that of Collin et al. (1999), where tissue was fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH, 7.4), and embedded in araldite before being sectioned on an LKB rotary ultramicrotome. Ultrathin sections stained with lead citrate and uranyl acetate were examined on either a Phillips 410 or a Phillips CM10 transmission electron microscope set at 80 kV.

Microspectrophotometry

Four dark-adapted individuals of both downstream and upstream migrants of *G. australis* were euthanased with an overdose of MS222 (1:2000) and their eyes removed. Retinae were dissected out under infrared illumination, cut into small pieces (*ca.* 1–2 mm^2), and mounted in a solution of 275 mOsmol kg^{-1} phosphate-buffered saline containing 10% dextran. Absorbance spectra of individual photoreceptor outer segments were measured using a single-beam, wavelength-scanning, computer-controlled microspectrophotometer (MSP), as described by Shand et al. (2002). The absorbance spectra were analyzed using the method of Govardovskii et al. (2000) to estimate the λ_{max} of the visual pigment. Spectra were fitted with both A_1 and A_2-based visual pigment templates (Govardovskii et al., 2000) to establish which type of chromophore was present. Measured outer segments were bleached with full spectrum white-light in order to confirm that the visual pigments were photolabile. Yellow pigments located in the inner segment and myoid were also examined using MSP and were found to be photostable after attempts to bleach the pigment were unsuccessful. These pigments even failed to bleach after hours of bright-field illumination of fixed and unfixed retinae.

Calculation of photoreceptor spectral sensitivities

Relative quantal spectral sensitivities were calculated for each photoreceptor type in both downstream and upstream migrants. Visual pigment spectral absorptance was modelled using mathematical templates of the appropriate λ_{max} (Govardovskii et al.,

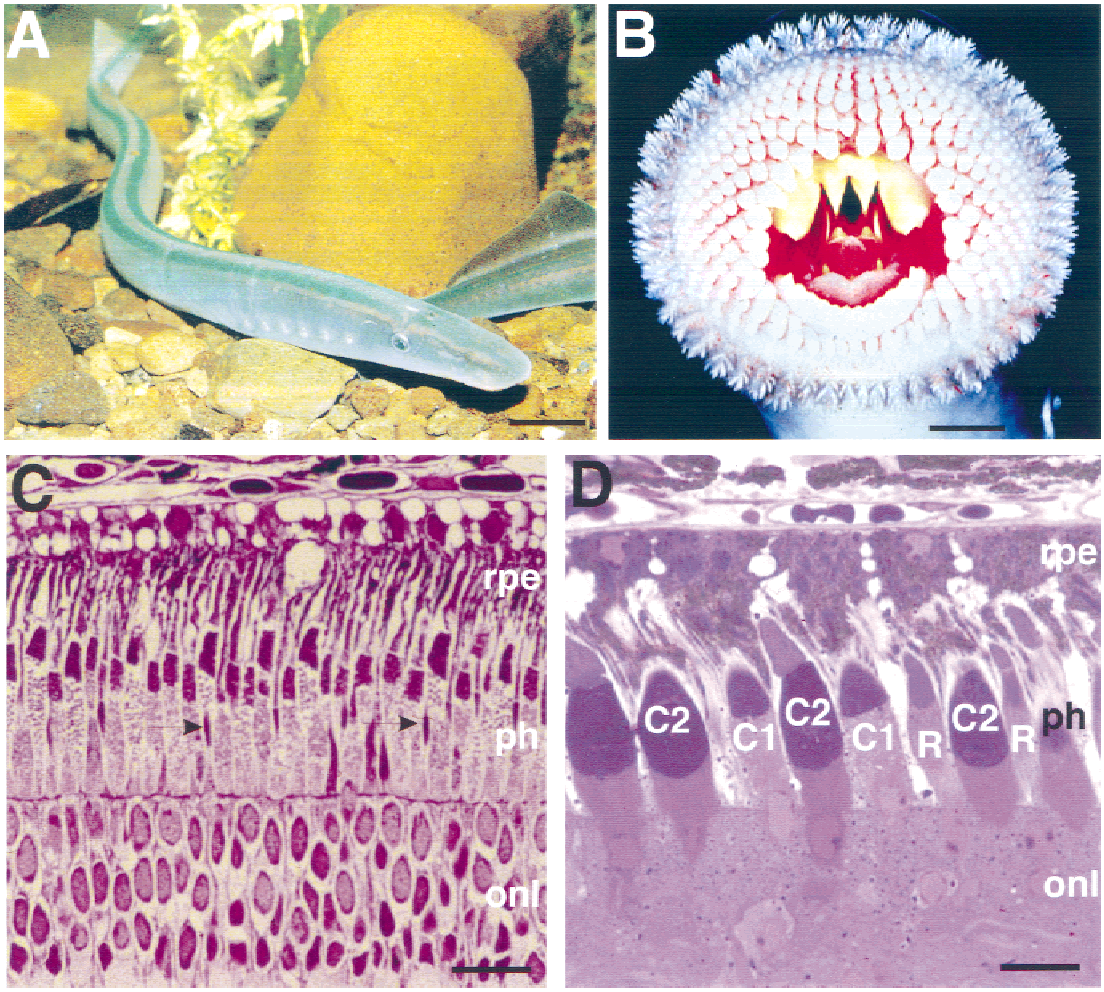

Fig. 1. (A) Upstream migrant of the southern hemisphere lamprey *Geotria australis*. Note the blue/silver counter shading, typically found in aquatic surface dwellers. Scale bar = 30 mm. (B) Suctorial disc of the upstream migrant that is used for attachment to its hosts during the marine phase of its life cycle. Scale bar = 10 mm. (C, D) Light micrographs of 1-μm (transverse) sections of the retina stained with toluidine blue in downstream (C) and upstream (D) migrants photographed at the same magnification and thus showing the marked changes in both photoreceptor size and morphology. Arrowheads in (C) depict rods. C1: long-wavelength-sensitive cone; C2: medium-wavelength-sensitive cone; onl: outer nuclear layer; ph: photoreceptor layer; R: medium-wavelength-sensitive rod; and rpe: retinal pigment epithelium. Scale bars = 15 μm.

2000). The specific absorbance used for the visual pigment was 0.015 μm^{-1} for all cells (Rodieck, 1973) and lengths of outer segments are given in Table 1. Mean absorptance spectra (fitted with an 11 point unweighted running average) of the regions of the inner segments occupied by the screening pigment or ellipsosome were corrected for the fact that microspectrophotometric measurements were made transversely not axially (dimensions listed in Table 1).

Results

Light microscopy and transmission electron microscopy were used to examine the ultrastructure of the three photoreceptor types in upstream migrants of *G. australis* (previously identified in the downstream migrants, Collin et al., 1999) and to characterize them as either rods or cones. Microspectrophotometric analysis of the visual pigments and various intracellular inclusions was also performed in order to predict the spectral sensitivity of each of the three retinal receptor types in both downstream and upstream migrants. All visual pigment absorbance spectra were best-fitted by an A_2 (porphyropsin) template, suggesting that the chromophore used by both downstream and upstream migrants was 3,4-didehydroretinal.

Long-wavelength-sensitive cones (C1)

At the commencement of the marine phase of *G. australis*, the first cone type (C1) has a tapered outer segment, an ellipsoid with densely packed mitochondria, a myoid containing aggregations of distended endoplasmic reticula, and a pedicle-shaped (cone-like) synaptic terminal with up to five synaptic ribbons (Collin et al., 1999; Fig. 1C). Apart from a marked increase in size, new morphological data reveals that the C1 cone in the fully grown adult, which has reentered rivers on its spawning run, is the same as that described at the beginning of the marine phase (Figs. 1C, 2A, & 3A). However, this C1 cone increases in length (25 $\pm$ 3.2 μm to

Table 1. *Summary of the microspectrophotometric measurements of the three photoreceptor types in both downstream and upstream migrant phases*[a]

Downstream *Geotria australis*	LWS cone (C1)	MWS cone (C2)	Rod
Mean prebleach λ_{max} (nm)	610.0 ± 9.7	515.4 ± 5.9	506.1 ± 4.9
λ_{max} of mean prebleach spectrum (nm)	611.1	516.8	507.0
Mean difference spectrum λ_{max} (nm)	605.7 ± 12.5	519.4 ± 6.3	513.2 ± 3.5
λ_{max} of mean difference spectrum (nm)	610.5	521.5	511.7
Number of cells	7	5	2
Mean transverse measured absorbance at λ_{max} of difference spectrum	0.011 ± 0.005	0.012 ± 0.005	0.009 ± 0.006
Calculated quantal spectral sensitivity	614	552	507
Upstream *Geotria australis*	LWS cone (C1)	MWS cone (C2)	Rod
Mean prebleach λ_{max} (nm)	616.4 ± 4.7	515.2 ± 3.0	499.7 ± 6.4
λ_{max} of mean prebleach spectrum (nm)	616.7	515.0	500.8
Mean difference spectrum λ_{max} (nm)	615.3 ± 6.6	517.7 ± 3.8	503.2 ± 5.8
λ_{max} of mean difference spectrum (nm)	616.4	517.4	506.9
Number of cells	5	20	7
Mean transverse measured absorbance at λ_{max} of difference spectrum	0.023 ± 0.006	0.031 ± 0.012	0.014 ± 0.004
Calculated quantal spectral sensitivity	618	517	503

[a]Quantal spectral sensitivity was calculated by modelling the absorptance of the visual pigment in the outer segment and the spectral transmittance of the photostable pigment located in the myoid region (see Methods). Values for the mean prebleach and difference spectra λ_{max} and mean transverse measured absorbances are ± one standard deviation.

58.9 ± 5.7 μm) and width (3.3 ± 0.2 μm to 11.2 ± 2.7 μm) during the period between its downstream and upstream migration (Figs. 1C & 1D). In unfixed retinae of both downstream and upstream migrants, the receptor regions occupied by the distended endoplasmic reticula in the myoid contain short-wavelength-absorbing pigments (Figs. 2A & 2D). The pigment in the downstream phase appears yellow, while that in the upstream phase appears more orange (Figs. 2A & 2D). Microspectrophotometry (MSP) demonstrates that both of these pigments are photostable and absorb strongly below about 550 nm (Figs. 4C & 4G). Although not examined biochemically, it is thought that these colored filters may comprise the same pigment in both migrant phases but occurring in different concentrations, as has been found for the orange and red oil droplets in the avian retina (Goldsmith et al., 1984). The mean wavelengths of maximum absorbance (λ_{max}) of the photosensitive visual pigments in the C1 cones of downstream and upstream migrants were very similar at 610 nm and 616 nm, respectively (Figs. 4A, 4B, & 4E–4F; Table 1). When the quantal spectral sensitivity is calculated for the whole photoreceptor (Figs. 4D & 4H), the comparable peaks at 614 nm and 618 nm show that the photostable pigments within the myoid would simply prevent photostimulation of the beta-band of the visual pigment, as is the case with some other short-wavelength-absorbing spectral filters (Muntz, 1973).

Medium-wavelength-sensitive cones (C2)

In the downstream migrants, the second type of cone photoreceptor (C2) has a tapered outer segment, a pedicle-shaped receptor terminal, and a myoid containing a yellow photostable pigment (Figs. 1C & 2B). The outer segment contains a photosensitive visual pigment with a mean λ_{max} at 515 nm (Figs. 5A & 5B). Although the spectral absorption characteristics of the visual pigment remain unchanged in upstream migrants (Figs. 5E & 5F), the yellow photostable pigment (Fig. 5C) has been replaced by a large unpigmented ellipsosome (Figs. 1D, 2E, 3B, & 5G). The development of the ellipsosome in the upstream phase coincides with an increase in both length (25 ± 3.7 to 60.1 ± 5.1 μm) and width (2.5 ± 0.5 to 11.2 ± 2.7 μm) (Figs. 1C & 1D). In the upstream phase, the C2 receptors also possess a tapered outer segment and a pedicle-shaped receptor terminal but, unlike the situation in the downstream phase, the endoplasmic reticula are not distended and the yellow pigment in the myoid has been lost and gives way to the large, essentially transparent, ellipsosome of mitochondrial origin. MSP confirms the lack of any pigment within the ellipsosome (Fig. 5G), which may function to focus light onto the outer segment rather than providing the capacity for any spectral filtering. However, in contrast to the C1 cone, the presence of the yellow screening pigment within the downstream C2 myoid shifts the calculated peak spectral sensitivity of the cell to a wavelength (543 nm) longer than the λ_{max} of the visual pigment.

Medium-wavelength-sensitive rods

As is typical of other vertebrate rod photoreceptors, the rod of the downstream migrant of *G. australis* contains a cylindrical outer segment with only a moderate taper and an ellipsoid containing numerous mitochondria (Fig. 1C). Like the C1 cone, the ellipsoid is filled with distended endoplasmic reticula that contain a pale yellow pigment (Fig. 2C). During the period between the downstream and upstream migrations, the rods increase in both length (15 ± 0.3 μm to 55.6 ± 1.9 μm) and width (1.7 ± 0.3 μm to 4.2 ± 1.5 μm) (Figs. 1C, 1D, 2C, 2F, & 3C). The rod visual pigments have a mean λ_{max} at 506 and 500 nm in the downstream and upstream migrants, respectively, and during both migrations, the myoid region contains low concentrations of a short-wavelength-

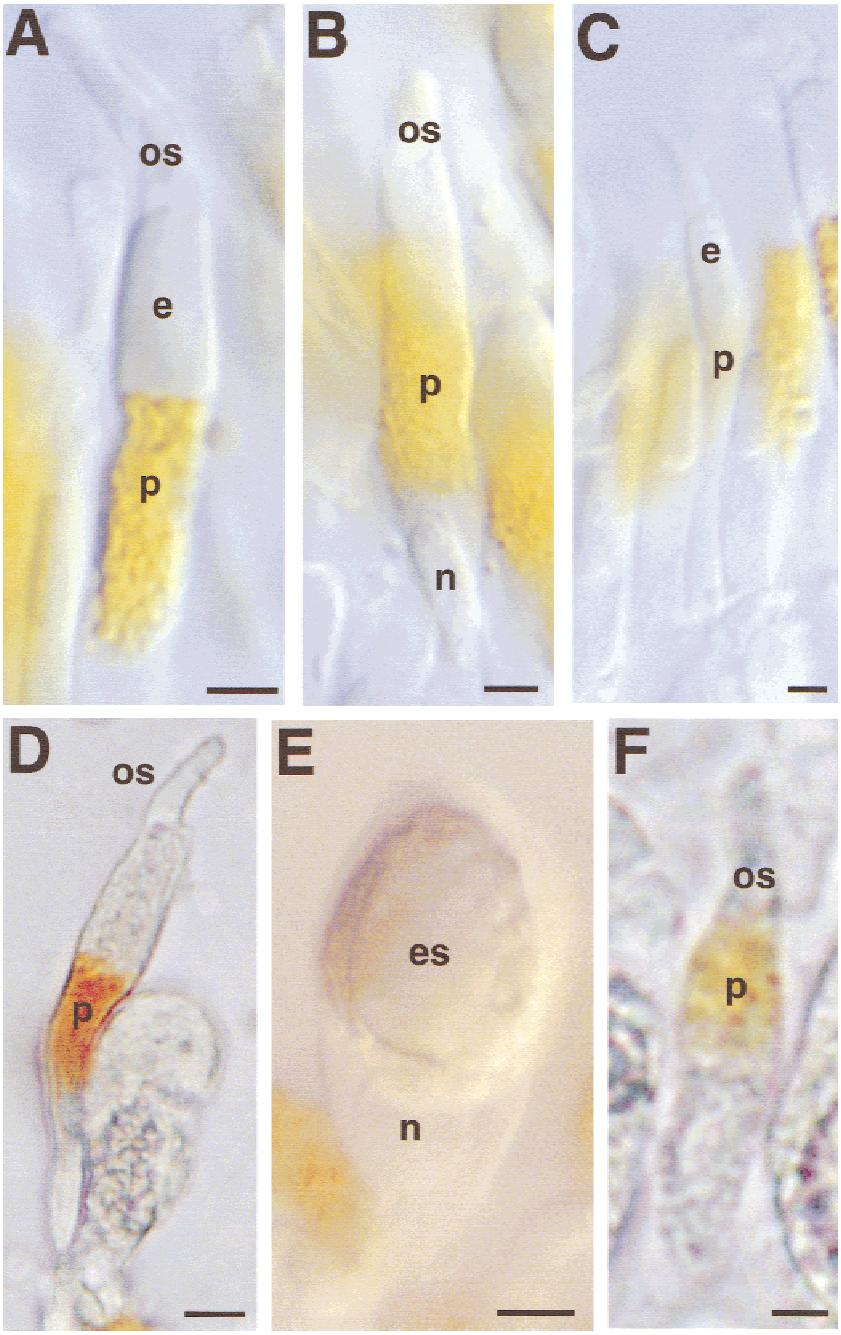

Fig. 2. Morphological characterization of the three types of photoreceptors in downstream (A–C) and upstream (D–E) migrants of *Geotria australis* photographed using differential interference contrast (Nomarski) optics in unfixed preparations. (A, D) The C1 cone. (B, E) The C2 cone. (C, F) The rod. Note the dense aggregations of yellow and orange pigment (p) in the myoid regions. The ellipsoid region (e) also contains low concentrations of pigment. The pigment in the myoid region of the C2 cone in downstream migrants (B) is replaced by a large ellipsosome (es) in the upstream migrants (E). n: nucleus; and os: outer segment. Scale bars: 3 μm (A); 2 μm (B); 2 μm (C); 6 μm (D); 4 μm (E); and 2 μm (F).

absorbing "yellow" photostable pigment that absorbs wavelengths below about 550 nm (Fig. 6). In both the downstream and upstream migrants, absorption by the yellow myoid pigment does not cause an appreciable shift in the calculated peak spectral sensitivity of the cell from that conferred by the visual pigment (Fig. 6).

Discussion

Ultrastructural and microspectrophotometric examinations of the retinal photoreceptors in downstream and upstream migrating adults of the southern hemisphere lamprey *G. australis* show that the retinae of both life cycle stages contain two types of cone and one type of rod. Each cone photoreceptor type has a different spectral sensitivity, strongly suggesting the possibility of a cone-based color vision system.

On the basis of the goodness-of-fit of the mean absorbance spectra to mathematical visual pigment templates (Govardovskii et al., 2000), each of the three visual pigments in both the downstream and upstream migrants of *G. australis* is a porphyropsin (where the chromophore conjugated with the opsin protein is 3, 4-didehydroretinal, an aldehyde of vitamin A_2). The possession of an A_2-based visual pigment by *G. australis* at the time this species enters the sea contrasts with the situation in the comparable stage of *Petromyzon marinus* and in marine teleosts and elasmobranchs, which, generally, have vitamin A_1-based visual pigments (rhodopsins) (Bowmaker, 1990 but see Cummings & Partridge, 2001).

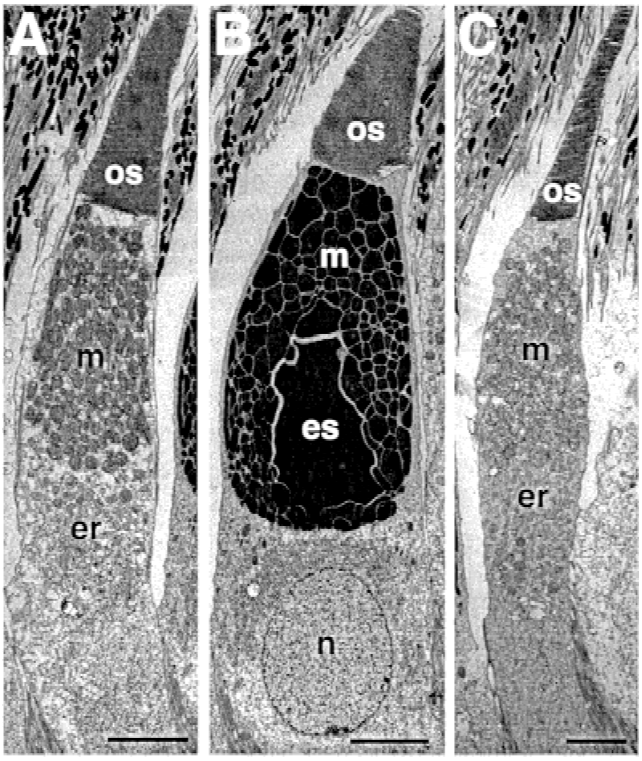

Fig. 3. Ultrastructural characterization of the photoreceptors in the retina of the upstream phase in *Geotria australis*. (A) C1 cone. (B) C2 cone. (C) Rod. Note the dense packing of distended endoplasmic reticula (er) in the myoid region of both the C1 cone and rod photoreceptors. The densely stained inner segment of the C2 cone possesses a developing ellipsosome (es) surrounded by mitochondria (m). n: nucleus; and os: outer segment. Scale bars = 4 μm.

Interestingly, during the upstream migration of *P. marinus*, the chromophore becomes A_2-based (Wald, 1942; Harosi & Kleinschmidt, 1993), as is typically the case in freshwater teleosts (Bowmaker, 1990). In contrast, the chromophore of the upstream migrating *Lampetra fluviatilis* is A_1-based, which almost certainly accounts for the short wavelength shifted λ_{max} (555 nm) of its cone visual pigment compared to *P. marinus* (600 nm) and the C1 cone of *G. australis* (610/616 nm in the downstream and upstream migrants, respectively). When considered together with the finding of the entire complement of photoreceptors possessing visual pigments incorporating a chromophore based on vitamin A_2 in the anadromous white sturgeon *Acipenser transmontanus*, it appears that migration between freshwater and saltwater may not be sufficient to induce a paired A_1/A_2 visual pigment system (Whitmore & Bowmaker, 1989; Sillman et al., 1995).

The λ_{max} of the visual pigments in the C1 cone of *G. australis* (610 and 616 nm in the downstream and upstream migrants, respectively) and the single cone types of *P. marinus* and *L. fluviatilis* lie within the range of the λ_{max} for the visual pigments in the long-wavelength-sensitive (LWS) cones of freshwater gnathostomatous fishes (Bowmaker, 1990). Therefore, the λ_{max} of the visual pigment of the C2 cone in *G. australis* (515 and 515 nm in the downstream and upstream migrants, respectively) falls outside the range for LWS cones in freshwater fishes and is more typical of a MWS cone.

The λ_{max} for the rod pigment (506 and 500 nm in the downstream and upstream migrants, respectively) of *G. australis* occurs at a shorter wavelength than the visual pigments in the rod photoreceptors of upstream migrants of the northern hemisphere lampreys *L. fluviatilis* (517 nm, Govardovskii & Lychakov, 1984) and *P. marinus* (525 nm, Harosi & Kleinschmidt 1993). The rod λ_{max} for *G. australis* is thus more similar to the rod λ_{max} of gnathostomatous vertebrates, especially marine teleosts (Bowmaker, 1990). Another interesting feature of the rod photoreceptors in *Geotria* is the yellow photostable pigment found in the inner segment. While spectral filters occur in the cone photoreceptors of some jawed fishes, reptiles, birds, and mammals (albeit packaged differently), this is the first report of an intracellular spectral filter in a rod photoreceptor. This raises the question of whether the rods are operating under light levels similar to those of the cone photoreceptors, as has been suggested to be the case in the northern hemisphere lamprey *Lampetra fluviatilis* (Govardovskii & Lychakov, 1984).

Why should *G. australis* have retained or evolved a second cone type which, unlike the other cone of this species and the single cone of other lamprey species, is medium wavelength sensitive (MWS)? Since *G. australis* spends only a short time migrating downstream, travelling at night, and not feeding, the characteristics of the retina of downstream migrants presumably represent a preadaptation for life in the ocean. During its marine trophic phase, *G. australis* is particularly susceptible to avian predation. This view is based on the very large numbers of adult *G. australis* that are caught by the grey-headed albatross *Diomedia chrysoma* (Potter et al., 1979). Since albatrosses feed in an average water depth of only 0.74 m (Huin & Prince, 1997), the adults of *G. australis* must spend much of their day in the well-lit surface marine waters of the Southern Ocean, in contrast to species such as *P. marinus*, which often occupy deep waters (Beamish, 1980; Halliday, 1991). Consequently, it may be advantageous for *G. australis* to be able to detect visually not only prey but avian predators in surface waters, which, during the austral summer, remain brightly lit for almost 24 h.

Having two cone types, one of which is LWS and the other MWS (Fig. 7), may increase the ability to detect the achromatic contrast (brightness) between objects observed against background illumination and thus improve the ability of *G. australis* to detect the silhouettes of avian predators against the sky or fish against the water (Lythgoe, 1979). Sillman et al. (1999) have suggested a similar function for the multiple cone pigments in the shovelnose sturgeon *Scaphirhynchus platorynchus* and the paddlefish *Polyodon spathula*, which both live in an environment where light of all wavelengths is limited. In brightly lit water, Maximov (2000) has suggested that selection pressures to overcome the visual problems caused by light flicker at the surface of the ocean may have led to the evolution of two such types of cone visual pigment as long ago as 500 million years.

It is also possible that the outputs from the two cones are compared by the visual system of this lamprey in order to analyze chromatic (color) information in the environment in addition to brightness. At least two types of horizontal cells, which are the first level of visual processing in the retina, have been revealed ultrastructurally (Collin et al., 1999) and immunohistochemically (S. P. Collin and M. Kalloniatus, unpublished data) in *G. australis*, and so the neural substrate for opponent interactions between different photoreceptors exists. Moreover, both types of cone (and the rods) are known, from topographical analysis, to be distributed throughout the entire retina with approximately three times more rods than cones (the numbers of C1 and C2 cones are comparable, K. Wallace and S. P. Collin, unpublished data). Of course, if the rod

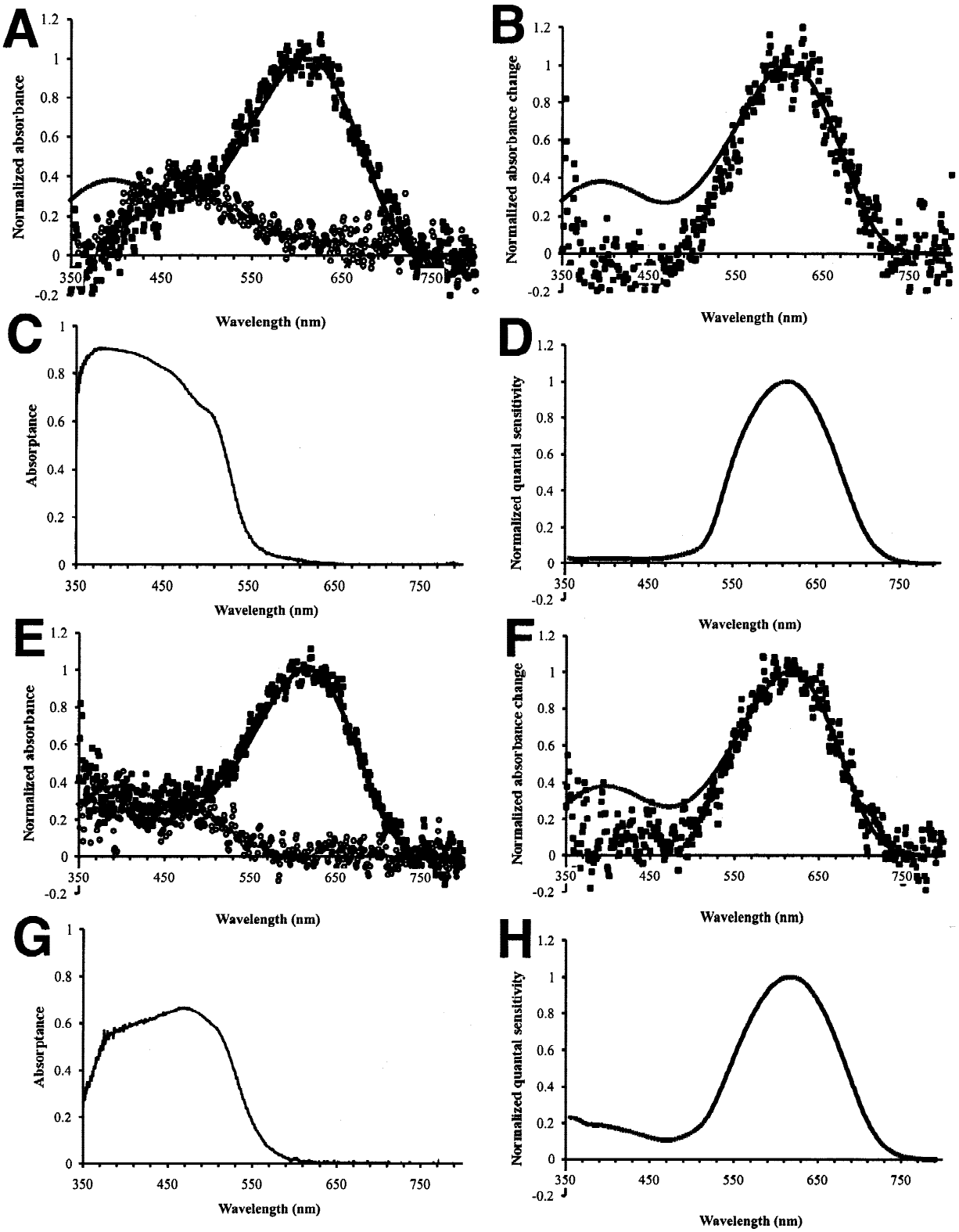

Fig. 4. Spectral characteristics of the LWS (C1) cone in downstream (A–D) and upstream (E–H) migrants of *Geotria australis*. (A, E) Prebleach and postbleach absorbance spectra. Filled squares indicate the mean prebleach absorbance spectrum fitted with the template spectrum of Govardovskii et al. (2000) (thick line). The open circles represent the mean postbleach absorbance spectrum fitted with an unweighted running average (thin line). (B, F) Mean difference spectra (filled squares) fitted with a template spectrum of Govardovskii et al. (2000) (thick line). (C, G) Mean absorptance spectrum of the photostable pigment in the myoid region fitted with an unweighted running average (smooth line). (D, H) The relative quantal spectral sensitivity of the whole photoreceptor for the downstream (λ_{max} = 610 nm) and upstream (λ_{max} = 616 nm) migrants, based on both the visual pigment and screening pigment spectra and the dimensions of the inner and outer segments.

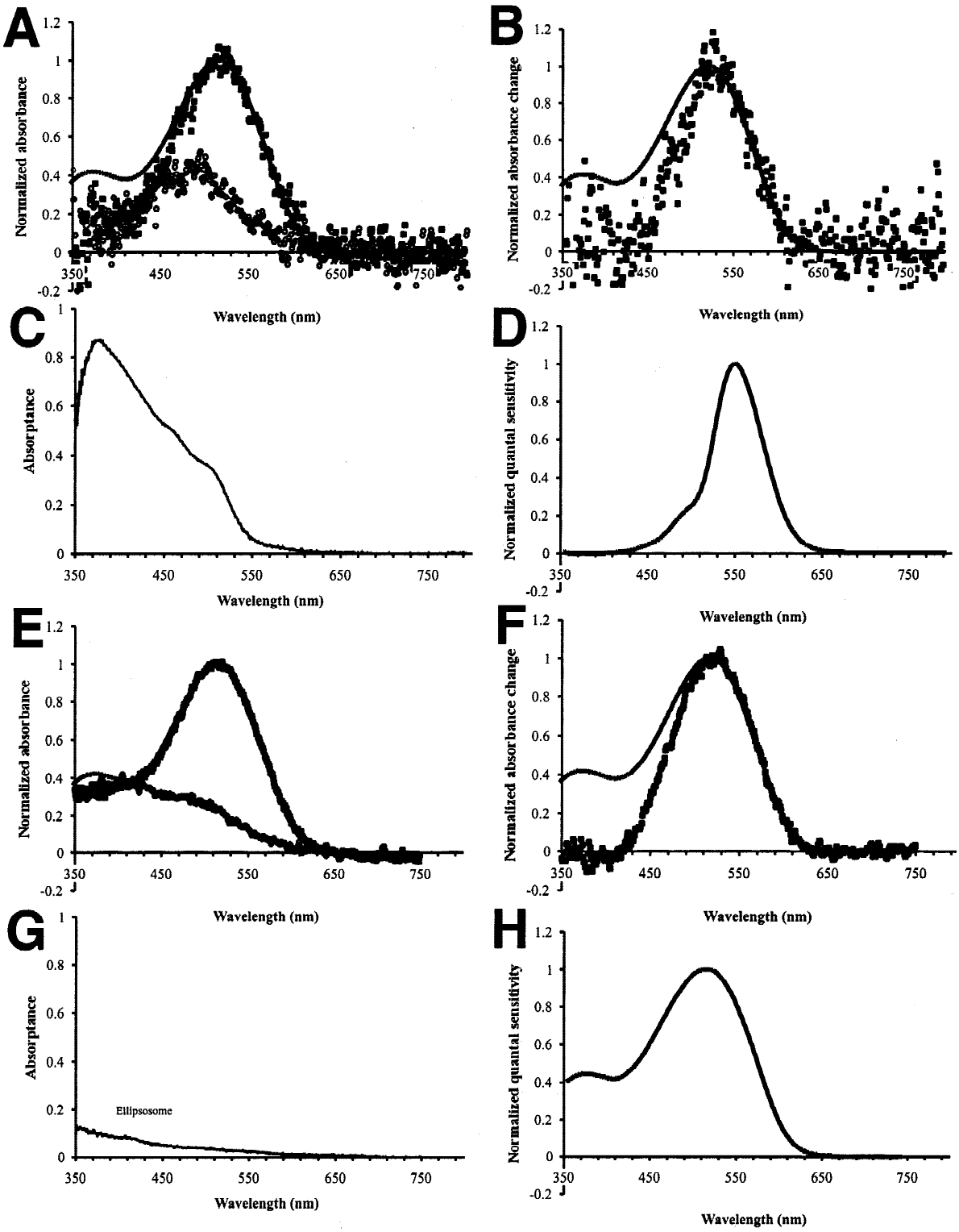

Fig. 5. Spectral characteristics of the MWS (C2) cone in downstream (A–D) and upstream (E–H) migrants of *Geotria australis*. (A, E) Prebleach and postbleach absorbance spectra. Filled squares indicate the mean prebleach absorbance spectrum fitted with the template spectrum of Govardovskii et al. (2000) (thick line). Open circles represent the mean postbleach absorbance spectrum fitted with an unweighted running average (thin line). (B, F) Mean difference spectra (filled squares) fitted with a template spectrum of Govardovskii et al. (2000) (thick line). (C, G) Mean absorptance spectrum of the photostable pigment in the myoid region fitted with an unweighted running average (smooth line). Note high concentration of pigment in the myoid region of the downstream migrant cuts off short wavelengths below approximately 550 nm and is replaced, in the upstream migrant, by an ellipsosome, which contains no pigment. (D, H) The relative quantal spectral sensitivity of the whole photoreceptor of downstream (λ_{max} = 515 nm) and upstream (λ_{max} = 515 nm) migrants based on both the visual pigment and screening pigment spectra and the dimensions of the inner and outer segments. The myoidal screening pigment in the downstream migrant shifts the peak spectral sensitivity from 515 nm to 543 nm.

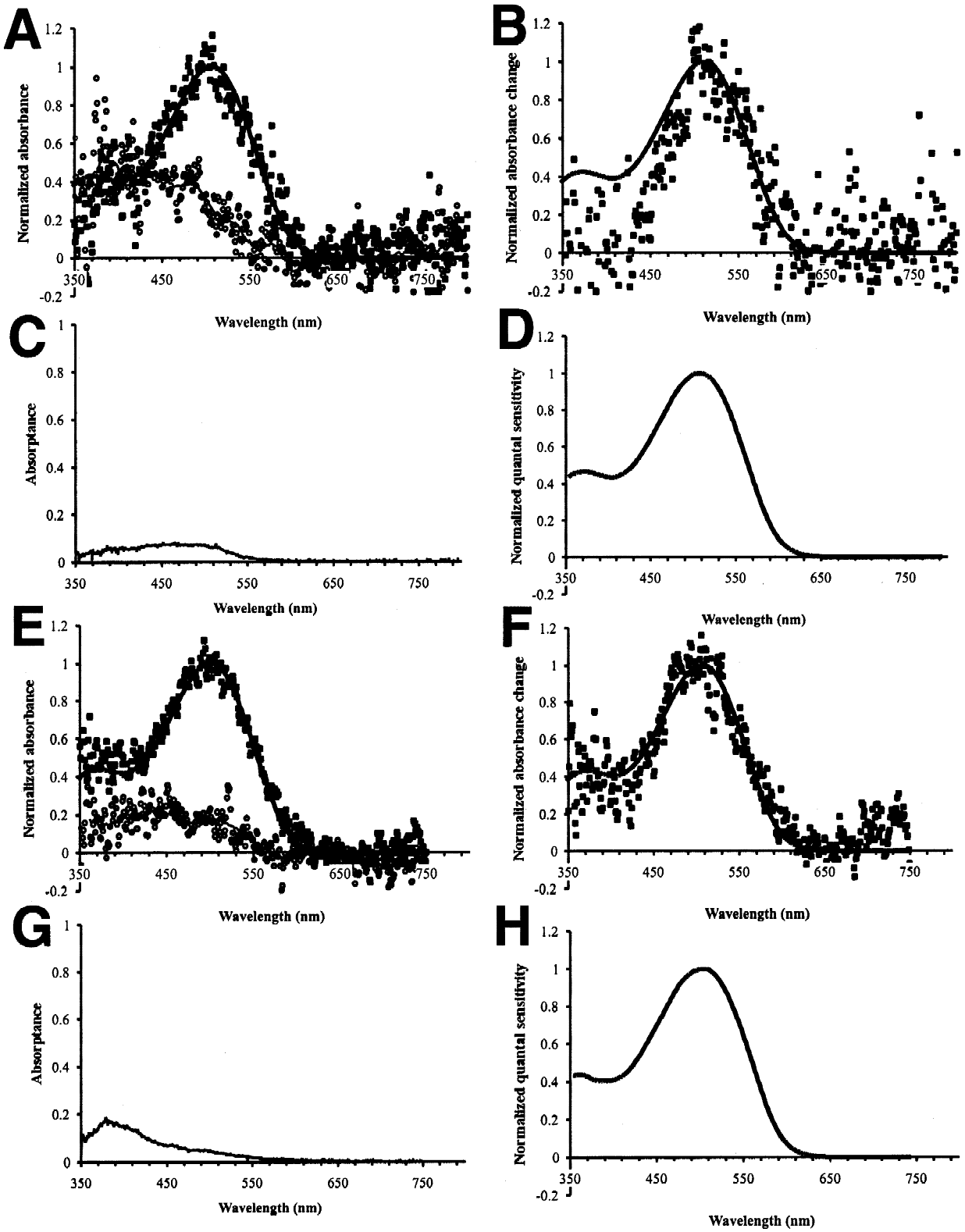

Fig. 6. Spectral characteristics of the MWS rod in the downstream (A–D) and upstream (E–H) migrants of *Geotria australis*. (A, E) Prebleach and postbleach absorbance spectra. Filled squares indicate the mean prebleach absorbance spectrum fitted with the template spectrum of Govardovskii et al. (2000) (thick line). Open circles represent the mean postbleach absorbance spectrum fitted with an unweighted running average (thin line). (B, F) Mean difference spectra (filled squares) fitted with a template spectrum of Govardovskii et al. (2000) (thick line). (C, G) Mean absorptance spectrum of the photostable pigment in the myoid region fitted with an unweighted running average (smooth line). Note lower concentration of pigment in the myoid region in both stages. (D, H) The relative quantal spectral sensitivity of the whole photoreceptor for the downstream (λ_{max} = 506 nm) and upstream (λ_{max} = 500 nm) migrants based on both the visual pigment and screening pigment spectra and the dimensions of the inner and outer segments.

photoreceptors of *G. australis* are also functioning under the same light levels as the cones (as in *Lampetra fluviatilis*; Govardovskii & Lychakov, 1984) there is the potential for a trichromatic, rather than simply a dichromatic, color vision system. Behavioral studies must be performed to investigate this possibility.

The presence of a large ellipsosome within the ellipsoid region of the C2 (MWS) cones in upstream, but not downstream, migrants of *G. australis*, which is the first report of such a structure in lampreys, suggests that the need for spectral filtering of short wavelengths decreases markedly during the adult phase of this species. Described by Franz (1932) as false-oil droplets, these large globules of mitochondrial origin have been previously recorded in cone photoreceptors in a number of vertebrate classes, including teleosts (Walls, 1942; Ishikawa & Yamada, 1969; Borwein & Hollenberg, 1973; Kunz & Regan, 1973; Kunz & Wise, 1973; Anctil & Ali, 1976; MacNichol et al., 1978; Nag & Bhattacharjee, 1989, 1995; Nag, 1995) and mammals (Knabe et al., 1997) including primates (Bowmaker, 1991). Although the ellipsosomes in upstream migrants of *G. australis* do not act as spectral filters, similarly large mitochondria or ellipsosomes in fish have been shown to possess a spectral absorbance that is characteristic of a dense heme pigment, similar to that of reduced cytochrome c (Avery & Bowmaker, 1982; Bowmaker, 1990). This heme pigment has been found to absorb light within a narrow band in the violet region of the spectrum (around 415 nm), thereby reducing to shorter wavelengths the spectral sensitivity of the visual pigments housed within the outer segments (MacNichol et al., 1978; Avery & Bowmaker, 1982; Bowmaker, 1990). The loss by upstream migrants of *G. australis* of the yellow screening pigment in the C2 photoreceptor and its replacement by a transparent ellipsosome is consistent with the fact that these individuals migrate at night. The spherical C2 ellipsosome may play a role in trapping photons and focusing them onto the visual pigment in the outer segment, thereby enhancing visual sensitivity (Young & Martin, 1984). The subsequent loss of the screening pigment by the MWS photoreceptor in the upstream migrant also shifts its spectral sensitivity to shorter wavelengths to align closely with the λ_{max} of the rod photoreceptor (Fig. 7). Physiological analysis is required to indicate the range of light levels in which the rods are operating and whether the close proximity of the spectral sensitivity curves of the MWS receptor and the rod, and the change in the relative spacing of the three receptor sensitivities, decreases this species' capacity for color discrimination.

Among lampreys, the yellow photostable pigment found in all three photoreceptor cell types is unique to *G. australis*, and presumably evolved in response to the need to filter out the potentially damaging short-wavelength light to which the eye is constantly being subjected when this species is in surface marine waters. Interestingly, a type of single cone in the ornate lizard *Ctenophorous ornatus*, which also inhabits a brightly lit environment, has recently been found to possess a similar yellow pigment (Barbour et al., 2002). Based on the spectral absorptance properties of the yellow pigments in *G. australis*, we suspect that they may be carotenoids and similar to the screening pigments identi-

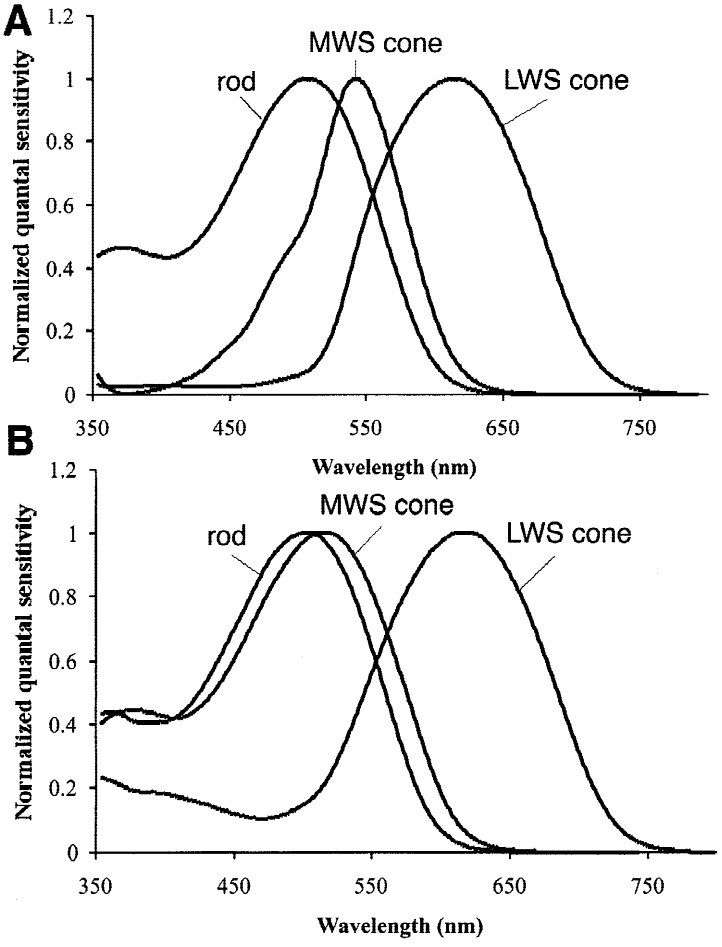

Fig. 7. Summary of the calculated photoreceptor quantal spectral sensitivity curves for the three types of photoreceptors in downstream (A) and upstream (B) migrants of *Geotria australis*. The effect of the myoidal screening pigment on spectral sensitivity is greatest in the medium-wavelength-sensitive (C2) cones of the downstream migrants [and thus presumably of marine phase animals] where it shifts the spectral sensitivity of the cell to longer wavelengths (peak 543 nm) compared to the λ_{max} of the visual pigment (515 nm), a value intermediate between the rod and the long-wavelength-sensitive (C1) cone.

fied in the cornea and lens of a number of species that frequent the upper regions of the water column (Douglas & Marshall, 1999; Siebeck & Marshall, 2001; Collin & Collin, 2001). The removal of short-wavelength light would also prevent photostimulation of the beta-band of the visual pigments (potentially improving wavelength discrimination) and increase acuity by absorbing short-wavelength light scattered by the atmosphere or ocular structures of the eye (Muntz, 1973).

Whether increasing the range of wavelengths available for cone-based vision and/or the need to sample the visual world chromatically was the selective force behind the evolution (or retention) of a second cone type by *G. australis* is not known. However, the implications of our findings suggest that the early vertebrates may hold important clues to the selection pressure(s) underlying the plasticity of photoreception and the evolution of color vision. The molecular genetic basis of photoreception in lampreys is now the subject of intense study in our laboratory.

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