

Early evolution of multifocal optics for well-focused colour vision in vertebrates

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SUMMARY

Jawless fishes (Agnatha; lampreys and hagfishes) most closely resemble the earliest stage in vertebrate evolution and lamprey-like animals already existed in the Lower Cambrian [about 540 million years ago (MYA)]. Agnathans are thought to have separated from the main vertebrate lineage at least 500 MYA. Hagfishes have primitive eyes, but the eyes of adult lampreys are well-developed. The southern hemisphere lamprey, *Geotria australis*, possesses five types of opsin genes, three of which are clearly orthologous to the opsin genes of jawed vertebrates. This suggests that the last common ancestor of all vertebrate lineages possessed a complex colour vision system. In the eyes of many bony fishes and tetrapods, well-focused colour images are created by multifocal crystalline lenses that compensate for longitudinal chromatic aberration. To trace the evolutionary origins of multifocal lenses, we studied the optical properties of the lenses in four species of lamprey (*Geotria australis*, *Mordacia praecox*, *Lampetra fluviatilis* and *Petromyzon marinus*), with representatives from all three of the extant lamprey families. Multifocal lenses are present in all lampreys studied. This suggests that the ability to create well-focused colour images with multifocal optical systems also evolved very early.

Key words: longitudinal spherical aberration, longitudinal chromatic aberration, lens, multifocal, colour vision, evolution, lamprey.

INTRODUCTION

The focal length of a simple lens varies as a function of the wavelength of light. Short wavelengths (blue) are focused at shorter distances from the lens, whereas long wavelengths (red) are focused at longer distances from the lens (Hecht, 2002). This longitudinal chromatic aberration (LCA) leads to chromatic blur that degrades image quality. In manufactured optical systems, LCA is minimized by using a combination of several lenses composed of different refractive materials (Hecht, 2002).

The biological solution to LCA is the multifocal lens. Such a lens has several distinct zones that focus monochromatic light of a particular wavelength at different distances. Since the refractive power of the multifocal lens is also a function of the wavelength of light, each of the zones of different focal length for monochromatic light focuses a different band of wavelengths on the same plane in the retina. A sharp colour image is created on the background of defocused light. This solution seems at first sight to be inferior to the technical solution, but has the important advantage that the optical system is considerably smaller, which is of energetic benefit to the animal.

Multifocal lenses were first discovered in the African cichlid fish *Astatotilapia* (formerly *Haplochromis*) *burtoni* (Kröger et al., 1999), where each of the three focal lengths of the lens focuses wavelengths that closely match the wavelength of maximum absorbance (λ_{\max}) of one of the three cone photoreceptor types present in the retina. Multifocal lenses are now known to be present in other species of bony fish (Malkki and Kröger, 2005; Karpesta et al., 2007) and this mechanism for minimizing LCA was retained when the cornea came into play as a refractive element during the evolutionary transition from aquatic to terrestrial life. The success of this optical design is reflected in the multifocal systems present in a variety of amphibians, reptiles and mammals (including primates) (Malmström

and Kröger, 2006). The evolutionary origins of this lens design, however, are unknown.

It is known that the ability to discriminate between different wavelengths of light (colour vision) evolved very early. The southern hemisphere lamprey *Geotria australis* possesses five photopigments with λ_{\max} of 359 nm, 439 nm, 497 nm, 492 nm and 560 nm (Davies et al., 2007). At least three of the five visual pigments [long wavelength sensitive (LWS), and short wavelength sensitive types 1 and 2 (SWS1 and SWS2)] are orthologous to the visual pigments in jawed vertebrates (Collin et al., 2003a). Although rod opsin-like opsin type A (RhA) and type B (RhB) of lampreys share similarities with the rod opsin (Rh1) and Rh1-like cone opsin (Rh2) of jawed vertebrates, respectively (Collin et al., 2003b; Collin and Trezise, 2004; Pisani et al., 2006; Collin and Trezise, 2006), the functional identity of the receptors housing these visual pigments remains elusive. It has, however, been suggested that all five photoreceptors in *G. australis* have close affinities to those of cones in gnathostomes [jawed vertebrates (Lamb et al., 2007)].

Lampreys and hagfishes are the only extant jawless fishes (Agnatha) and they most closely resemble the earliest stage in vertebrate evolution. Fossils of similar animals have been found in layers dating from the early Cambrian [about 540 million years ago (MYA)] (Shu et al., 2003). The separation of agnathans from the main vertebrate lineage is thought to have occurred at least 500 MYA (Gess et al., 2006). Although the eyes of hagfishes are rudimentary, they may hold important clues about the early evolution of the vertebrate eye (Lamb et al., 2007). However, since they lack a lens and further research is required to assess the differentiation of the retinal photoreceptor types (Holmberg, 1977), an analysis of the eyes of adult lampreys, which possess well-developed eyes that closely follow the eye design of jawed vertebrates (Walls, 1942;

Duke-Elder, 1958), may hold more promise in tracing the origin of multifocal optical systems.

In order to investigate whether the early evolution of a wide range of chromatic sampling at the level of the photoreceptors (Collin et al., 2003b) was paralleled by the optical system of the eye, we studied lens optics in four species of lamprey (Fig. 1). The 38 known species of lampreys have an anti-tropical distribution with 34 species occurring in the northern hemisphere and four species in the southern hemisphere (Gill et al., 2003). We studied two species from the northern hemisphere: *Lampetra fluviatilis* and *Petromyzon marinus*, and two species from the southern hemisphere: *Mordacia*

praecox and *Geotria australis*. This covered all three extant families of lamprey (Petromyzontidae, Mordaciidae and Geotriidae).

Lampreys spend several years as larvae (ammocoetes) buried in river beds, filter-feeding and with their developing eyes covered by pigmented skin. The eyes then rapidly become functional at the end of the larval period. Some lampreys are anadromous, i.e. after metamorphosis they migrate from their natal freshwater river into the sea to return years later and ascend the river for breeding. The adults of these lamprey species parasitize bony fishes, digesting blood and/or flesh from their hosts using horny teeth housed within their specialized mouthparts. About half of the extant species of lampreys do not have a parasitic stage, but spawn directly after transformation from ammocoete to adult (Hardisty and Potter, 1971) and some populations have become land-locked in freshwater systems.

The complement of photoreceptor types in the retina of each species of lamprey examined is predicted to be an important factor in the selection pressures underlying the evolution of multifocal lenses. In *G. australis* there are five morphologically distinct photoreceptors (all with cone-like characteristics), each expressing a different photopigment (Davies et al., 2007). The holarctic lampreys *L. fluviatilis* and *P. marinus* each possess two morphological types [both considered to be cones by Dickson and Graves (Dickson and Graves, 1979)] with λ_{\max} values of 525 nm and 600 nm, and 517 nm and 555 nm, respectively (Govardovskii and Lychakov, 1984; Harosi and Kleinschmidt, 1993). Only one morphological type (seemingly a hybrid between a rod and a cone) has been described for *M. mordax*, which is the parasitic homologue of *M. praecox*, with a λ_{\max} of 514 nm (Collin et al., 2004). Presumably being a monochromat, *M. praecox* might do best with a monofocal lens, whereas in the other species the spectral separation between visual pigments housed within the outer segments of the different photoreceptor types is wide enough to make multifocal lenses advantageous.

MATERIALS AND METHODS

Animals

Four species of lampreys were studied, two species from the northern hemisphere (*Lampetra fluviatilis* L. and *Petromyzon marinus* L.) and two species from the southern hemisphere (*Mordacia praecox* Potter 1968 and *Geotria australis* Gray 1851). *L. fluviatilis* (Fig. 1A) was from the Baltic Sea and was sampled during the upstream migration in the Ljusnan River, Sweden. *P. marinus* (Fig. 1B) was sampled from the land-locked population in Lake Michigan, USA. *M. praecox* (Fig. 1C) is not anadromous and was sampled from the Yarra River in south east Australia. *G. australis* (Fig. 1D) was sampled from the Warren River in south west Australia during the downstream migration. For the numbers of lenses, animals and total animal body lengths see Table 1.

Three complementary methods were used to study the optical properties of the crystalline lenses: eccentric slope-based infrared photoretinoscopy, schlieren photography and laser scanning (Malkki and Kröger, 2005).

Photoretinoscopy

The animals were lightly anaesthetized with 3-aminobenzoic acid ethyl ester (MS-222, 100 mg l⁻¹; Sigma, St Louis, MO, USA) in a small water-filled aquarium at 10°C. After allowing the lamprey to acclimatize for about 5 min, each eye was videotaped with a retinoscope for approximately 1–1.5 min and some typical frames exported to a computer using Adobe Premiere 6.0 software. Developed by Schaeffel and coworkers (Schaeffel et al., 1987; Schaeffel et al.,

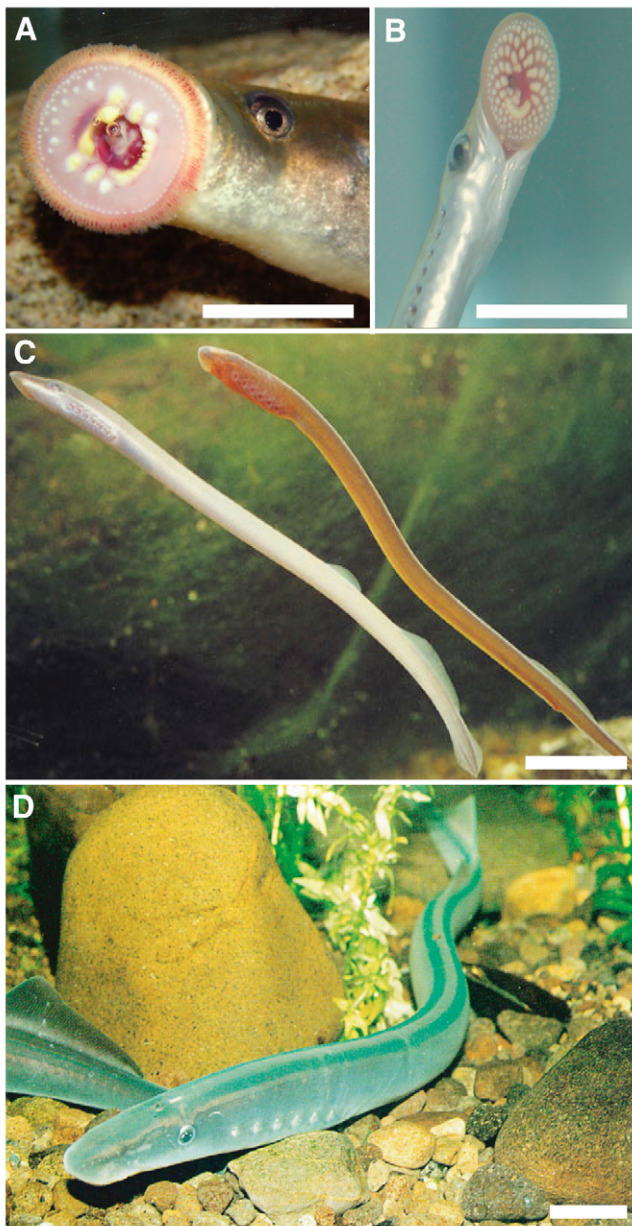


Fig. 1. Images of the four species of lamprey used in this study. (A) *Lampetra fluviatilis* (river lamprey). (B) *Petromyzon marinus* (sea lamprey). (C) *Mordacia mordax* [the parasitic homologue of the non-parasitic *Mordacia praecox*, from Collin et al. (Collin et al., 2004)]. (D) *Geotria australis* (pouched lamprey) [from Collin et al. (Collin et al., 2003b)]. Scale bars, 20 mm. Note that the eyes are well developed in all species.

1993), eccentric slope-based infrared photoretinoscopy (Fig. 2A) provided an indication of the distance, relative to the camera, at which the eye is focused. If an eye has multiple focal lengths, ring-like patterns varying in brightness appear in the pupil. Infrared light was used to avoid interference with the animals.

Schlieren photography

After photoretinoscopy was performed and the eyes were found to be normal for the species, the animal was sacrificed by decapitation under deep anaesthesia with MS-222 (500 mg l⁻¹). Immediately after enucleation, the crystalline lens was dissected free from one eye and suspended using a pair of forceps which held the equatorial membrane that supports the lens in the eyecup. The lens was then immersed in a small plastic tank containing Hepes-buffered Ringer solution (NaCl 138 mmol l⁻¹, KCl 2.1 mmol l⁻¹, MgCl₂·6H₂O 1.2 mmol l⁻¹, glucose 4 mmol l⁻¹, Hepes 2 mmol l⁻¹, CaCl₂·2H₂O 1.8 mmol l⁻¹; pH 7.4) in order to examine its optical properties by schlieren photography (Fig. 2B) using the white light of a cold light source. Photographs of the immersed lens were taken with a digital camera (Sony DSC-F 707). The double-pass design allowed for correct focusing of the lens and the resultant images gave indications of the spectral ranges of light being brought to focus. Characteristic coloured rings appeared if the lens was multifocal (Malkki and Kröger, 2005).

Laser scanning

We used a 537 nm diode-pumped solid-state laser in a scanning setup (Fig. 2C) to determine quantitatively the longitudinal spherical

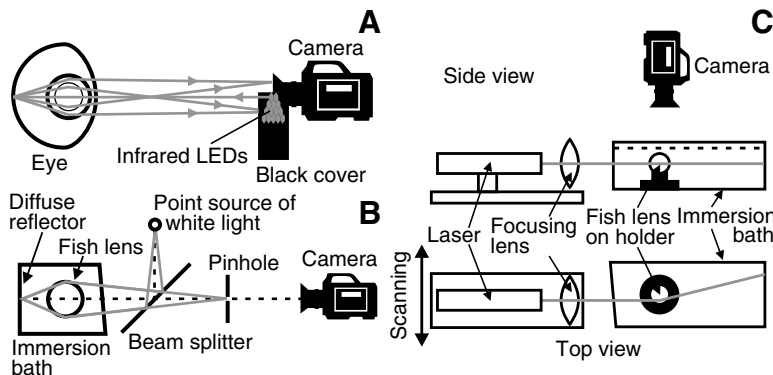


Fig. 2. Schematic representations of the three methods used to study the optical properties of the lamprey eyes. (A) Photoretinoscopy. The lower half of the objective of an infrared-sensitive digital camera is covered by a black occluder holding an array of infrared light-emitting diodes (LEDs). Light reflected back towards the camera from the fundus of the eye is spatially filtered by the occluder, which leads to a light upper half of the pupil if the eye is focused behind the camera and a light lower half of the pupil if the eye is focused in front of the camera. Multifocal optical systems lead to alternating light and dark regions in the pupil. (B) Schlieren photography (setup seen from above). White light is reflected via a beam splitter onto the lamprey lens. The light is focused by the lamprey lens onto a diffuse reflector. Reflected light is focused by the lamprey lens onto a small aperture (pinhole) mounted in front of a digital colour camera. Only light that has passed through the pinhole can be used to take a photograph of the lamprey lens. (C) Laser scanning. The beam of a green laser is focused to reduce beam diameter and scanned through a meridional plane of the lens. Beam paths are recorded with a digital video camera. Longitudinal spherical aberration (LSA) is determined from exported frames by a custom-written program.

Table 1. Numbers of lenses and animals used, average total lengths of the animals, average lens diameters and average focal lengths

Species	Number of lenses (animals)	Total body length (cm)	Lens diameter (mm)	Focal length (537 nm; R)
<i>Lampetra fluviatilis</i>	16 (9)	29.9±1.95	1.99±0.07	2.31
<i>Petromyzon marinus</i>	18 (10)	15.5±0.70	1.75±0.05	2.31
<i>Mordacia praecox</i>	5 (3)	10.8±0.53	1.00±0.10	2.32
<i>Geotria australis</i>	5 (3)	10.17±0.3	0.87±0.06	3.04

Values are mean ± standard deviation (s.d.). Focal lengths are normalized to lens radius (R).

aberration (LSA) of the lens (Campbell and Hughes, 1981; Sivak, 1982; Kröger et al., 1994; Malkki and Kröger, 2005). Polystyrene microbeads (diameter 100 nm) were added to the Ringer solution to enhance the visibility of the laser beam. The beam was focused in front of the lamprey lens using a lens with a focal length of 50 mm, to reduce beam diameter, and scanned through a meridional plane of the lamprey lens. Each lens was scanned twice and the scans were videotaped from above with a digital camera (Sony DCR-TRV 730E PAL). After the optical experiments had been carried out, lens diameter was measured with callipers to the nearest 0.1 mm. The entire procedure for both lenses of an animal lasted between 45 and 60 min.

From the video sequence of each scan, 200 frames were exported using Adobe Premiere 6.0. From these frames, the LSA was determined by using custom-written software (Malkki and Kröger, 2005). We were only interested in spherical aberration, which is a symmetrical aberration, and therefore we averaged the LSA curves across the optical axis over both halves of each lens. Each lens was treated as an independent measurement since intra-animal variance is higher than inter-animal variance using this technique (Kröger et al., 2001). The results were compared with schlieren images in which variation in focal length is indicated by variation in colour (Fig. 3C–F). Mean focal lengths normalized to lens radius (R) were calculated as weighted means $[\sum(BCD \cdot BEP) / \sum BEP]$ with $0.01 \leq BEP \leq 0.95R$ (BCD, back centre distance; BEP, beam entrance position). These limits were used because the laser-scanning method has low accuracy for small BEPs (Malkki and Kröger, 2005) and most of the energy is reflected for BEPs larger than $0.95R$ (Sroczyński, 1977).

RESULTS

All four species of lamprey were found to have multifocal lenses, optically similar to those of bony fishes. The holarctic (northern hemisphere) lampreys, were shown to have multifocal lenses using the *in vivo* method of photoretinoscopy (Fig. 3A,B). Ring-like patterns varying in brightness clearly appeared in the pupils. Unfortunately, the small lens sizes of *M. praecox* and the downstream migrants of *G. australis* (Table 1) prohibited the use of this method on the southern hemisphere lampreys.

Schlieren photography confirmed the photoretinoscopic results from the holarctic lampreys (Fig. 3C,D) and indicated that the southern hemisphere lampreys also have multifocal lenses (Fig. 3E,F). The findings were clearest for the holarctic lampreys, where several zones of different focal lengths were present, including a peripheral zone of long focal length that appeared dark on schlieren photographs and may be used

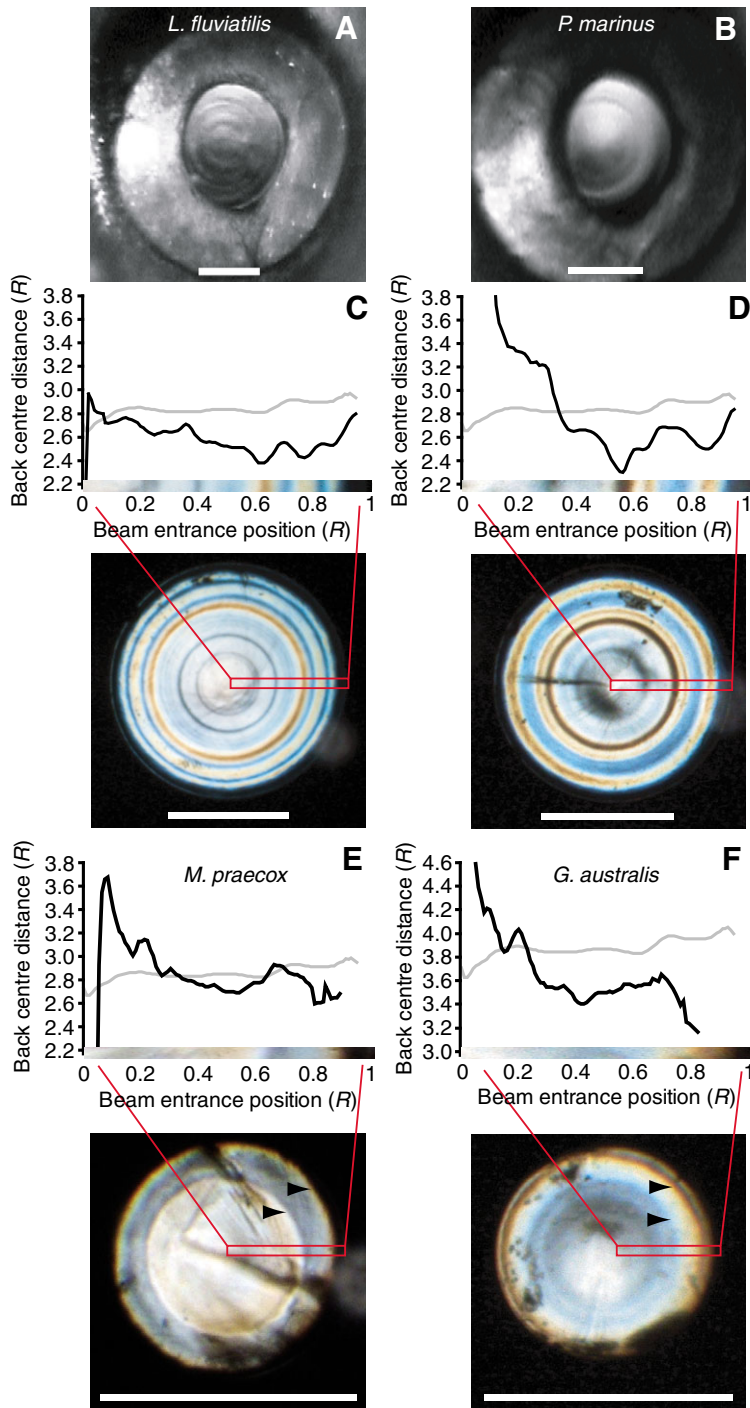


Fig. 3. Retinoscopic photographs (A,B) and comparisons of longitudinal spherical aberration (LSA) curves with schlieren photographs for individual lamprey lenses (C–F). (C) Comparisons between the LSA curve and a schlieren photograph of a representative *L. fluviatilis* lens. The grey line is the mean LSA curve of 21 lenses from *Astatotilapia burtoni*, the species in which multifocal lenses were discovered (Kröger et al., 1999). The *A. burtoni* curve was scaled for easier comparison and shows less variation because averaging smoothes the data somewhat. The graph (LSA) shows the axial distance between the centre of the lens and where the beam intercepts the optical axis (back centre distance, BCD) as a function of the lateral distance between the optical axis and the undeflected entrance beam (beam entrance position; BEP) in units of lens radius (R). The curves were terminated at $0.95R$ because most of the energy incident on a fish lens at higher BEPs is reflected (Sroczyński, 1977). Close to the optical axis the laser-scanning method has low accuracy (Malkki and Kröger, 2005). (D) Comparisons as in C for *P. marinus*. (E) Comparisons as in C for *M. praecox*. (F) Comparisons as in C for *G. australis*. Note that the BCDs are short for BEPs corresponding to radial positions in the lens with reddish areas in the schlieren photographs. By contrast, the BCDs peak at positions corresponding to bluish areas in the schlieren photographs. The results obtained with both methods therefore consistently indicate that lamprey lenses are multifocal and compensate for LCA. Zones that are dark on the schlieren photographs may focus either infrared (short BCDs) or ultraviolet (long BCDs) light. Note that the scale of the y axis is different for *G. australis*. Scale bars, 1 mm.

Mean normalized focal lengths of the lenses of the holarctic species and *M. praecox* were about $2.3R$, whereas *G. australis* lenses had longer focal lengths of about $3.0R$ (Table 1).

DISCUSSION

Early colour vision and multifocal lenses

This is the first study to show that the eyes of lampreys (Agnatha), extant representatives of the earliest vertebrates, possess multifocal lenses and therefore the optical apparatus for well-focused colour vision. However, a more detailed analysis of the correlation between the multifocal properties of these lamprey lenses and the absorbance maxima of their photopigments, such as was previously performed for the teleost *Astatotilapia* (formerly *Haplochromis*) *burtoni* (Kröger et al., 1999), was not possible. The dispersive properties of lamprey lenses are currently unknown and measuring LCA with several lasers of different wavelengths was beyond the scope of this study. All lenses studied were small (Table 1), which led to large uncertainties in the mean LSA curves. Furthermore, only a few lenses could be studied in the southern hemisphere lampreys.

A monofocal lens has little variation in back centre distance (BCD) as a function of beam entrance position (BEP) (Kröger and Gislén, 2004) and would appear unicoloured on schlieren images. A homogenous spherical lens would have such severe spherical aberration that no useful image would be created. A lens poorly corrected for LSA would focus long wavelengths with its periphery and short wavelengths with its central region. Furthermore, the LSA curve would have continually decreasing BCDs for increasing BEPs. Such results were not obtained from any of the lenses studied, which strongly suggests that all species studied possess multifocal lenses.

The findings are clearest in the holarctic species. The small lens sizes of the southern hemisphere lampreys (Table 1) prohibited the

to focus ultraviolet light on the retina. Schlieren photographs of *M. praecox* lenses revealed three well-defined zones (Fig. 3E). In *G. australis*, the zones were more diffusely demarcated, but equally different in focal length (Fig. 3F). *M. praecox* and *G. australis* lenses had zones of short focal length in the periphery.

The averaged results from laser scanning show that each species has a specific LSA curve, with sufficient variation in focal length across the aperture of the lens (Fig. 4) that all lenses can be classified as multifocal. The 90% confidence intervals of the LSA curves are separated at several places (arrowheads in Fig. 4), which means that the curves are significantly different at $P < 0.05$.

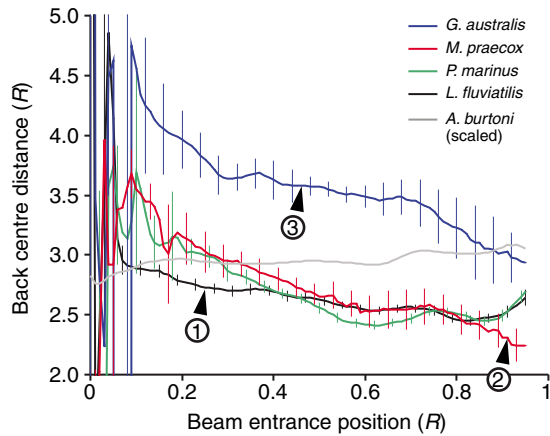


Fig. 4. Mean longitudinal spherical aberration (LSA) curves with 90% confidence intervals for the four species of lamprey and the mean curve of *A. burtoni* (grey line). Averaging the results from several lenses (for numbers see Table 1) leads to some smoothing of the curves (compare with curves in Fig. 3). Note that the confidence intervals do not overlap in some regions (arrowheads), which means that the curves are different at the $P < 0.05$ level. The laser-scanning method has low accuracy for small beam entrance positions (BEPs; close to the optical axis) because of technical reasons (Malkki and Kröger, 2005). This leads to very large error margins. All curves have been terminated at $0.95R$ (see Fig. 3 legend). The *A. burtoni* curve has been scaled for easier comparison. Note that the LSA curves of the lampreys have at least as much variation in back centre distance (BCD) as the curve of *A. burtoni* lenses (grey line) which are known to be multifocal. (1) The LSA curve of *L. fluviatilis* is different from all other curves. (2) The *M. praecox* curve is different from all other curves. (3) *G. australis* lenses had longer normalized focal lengths than the lenses of all other species studied, which is indicated by the vertical shift of the LSA curve of *G. australis*.

use of photoretinoscopy and limited the spatial and spectral resolutions of the results obtained with schlieren photography and laser scanning. Small lens size is probably the main reason for the somewhat diffuse results obtained from the southern hemisphere species. There may, however, also be biological reasons for these results. Firstly, photoreceptor diameter is large relative to focal length in a small eye. This means that depth of focus is long, which in turn reduces the necessity to compensate for LCA. Secondly, small lenses consist of few cell layers, such that it may not be possible to accommodate several sharply bordered regions of different focal lengths within the lens diameter. This effect would be expected to be most severe in *G. australis* that has five spectrally different photopigments and this may explain why the most diffuse results were obtained from this species. However, both *M. praecox* and *G. australis* lenses show sufficient variation in BCD across the aperture (Fig. 4) to be considered multifocal.

As in the lampreys studied, teleost lenses may also have various combinations of refractive zones. Diurnal planktivores of the Red Sea [three species studied by Karpestam et al. (Karpestam et al., 2007)] and the crucian carp [*Carassius carassius* (Malkki and Kröger, 2005)] have outer zones of long focal lengths, similar to the lenses of *L. fluviatilis* and *P. marinus*. Several rings alternating between two colours (Fig. 3C,D) have been observed in teleost lenses (unpublished results), but are unusual. Three species of diurnal herbivores and two predatory species of the Red Sea were found to have outer zones of long focal lengths (Karpestam et al., 2007), as in the lenses of *M. praecox* and *G. australis*. Interestingly, schlieren images of *M. praecox* lenses could easily be confused with schlieren images obtained from the diurnal planktivores of the Red Sea. The

LSA curves, however, reveal an important difference: the outer, almost invisible zones have long focal lengths in the planktivores and are thought to be used to focus UV light (Karpestam et al., 2007), the corresponding zone in *M. praecox* lenses has short focal length and its functional significance is unclear. Sensitivity to infra-red (IR) light is unlikely, and even if it were present, there is little difference in focal length of the lens between long visible and near-IR wavelengths. Instead, for optical reasons, the zone may come about by constraints on the cellular compositions of these small lenses.

The lenses of *P. marinus*, *M. praecox* and *G. australis* have in common that back centre distance increases with decreasing beam entrance position below $0.4R$ (Figs 3 and 4). This means that light passing through the central region of the lens is defocused. However, the effects on image quality are minor, since the area of the central region is small, such that little light passes through it. Furthermore, because of the small diameter of the region, depth of focus is long such that the tolerable amount of defocus is higher than in more peripheral regions of the lens.

The holarctic lampreys *L. fluviatilis* and *P. marinus* each possess two morphological types of photoreceptor with different λ_{\max} values (Govardovskii and Lychakov, 1984; Harosi and Kleinschmidt, 1993) and although controversy still surrounds the identity of these two receptor types (for a review, see Collin and Trezise, 2006), the molecular basis for colour discrimination is certainly present. Only one morphological type has been described for *M. mordax* (Collin and Potter, 2000; Collin et al., 2004), which is somewhat surprising, given that our results suggest that the sister species *M. praecox* has some form of colour vision. *G. australis*, with five types of photoreceptor characterized by anatomical, spectral and molecular criteria would be predicted to possess the most advanced level of colour discrimination of the four species examined here (Collin et al., 2003b; Collin and Trezise, 2004; Collin and Trezise, 2006; Davies et al., 2007). However, in the small eyes that we could study, complex multifocal lenses may not be necessary because of limited spatial resolution and thus long depth of focus of such eyes. The long normalized focal lengths of *G. australis* lenses (Table 1) increase depth of focus even further. Fully developed multifocal lenses with sharply demarcated zones of different focal lengths may therefore become necessary first when the eyes have grown larger.

The mean normalised focal lengths of the lenses are $2.31R$ in both *L. fluviatilis* and *P. marinus*, and $2.32R$ in *M. praecox*. This is in the lower range of the focal lengths typical for teleost lenses ($2.2\text{--}2.8R$) (Matthiessen, 1882; Kröger and Campbell, 1996), whereas the focal length of the lens is considerably longer in *G. australis* ($3.04R$). The adults of *M. praecox* (found in downstream regions of rivers), *L. fluviatilis* (Baltic Sea) and *P. marinus* (Lake Michigan) live in deep or turbid waters, whereas *G. australis* occurs close to the surface in clear water (Southern Ocean) (Potter and Hilliard, 1987; Collin et al., 2003b). It is therefore not surprising that *G. australis* has the most advanced system of colour vision with five different visual pigments and the longest focal length relative to lens size. Long focal length increases image magnification and thus resolution, while it decreases light gathering ability and thus sensitivity (Land and Nilsson, 2002).

The evolution of multifocality

The presence of multifocal lenses in the eyes of lampreys confirms the early origins of colour vision in vertebrates, and suggests that other aquatic vertebrates and tetrapods have retained this optical feature, the latter despite the transition from an aquatic environment to a terrestrial habitat, where the cornea comes into play as an additional refractive element. Therefore, in vertebrates, multifocal

optical systems seem to be evolutionarily older than monofocal systems. Monofocality is present mainly in diurnal tetrapods, such as humans, which have pupils that are small relative to the focal length of the eye (Malmström and Kröger, 2006). In such an eye, depth of focus is long and LCA produces little chromatic blur (Kröger, 2000; Land and Nilsson, 2002).

However, there are interesting examples of well-developed camera-type eyes with large pupils that possess monofocal lenses, such as in cephalopods (Land and Nilsson, 2002). The firefly squid *Watasenia scintillans* is one of the few cephalopods known to have the molecular basis for colour vision (Seidou, 1990) and possesses a monofocal lens. The problem of chromatic defocus is solved instead by a banked retina (Kröger and Gislén, 2004). The presence of multifocal lenses in representatives of all vertebrate classes studied thus far and their absence in cephalopods suggests a monophyletic origin for this lens design. Convergent evolution in so many vertebrate lineages is unlikely.

The evolution of camera-type eyes capable of forming an image and providing directional information from a distance (Nilsson, 1996; Land and Nilsson, 2002) is thought to have been an important factor in the 'explosion' in species diversity, i.e. the sudden appearance of many highly motile species with hard skeletons (Budd, 2003) in the Cambrian period (about 540 MYA). The presence of at least four visual pigments [long wavelength sensitive (LWS), short wavelength sensitive type 1 and 2 (SWS1, SWS2) and an rod opsin (Rh) class of visual pigment (Collin et al., 2003a)] in the last common ancestor of jawed and jawless vertebrates suggests that the earliest vertebrates were able to sample a rich spectral light environment and enjoy the many advantages of colour vision. Our findings suggest that the eyes of these ancient animals were capable of forming well-focused colour images. This visual ability may have been critical for the evolutionary success of the lineage leading to gnathostomes at a time when optimizing the visualization of either food or predator had become of utmost importance.

LIST OF ABBREVIATIONS

BCD	back centre distance
BEP	beam entrance position
LCA	longitudinal chromatic aberration
LSA	longitudinal spherical aberration
LWS	long wavelength sensitive
MS 222	3-aminobenzoic acid ethyl ester
MYA	million years ago
R	lens radius
Rh1	rod opsin
Rh2	Rh1-like cone opsin
RhA	Rh1-like opsin type A
RhB	Rh1-like opsin type B
SWS1	short wavelength sensitive type 1
SWS2	short wavelength sensitive type 2
λ_{\max}	wavelength of maximum absorbance

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