Cone monochromacy and visual pigment spectral tuning in wobbegong sharks

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Much is known regarding the evolution of colour vision in nearly every vertebrate class, with the notable exception of the elasmobranchs. While multiple spectrally distinct cone types are found in some rays, sharks appear to possess only a single class of cone and, therefore, may be colour blind. In this study, the visual opsin genes of two wobbegong species, 	extit{Orectolobus maculatus} and 	extit{Orectolobus ornatus}, were isolated to verify the molecular basis of their monochromacy. In both species, only two opsin genes are present, RH1 (rod) and LWS (cone), which provide further evidence to support the concept that sharks possess only a single cone type. Examination of the coding sequences revealed substitutions that account for interspecific variation in the photopigment absorbance spectra, which may reflect the difference in visual ecology between these species.

Keywords: shark; monochromacy; opsin; rod; cone

1. INTRODUCTION

Studies of the evolution of colour vision have now been extended to most vertebrate classes [1,2], with the notable exception of the elasmobranchs (sharks, skates and rays). To date, the RH1 (rod) opsin coding sequence has been obtained for only four elasmobranch species [3,4], but no cone sequences have been generated. Although some rays possess multiple spectrally distinct cone types [5,6], it was thought that most sharks possess rod-only retinae, with only a few species having a single class of cone photoreceptor [7], suggesting that all shark species may lack colour vision. In the spotted wobbegong shark, 	extit{Orectolobus maculatus}, and the ornate wobbegong shark, 	extit{Orectolobus ornatus}, only a rod and a single cone class were identified [7], although their visual pigments show interspecific differences in spectral absorbance: the wavelength of maximum absorbance ($\lambda_{\text{max}}$) of the rods and cones of 	extit{O. maculatus} ($\lambda_{\text{max}}$ at 484 and 553 nm, respectively) are considerably short-wavelength-shifted compared to 	extit{O. ornatus} ($\lambda_{\text{max}}$ at 498 and 561 nm, respectively).

The presence of spectrally distinct cone photoreceptors in several shark species [8–10] raises the questions of which cone opsins are retained and what tuning mechanisms govern photopigment spectral absorbance. In this study, we provide, for the first time, an understanding of the genetic basis and spectral tuning of the shark visual system that expands our knowledge of the evolution of photopic vision in ancient vertebrates.

2. MATERIAL AND METHODS

Coding sequences were PCR amplified from cDNA (expressed transcripts) and gDNA (gene complement) derived from two wobbegong sharks (\textit{O. maculatus} and \textit{O. ornatus}), using degenerate primers designed from sequence alignments of all opsin classes across many vertebrate groups (see the electronic supplementary material, table S1) [11,12], and extended by 5' - and 3’-RACE. Neighbour-joining phylogenetic analysis [13] was performed by applying a Kimura two-parameter substitution matrix [14] with the \textit{Petromyzon marinus} vertebrate ancient opsin (GenBank: AF006524) as an outgroup (see the electronic supplementary material).

3. RESULTS

Extensive PCR amplifications were carried out; however, only RH1 and LWS genes were found in the retinae and genome of both species of shark. Phylogenetic analysis showed that these sequences are orthologues of the RH1 and LWS opsin gene classes (figure 1; GenBank: JX534163–JX534166). The position of the wobbegong LWS sequences shows that they are more closely related to the LWS2 orthologue of the elephant shark (\textit{Callorhinus milii}) than the short-wavelength-shifted LWS1 variant of the same species [16], indicating that the LWS gene duplication identified in \textit{C. milii} may have occurred early in the evolution of the cartilaginous fishes, with the LWS1 pigment lost in the elasmobranch lineage.

The amino acids important for spectral tuning of rod pigments ($\lambda_{\text{max}}$ at approx. 500 nm) have previously been described [17]. The tuning sites of the RH1 pigment in both species of wobbegongs are identical to each other and to the bovine sequence at all but sites 292 and 299 (table 1). The \textit{O. maculatus} sequence encodes an Ala292Ser substitution, which is known to cause a 10 nm short-wavelength shift in the $\lambda_{\text{max}}$ [16,18,19]. This is consistent with the spectral absorbance of the \textit{O. maculatus} rod pigment, which has a $\lambda_{\text{max}}$ at 484 nm compared with 498 nm for \textit{O. ornatus} (figure 1) [7]. The \textit{O. ornatus} RH1 gene encodes an Ala299Ser substitution that has been implicated in the spectral shifts observed in deep-sea teleost rod pigments. Other key sites for RH1 pigments are sites 83 and 122 [20,21], but these are not substituted in the shark pigments.

The spectral tuning of LWS pigments follows the ‘five-sites’ rule, whereby the residues at sites 180, 197, 277, 285 and 308 together determine the $\lambda_{\text{max}}$ of the pigment [22]. For the \textit{O. maculatus} and \textit{O. ornatus} cone pigments, these sites are occupied by Ser180, His197, Phe277, Thr285 and Ala308 (table 2). This is the same combination of residues seen in the LWS opsin of the harbour seal, \textit{Phoca vitulina} [23], which yields a pigment with a spectral maximum at 548 nm. The $\lambda_{\text{max}}$ at 553 nm seen in the \textit{O. maculatus}
cone pigment [7] is similar to that of\( P. \)\( \textit{vitulina} \), whereas the\( O. \)\( \textit{ornatus} \) LWS pigment is long-wavelength-shifted to 561 nm (figure 1) [7]. The LWS opsin sequences differ at only two sites, 97, where Ile in\( O. \)\( \textit{maculatus} \) is replaced by Val in\( O. \)\( \textit{ornatus} \), and 293, where Val in\( O. \)\( \textit{maculatus} \) is replaced by Leu in\( O. \)\( \textit{ornatus} \). Site 97 is a part of the second transmembrane domain, but faces away from the chromophore binding pocket [24], and site 293 is found in the third extracellular loop between transmembrane domains 6 and 7. Therefore, neither site would be expected to contribute to the tuning of the pigment, although substitutions at one or other of these sites may cause a conformational change that would impact indirectly on the chromophore binding pocket and thereby cause a spectral shift.

4. DISCUSSION

Information on cartilaginous fish visual pigments is limited. In the holocephalian elephant shark, three cone opsin genes (RH2, LWS1 and LWS2) are found in addition to a rod (RHI) opsin gene [16], and several spectrally distinct cone classes have been identified in rays [5,6]. Therefore, multiple cone opsin classes have been retained by some cartilaginous fishes, although the molecular identity of the pigments expressed in these cone classes, and their conservation throughout this vertebrate
group, is not known. Here, we identify, for the first time, the genetic basis for cone photopigments in an elasmobranch, using two wobbegong sharks as representative species. The only expressed cone opsin gene in the retina of these species belongs to the LWS class, and this is consistent with the presence, in wobbegong sharks and in several other shark species, of only a single class of cone photoreceptor that peaks in the long-wavelength region of the spectrum [7]. It would appear, therefore, that only the LWS cone opsin gene (plus the RH1 rod opsin gene) has been retained in sharks.

Interspecific differences in rod $\lambda_{\text{max}}$ values between these wobbegong sharks may be related to habitat depth [15,25]: O. maculatus is sometimes found in deeper waters (more than 200 m) than O. ornatus, which prefers clear, shallow water (less than 50 m) [26]. The former may, therefore, benefit from a short-wavelength-shifted rod photopigment as an adaptation to downwelling light that is restricted to short- to middle-wavelengths at greater depths [27], as observed in many deep-sea teleosts [21]. Similar to coastal teleosts [28], the LWS cone pigments of both wobbegong species (with $\lambda_{\text{max}}$ in the range of 533–561 nm) are long-wavelength-shifted compared with shark species with $\lambda_{\text{max}}$ in the range of 532–534 nm that dwell in reefs or open water; this shift may be an adaptation for detecting and ambushing benthic prey within the mesopic range of the spectrum [7], although the behavioural significance of the small interspecific difference in $\lambda_{\text{max}}$ of these cones remains unclear.

Taken together with microspectrophotometric data [7], there is now strong evidence to suggest that at least some shark species are cone monochromats. As two or more cone photoreceptors are essential for colour opponency, owing to the univariance properties of photoreceptors, monochromatic species are predicted to be completely colour blind. Both wobbegong species are at least partially nocturnal [26]: O. maculatus is fully active at night and possesses a visual system that is well-suited to dim-light conditions, whereas O. ornatus possesses visual characteristics that are consistent with partial nocturnality [15]. Under mesopic conditions, it is possible that both cones and rods are active, and the addition of a rod-mediated middle-wavelength colour channel would result in conditional dichromacy [29] and the enhancement of colour vision in both species. Interestingly, many large marine predators (e.g. whales and dolphins) are also LWS cone monochromats [23]. Thus, the presence of monochromacy and the potential for conditional dichromacy under mesopic conditions in sharks implies that such a chromatic state may be widespread in marine species and important for their visual ecology.

We thank John Page for help with animal collection, and Livia Carvalho, Jill Cowing and Susan Wilkie for technical advice. This work was funded by a UQ Graduate School Research Travel Grant, an ARC QEII Fellowship and an ARC Discovery Project Grant. S.M.T. also like to acknowledge the support of Ron Johnstone.


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