Cone monochromacy and visual pigment spectral tuning in wobbegong sharks

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Key words: shark; monochromacy; opsin; rod; cone

Running title: Visual pigments in wobbegong sharks

ACKNOWLEDGEMENTS

The authors would like to 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 (DP0558681) and an ARC
Discovery Project Grant (DP110103294). SMT would also like to acknowledge the support of Ron Johnstone.
SUPPLEMENTARY MATERIALS AND METHODS

Tissue collection and DNA generation

*O. maculatus* and *O. ornatus* were collected and spinal pithed with approval of the University of Queensland Animal Ethics Committee (ANAT/834/07/ARC and SBMS/613/08/ARC). Messenger RNA (mRNA) was isolated from retinal tissue stored in RNAlater™ (Ambion, Australia), using a QuickPrep™ Micro mRNA Purification Kit (GE Healthcare, UK), and converted to complementary DNA (cDNA) with a SuperScript™ III Reverse Transcriptase Kit (Invitrogen, UK) as previously described [1]. Genomic DNA (gDNA) was prepared from liver tissue according to standard procedures [2, 3].

Opsin sequence determination

Partial cDNA sequences were PCR amplified, on a number of independent occasions, using retinal-derived cDNA as a template and degenerate primers that were designed from alignments of opsin sequences across many vertebrate classes (table S1) [4]. First round PCR products were produced using AOASF1 (forward) and AOASR2 (reverse) primers and applying the following conditions: an initial denaturation of 94°C for 10 min; 50 cycles of 94°C for 30 s, 45°C for 1 min, 72°C for 1.5 min; and a final extension of 72°C for 10 min. Resulting PCR products, including the negative controls, were diluted 1 in 10 and used as a template in a second round hemi-nested or full-nested PCR using the following primer combinations: (i) AOASF1 (forward) and AOASR1 (reverse), (ii) AOASF2 (forward) and AOASR2 (reverse), and (iii) AOASF2 (forward) and AOASR1 (reverse). Conditions for the second round PCR were similar to the first round PCR, except for the use of an annealing temperature of 50°C [4]. The use of retinal-derived cDNA as template results in the detection of opsin sequences that are expressed in ocular tissue sampled at a particular development time point and may not reflect the full complement of opsin genes present in the genome. To
overcome this issue, opsin sequences were amplified from genome DNA, using a different set of degenerate primers, designed specifically to detect all classes of opsin from all vertebrate species (table S2). A PCR protocol identical to that used for cDNA experiments was utilised, with the exception that AOAS-GenF1 (forward) and AOAS-GenR (reverse) primers were included in a first-round reaction, before performing a second hemi-nested reaction with AOAS-GenF2 (forward) and AOAS-GenR (reverse) primers [5]. Amplified DNA fragments were sequenced using a Big Dye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM Genetic Analyzer (Applied Biosystems, UK), either directly or subsequent to cloning into a pGEM®-T Easy Vector Kit (Promega, UK). Plasmid DNA was isolated and purified using a GenElute Miniprep Purification Kit (Sigma, UK), according to the manufacturer’s instructions. The resulting sequences were then used to extend the sequences by 5'- and 3'- Rapid Amplification of cDNA Ends (RACE), where appropriate, using a second generation 5'/3'-RACE Kit (Roche Applied Sciences, USA).

**Phylogenetic analysis**

Neighbour-Joining [6] phylogenetic analysis, bootstrapped with 1000 replicates, was performed with the MEGA Version 4.0 computer package [7] on a codon-matched nucleotide alignment of all seven transmembrane domains (nucleotide sequence encoding amino acids 55 to 299), where wobbegong LWS and RH1 retinal opsin sequences were compared to the coding sequences of LWS, SWS1, SWS2, RHB/RH2, and RHA/RH1 opsin classes of other vertebrate species. Evolutionary distances were calculated to produce a Neighbour-Joining tree [6], by applying a Kimura 2-parameter substitution matrix [8], with complete deletion, a homogenous pattern of nucleotide substitution among lineages, and a uniform rate of nucleotide substitution across all sites. Sequences used for generating the tree were obtained from the following GenBank: NM000539; NM131084; Y17585; Y17586; U81514;
EF565167; AY366493. NM131253; EF565168; AY366494; NM131192; AY366492; NM001708; NM131319; AY366495; NM020061; NM131175; EF565165; EF565166; and AY366491. The *Petromyzon marinus* vertebrate ancient (VA) opsin (GenBank: AH006524) was used as an outgroup.
**SUPPLEMENTARY TABLE 1**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' to 3')</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAASF1</td>
<td>CCGCGAGAGATACATNGTNRTNTGYAARCC</td>
<td>cDNA</td>
</tr>
<tr>
<td>AOAASF2</td>
<td>ATTTTAGAAGGTCTGCCRGWSNTCNTGYGG</td>
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</tr>
<tr>
<td>AOAASR1</td>
<td>ATTGGTCACCTCCTTYTCNGCYYTTYGTNGT</td>
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<tr>
<td>AOAASR2</td>
<td>CCCGGAAGACGTAGATGANNNGGRTTRWANA</td>
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<td>AOAAS-GenF1</td>
<td>AAAAGAATCAGCCTCCACNCARMRRGCGNA</td>
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<td>AOAAS-GenF2</td>
<td>AGAGGTGACCACGATGGTNRTNNTNATGRT</td>
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</tr>
<tr>
<td>AOAAS-GenR</td>
<td>CCCGGAAGACGTAGATGANNNGGRTTRWANA</td>
<td>gDNA</td>
</tr>
</tbody>
</table>

Table S1. “All-opsin/all-species (AOAS)” degenerate oligonucleotide sequences used in nested-PCR to generate partial opsin sequences expressed in the retinae of two species of wobbegong shark (using cDNA as template) or present in the genome (using cDNA as template).
REFERENCES


