Genetic evidence for the ancestral loss of short-wavelength-sensitive cone pigments in mysticete and odontocete cetaceans

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All mammals ancestrally possessed two types of cone pigments, an arrangement that persists in nearly all contemporary species. However, the absence of one of these cone types, the short-wavelength-sensitive (SWS) cone, has recently been established in several delphinoid cetacean species, indicating that the loss of this pigment type may be widespread among cetaceans. To evaluate the functional condition of SWS cones in cetaceans, partial SWS cone-opsin gene sequences were obtained from nuclear DNA for 16 species representing 12 out of the 14 extant mysticete (baleen) and odontocete (toothed) families. For all these species one or more mutations were identified that indicate that their SWS cone-opsin genes are pseudogenes and thus do not code for functional visual pigment proteins. Parsimonious interpretation of the distribution of some of these mis-sense mutations indicates that the conversion of cetacean SWS cone-opsin genes to pseudogenes probably occurred before the divergences of the mysticete and odontocete suborders. Thus, in the absence of dramatic homoplasy, all modern cetaceans lack functional SWS cone visual pigments and, by extension, the visual capacities that such pigments typically support.

Keywords: Cetacea; short-wavelength-sensitive visual pigment; cone opsin; colour vision; missense mutation

1. INTRODUCTION

The transduction of light in vertebrate photoreceptors is performed by apoproteins known collectively as visual pigments (Wald 1968). Each visual-pigment molecule consists of a G-protein-coupled receptor, opsin, and a photosensitive chromophore, 11-*cis* retinal. Molecular genetic analyses of vertebrate opsins reveal that these proteins may be subdivided into five distinct retinal classes as well as one or more extra-retinal classes (see Yokoyama (2000) for a review). The retinal classes include the RH1 opsins, found in the rod photoreceptors, and four cone-opsin types: SWS1, SWS2, MWS and M/LWS. In general, these classes are characterized by the region of the spectrum to which their constituents are most sensitive. For instance, the SWS1 and SWS2 classes consist primarily of short-wavelength-sensitive (SWS) cone opsins, while the M/LWS class contains opsins maximally sensitive in the middle-to-long-wavelength region of the spectrum (Yokoyama & Yokoyama 1996; Bowmaker *et al.* 1997; Yokoyama 2000). Phylogenetic analyses of various opsin genes indicate that modern birds, fishes and other non-mammalian vertebrates may possess functional forms of all five retinal opsin types (Bowmaker *et al.* 1997; Yokoyama 2000). Within mammals, however, a maximum of three opsin genes are present in functional form (Yokoyama & Yokoyama 1996). These are the rod opsins, RH1, and two cone-opsin types, SWS1 and M/LWS. The other two cone pigment types, SWS2 and MWS, were lost some time before the divergence of modern mammals.

Behavioural and physiological examinations of a range of species confirm that almost all mammals possess functional forms of these two cone-opsin types as well as the corresponding ability for at least dichromatic colour vision (Jacobs 1993).

A limited number of mammalian species have been identified as cone monochromats. They lack functional SWS1 (SWS hereafter) cones and, therefore, have only a single functional cone visual pigment type, an M/LWS cone. In terrestrial mammals, this condition is unusual, although the nature of its distribution indicates that it has arisen independently in several different lineages including some primate (Jacobs *et al.* 1996), rodent (e.g. Szel *et al.* 1996; Peichl & Moutairou 1998) and carnivore species (Jacobs & Deegan 1992; Peichl & Moutairou 1998; Peichl & Pohl 2000). As essentially all these terrestrial cone monochromats are primarily active in dim nocturnal and/or subterranean habitats, the loss of SWS cones has been attributed to a lack of selective pressure for the maintenance of good photopic (cone-based) vision (Jacobs 1993; Peichl & Moutairou 1998; Peichl & Pohl 2000). By contrast, the absence of functional SWS cones has recently been documented in seven cetacean species, many or all of which are active in photopic conditions (Fasick *et al.* 1998; Peichl *et al.* 2001). Several amphibious mammals have also been found to lack SWS cones (Peichl & Moutairou 1998; Peichl *et al.* 2001). Thus, it has been suggested that at least in marine mammals the loss of SWS cones is adaptive, presumably for vision under water in photopic conditions, and consequently that all cetaceans lack SWS cone visual pigments (Peichl *et al.* 2001).

Presently, however, the cetacean species that have been evaluated are limited to closely related delphinoid

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Table 1. Nucleotide identity and non-synonymous substitution proportions for partial (*ca.* 391 bp) hippopotamus and cetacean SWS cone-opsin gene coding-sequences in comparison to the homologous bovine coding sequence (accession number U49760).

common name	species	nucleotide identity (coding only)	proportion of non-synonymous substitutions (no. of amino acid substitutions/no. of nucleotide substitutions)	indels	apparently functional?	accession number
river hippopotamus	<i>Hippopotamus amphibius</i>	0.936	0.16* (4/25)	0	yes	AF545497
common minke whale	<i>Balaenoptera acutorostrata</i>	0.938	0.50 (12/24)	1	no	AF545490
blue whale	<i>Balaenoptera musculus</i>	0.938	0.46 (11/24)	1	no	AF545484
humpback whale	<i>Megaptera novaeangliae</i>	0.938	0.42 (10/24)	1	no	AF545494
bowhead whale	<i>Balaena mysticetus</i>	0.933	0.54 (14/26)	1	no	AF545491
North Atlantic right whale	<i>Eubalaena glacialis</i>	0.930	0.56 (15/27)	1	no	AF545492
grey whale	<i>Eschrichtius robustus</i>	0.928	0.50 (14/28)	1	no	AF545493
sperm whale	<i>Physeter macrocephalus</i>	0.918	0.44 (14/32)	0	no	AF545482
pygmy sperm whale	<i>Kogia breviceps</i>	0.912	0.50 (17/34)	1	no	AF545486
Indian river dolphin	<i>Platanista gangetica</i>	0.920	0.58 (18/31)	1	no	AF545487
Sowerby's beaked whale	<i>Mesoplodon bidens</i>	0.929	0.48 (13/27)	2	no	AF545496
Amazon river dolphin	<i>Inia geoffrensis</i>	0.921	0.47 (14/30)	2	no	AF545495
franciscana	<i>Pontoporia blainvillei</i>	0.923	0.53 (16/30)	1	no	AF545483
common bottlenose dolphin ^a	<i>Tursiops truncatus</i>	0.909	0.57 (20/35)	3	no	U92557
beluga whale	<i>Delphinapterus leucas</i>	0.930	0.44 (12/27)	1	no	AF545485
vaquita	<i>Phocoena sinus</i>	0.936	0.44 (11/25)	1	no	AF545489
harbour porpoise ^a	<i>Phocoena phocoena</i>	0.928	0.46 (13/28)	1	no	AF545488

^a Previously identified as lacking SWS cones.* Significantly different from cetaceans, $p < 0.001$.

(dolphin and porpoise) species; no mysticete (baleen) whales have been examined, nor have any of the riverine dolphins or highly pelagic deep-diving odontocete (toothed) whales. To assess the extent to which SWS cones have been lost throughout the cetacean order, we have obtained DNA sequences to evaluate the SWS cone visual-pigment genes of a taxonomically diverse selection of mysticete and odontocete cetaceans. We chose a genetic approach as it reduces the potential for obtaining false-negative results by positively identifying the mutation(s) underlying the absence of the pigment type (Jacobs *et al.* 1996; Fasick *et al.* 1998). Moreover, as genetic techniques are not limited to the examination of live animals or even fresh tissue samples, it is possible to study a much broader range of species, allowing us to examine representatives of nearly every extant cetacean family.

2. METHODS

The 16 cetacean species evaluated are listed in table 1; these species represent 12 out of the 14 currently recognized cetacean families (Rice 1998). For genetic analysis, three individuals were examined from each species, except *Inia geoffrensis* and *Platanista gangetica*, where tissue availability was limited to a single individual. All procedures were performed on nuclear DNA extracted from frozen skin with lithium-chloride and chloroform (Hillis *et al.* 1996). Three river hippopotamuses (*Hippopotamus amphibius*), the closest living relative of cetaceans (Nikaido *et al.* 1999), were similarly examined for comparative purposes. The bottlenose dolphin (*Tursiops truncatus*) and the cow (*Bos taurus*) were examined solely to obtain comparative sequence data for

the region of the first intron. SWS cone 'opsin' coding sequence data for these species were obtained from GenBank, accession numbers U92557 and U49760, respectively (Chiu *et al.* 1994; Fasick & Robinson 1998).

Nucleotide sequence data were obtained using polymerase chain reaction (PCR) and the dideoxy-chain-terminator sequencing method (Sanger *et al.* 1977). For PCR, 1 µl of nuclear DNA (*ca.* 100 ng µl⁻¹) was amplified in a 50 µl mixture containing 37.75 µl of water, 5.0 µl of MgCl₂ polymerase buffer (2.0 mM), 3.0 µl of mixed deoxyribonucleoside triphosphates (dNTPs) (10 mM), 1.5 µl of sense and anti-sense oligonucleotide primer (10 µM) and 0.25 units of *Taq* DNA polymerase. Amplification was achieved through 35 cycles of denaturing at 90 °C for 45 s, annealing at 48 °C for 60 s and extension at 72 °C for 90 s, as well as a final extension period of 5 min at 72 °C. For some reactions annealing temperatures were adjusted between 42 and 62 °C to optimize primer performance. PCR products were analysed by size with ethidium-bromide-stained agarose-gel electrophoresis. Desired PCR products were purified and concentrated using Qiagen (Valencia, CA) Qiaquick PCR purification columns and then sequenced directly using Applied Biosystem's (ABI; Foster City, CA) Big Dye chemistry on an ABI 377 autosequencer. Both sense and anti-sense primer products were sequenced.

Oligonucleotide primers for PCR and sequencing were 14–22 bp in length, and were selected from conserved regions of the SWS cone-opsin genes of the bottlenose dolphin (Fasick & Robinson 1998), cow (Chiu *et al.* 1994) and human (accession number AH003620; Nathans *et al.* 1986). The primers were designed in pairs to amplify two overlapping sequence fragments beginning just upstream of the translation initiation codon and

including the entire first exon and intron as well as most of the second exon. After the sequences were obtained, introns were identified by homology using dolphin, bovine and human SWS cone-opsin sequences. Sequences were aligned for comparison using CLUSTALW (Thompson *et al.* 1994) and subsequently checked by eye. Quantitative evaluations of nucleotide and amino acid substitution rates were performed by eye while the sequences were aligned. Phylogenetic analyses, nucleotide identity values and Jukes–Cantor distances were calculated from the aligned sequences using PAUP (Swofford 2001).

3. RESULTS

Partial SWS cone-opsin gene sequences of 660–690 bp were obtained for the river hippopotamus and 15 cetacean species. Sequence data were also obtained for the first intron region of the SWS cone-opsin gene of a 16th cetacean species, *T. truncatus* (accession number AF545498), as well as the cow (accession number AF545499). Although each gene was sequenced at least six times for each species, no evidence was found for the presence of markedly different paralogous copies of the putative SWS cone-opsin gene in any individual of any species. Based on an evaluation of apparent polymorphisms in the raw sequence chromatogram data, intraspecific variability was found to be less than 1% in all cases. A small number of single-nucleotide polymorphisms were observed, but their paucity suggests that they were probably a consequence of allelic heterozygosity rather than the result of whole or partial gene duplication. Regardless of whether single or multiple copies of the SWS cone-opsin gene were present, no polymorphisms were seen for any individual at any of the functionally significant sites described below. A phylogenetic analysis of the complete dataset performed using parsimony is shown in figure 1.

The spliced cetacean and hippopotamus coding sequences exhibited nucleotide identity values with the bovine SWS cone-opsin gene of greater than 0.90 (table 1). Jukes–Cantor distances reflected similar differentiation: exonic distances between the cetaceans and the cow ranged from 0.063 to 0.103; the hippopotamus distance from the cow was intermediate at 0.066. Intronic Jukes–Cantor distances were higher, as expected, and ranged from 0.171 to 0.206, again with the hippopotamus intermediate at 0.179. However, while summary cetacean and hippopotamus mutation rates were apparently similar, there was a distinct difference in the nature of their respective mutational changes. As shown in table 1, all 16 cetacean species exhibited significantly higher proportions of non-synonymous substitutions than did the hippopotamus (table 1; Fisher's PLSD (protected least significant differences), d.f. = 15, all *p*-values < 0.001).

The primary structures of the sequenced cetacean and hippopotamus SWS cone opsins are shown in comparison with the functional cow opsin in figure 2. Both the cetacean and the hippopotamus sequences contain many of the highly conserved SWS cone residues (underlined in figure 2) identified by Chiu *et al.* (1994). Missense mutations are apparent in all the cetacean SWS cone-opsin genes; none can be seen in those of the hippopotamus or cow. In all six mysticete species, as well as in *Mesoplodon bidens* and *P. gangetica*, a mutation in the first exon creates a shift in the reading frame and introduces a

premature stop codon after residue 120 (using the bovine SWS sequence numbering). Furthermore, the substitution E111G, i.e. the substitution of glutamate at residue 111 with glycine, is apparent in all of the odontocete species examined that do not possess upstream frame-shifting mutations. Residue E111 of the SWS cone opsin corresponds to residue E113 of bovine rhodopsin, the essential Schiff's base linkage counter-ion that stabilizes the opsin–chromophore bond (Sakmar *et al.* 1989; Nathans 1990). The detrimental effect of the previously described frame-shifting deletion mutations of the bottlenose dolphin, *T. truncatus*, can also be seen (Fasick *et al.* 1998).

Nucleotide sequence data for the region of the SWS cone-opsin gene containing the majority of these missense mutations are presented in figure 3. As shown, the point mutation A → G at nucleotide 332 (using the bovine opsin numbering) responsible for the residue substitution E111G is present in all odontocetes, including those with upstream frame-shifting mutations that do not exhibit the aforementioned amino acid change. Similarly, the 4 bp indel mutation that disrupts the SWS cone-opsin genes of all the mysticetes and two of the odontocetes can be seen at nucleotides 262–265.

4. DISCUSSION

The results of the phylogenetic analysis of the obtained sequences are in general agreement with currently accepted cetacean systematics (Rice 1998; Nikaido *et al.* 1999, 2001). The tree structure is also largely consistent with both morphologic and genetic cetacean phylogenies (e.g. Barnes *et al.* 1985; Nikaido *et al.* 2001). Our results confirm the recently described paraphyletic status of the river dolphins (Hamilton *et al.* 2001; Nikaido *et al.* 2001; Yang *et al.* 2002) as well as the finding that the superfamily Physeteroidea (families Physeteridae and Kogiidae) and families Platanistidae and Ziphiidae are the three basal branches of the odontocete lineage (Nikaido *et al.* 2001). The only anomalous aspect of our obtained phylogeny is the placement of the grey whale, *Eschrichtius robustus*, within the balaenopterid family grouping (figure 1). However, while this result could simply be a consequence of limited sample size, the grouping was moderately robust. Forced grouping of Eschrichtiidae and Balaenopteridae into their respective families raised the number of required steps for the most parsimonious tree from 234 to 244. Indeed, similar findings have been reported elsewhere (e.g. Yang *et al.* 2002), indicating that further investigation of the relationships of these mysticetes may be merited.

The high degree of sequence identity with the bovine SWS cone opsin and the presence of many conserved SWS cone-opsin residues confirm that the hippopotamus and cetacean genes sequenced were members of the SWS1 cone visual-pigment class. In those sequences frame shifted by indel mutations, codons for many of the conserved residues can still be seen within the nucleotide data even though they are no longer reflected in the amino acid translation. The primary structure of the hippopotamus SWS cone opsin is very similar to that of the functional bovine opsin and contains no apparent missense mutations. Thus it seems likely that, like its close relative the pygmy hippopotamus (*Choeropsis liberiensis*; Peichl *et*

Figure 1. Bootstrap consensus tree of the cetacean, hippopotamus and bovine SWS cone opsin sequences obtained using parsimony (1000 replicates). Bootstrap values are shown for each node. Nodes with bootstrap values of less than 50% were collapsed. Current suborder and familial designations are indicated along the right margin (Rice 1998). Asterisk denotes river dolphin.

al. 2001), the river hippopotamus is capable of expressing functional SWS cone visual pigment. By contrast, all of the cetacean SWS cone-opsin genes appear to be pseudogenes. Every species examined was found to possess one or more missense mutations, and there was no indication from the results of PCR or sequencing analysis that any individual of any of the species examined possessed paralogous functional copies of this gene. The 4 bp deletion possessed by all six mysticetes, *M. bidens* and *P. gangetica* introduces a premature stop codon that dramatically truncates the encoded SWS opsin protein and eliminates several essential functional regions (Hargrave 1982). In those odontocetes whose genes are not disrupted by upstream frame-shifting missense mutations, a single nucleotide substitution in the second exon results in the loss of the glutamate counter-ion. Mutagenesis studies have unequivocally demonstrated that the elimination of this residue results in failed opsin-chromophore bonding (Nathans 1990). In *T. truncatus* and *Pontoporia blainvillei*,

additional frame-shifting indel mutations are present that further disrupt their respective SWS cone-opsin genes. Consequently, none of these cetaceans are capable of producing functional SWS cone visual pigment.

The distribution of these missense mutations among the species examined indicates that the loss of the SWS cone pigment type occurred relatively early in the evolution of modern cetaceans. For instance, the point mutation responsible for the elimination of the glutamate counter-ion is present in all the odontocete species examined, including the most basal lineage, the physeteroids. For this to occur, the missense character must have been present in the SWS cone-opsin genes of the ancestors of modern odontocetes when the suborder first began to diverge in the Oligocene, 25–38 Myr ago (Barnes *et al.* 1985; Nikaido *et al.* 2001). Similarly, nucleotides 262–265 have been deleted from the SWS cone-opsin genes of every individual of all six of the mysticete species that were examined. This indicates that this character was fixed in

	10	20	30	40	50	60	70	80	90	100	110	120	130
B. tau	MSKMSSEEEF	LLFKNLISVG	PWDGEQYHLA	PVWAHLQAV	FMGFVFFGT	ELNATLVAT	LYRKLEQPL	NYILNVSLG	GFYICISVF	IVFITSICY	FEVGRHVCAL	EATLGCTAGL	VTGWSLAFLA
H. amp													
B. acu													
B. mus													
M. nov													
E. rob													
B. mys													
E. gla													
P. mac													
K. bre													
P. gan													
M. bid													
I. geo													
P. bla													
T. tru													
D. leu													
P. pho													
P. sin													

Figure 2. A comparison of transcribed bovine, hippopotamus and cetacean SWS cone opsins. Note that non-frame-shifting gaps have been inserted as necessary to facilitate comparison. The disruptive effects of frame-shifting indel mutations can clearly be seen in many of the cetacean sequences. In all odontocetes not possessing upstream frame-shifting mutations, the non-synonymous change E111G (the replacement of glutamate at residue 111 with glycine), which disrupts the chromophore-binding Schiff's base linkage, is also apparent. Conserved SWS cone-opsin residues identified by Chiu *et al.* (1994) are underlined in the bovine sequence. The boxed area contains the region for which nucleotide data are presented in figure 3. Residue numbering is based on the bovine sequence. Residues identical to those of the bovine sequence are denoted by a dot; residue gaps are denoted by a dash; and stop codons are denoted by an asterisk.

then in the absence of dramatic homoplasy all modern cetaceans must lack SWS cones. The high proportions of non-synonymous substitutions seen in the species examined, a phenomenon characteristic of pseudogene evolution (Li *et al.* 1985), support the conclusion that these genes have been evolving outside of functional selection for some time. By comparison, the hippopotamus, which has accumulated a similar number of nucleotide substitutions to these cetaceans, has less than half the number of non-synonymous changes and exhibits no apparent missense mutations.

Indeed, it is not possible to determine when the original loss of SWS cones occurred in cetaceans. The apparently identical 4 bp deletion present in all six mysticetes and two odontocetes (figure 3) raises the possibility that the loss of SWS cones may even have preceded the separation of the two cetacean suborders. However, there was no single missense character found in all the cetaceans examined that would unequivocally support this claim. Similarly, it is also not possible to determine from these data whether any of the missense mutations discussed here were responsible for the initial conversion of cetacean SWS opsin genes to pseudogenes. These missense mutations might easily have arisen after the gene had converted to a pseudogene and was released from the constraints of functional selection. All that the presence and distribution of these mutations demonstrate is that the SWS cone-opsin genes of modern cetaceans no longer encode viable visual-pigment proteins. These results are supported directly by reports of the absence of SWS cones in *T. truncatus* (Fasick *et al.* 1998) and *Phocoena phocoena* (Peichl *et al.* 2001), and indirectly by similar findings in five other cetacean species (Peichl *et al.* 2001).

Cetaceans are the first order of mammals found to be entirely lacking one of the three ancestral photopigment types. They are also the only entire order of mammals known to lack the potential for cone-based colour vision (Jacobs 1993). While their nearly unique status as exclusively aquatic mammals seems likely to be involved, the adaptive significance of this loss of SWS cone sensitivity is not immediately apparent. In view of the relatively ancient nature of their loss of SWS cones, the monochromatic condition of cetaceans might simply be a non-adaptive artefact of their evolution, brought about by random

the SWS cone-opsin genes of the ancestors of modern mysticetes when they first diverged in the late Oligocene or early Miocene 15–25 Myr ago (Barnes *et al.* 1985; Nikaido *et al.* 2001). Because the distribution of these two missense mutations indicates that the loss of SWS cones is ancestral in both odontocete and mysticete cetaceans,

	250	260	270	280	290	300	310	320	330	340
B. tau	GGCTTCATCT	ACTGCATCTT	CTCTGTCTTC	ATCGTCTTCA	TCACCAGTTG	TTATGGGTAC	TTCGTCTTCG	GCCGCCATGT	CTGTGCCCTG	GAGGCCTTCC
H. amp			T G		C	CC	A	T	T	C
B. acu		C		T G		C	AA	T	A	TT
B. mus			T G		C		AA		T	
M. nov			T G	T	C		AA	T	A	T
E. rob			T G		C		AC	T	A	T
B. mys			T G		C		AA	T	T	T
E. gla			T G		C		GC	AA	T	T
P. mac			T G		C		A	T	T	C
K. bre			T G		C		A	T	A	T
P. gan			T G		C		A	T	A	T
M. bid	T	A		T G		C		A	T	A
I. geo			T G		C		A	T	T	T
P. bla			T G		C		A	T	T	T
T. tru			T G		C		A	T	T	T
D. leu			T G	A		C		A	T	T
P. pho	T		T G		C		A	T	T	T
P. sin			T G		C		A	T	T	T

Figure 3. Nucleotide sequences of a portion of the first and second exons of the SWS cone-opsin genes of 16 cetacean species, the hippopotamus and the cow. Both frame-shifting and non-frame-shifting nucleotide indels have been used to facilitate comparison. The frequency of nucleotide substitutions in the hippopotamus is similar to that of the cetaceans even though the relative number of amino acid changes is dramatically reduced (compare with figure 2). Two critical missense mutations are underlined: the 4 bp deletion at nucleotides 262–265, and the substitution A → G at nucleotide 332. Nucleotide numbering is based on the bovine sequence. Cetacean and hippopotamus nucleotides identical to those of the cow are marked with a dot; nucleotide gaps are marked with a dash.

mutation or limited selective pressure to maintain a SWS cone pigment in the dim conditions found under water. Alternatively, the concurrent loss of SWS cones in other marine mammals (Peichl & Moutairou 1998; Peichl *et al.* 2001) indicates that the absence of this cone type is adaptive and somehow beneficial for underwater vision in carnivorous marine mammals. Unlike its terrestrial relatives, in one cetacean, the bottlenose dolphin, the M/LWS cones are shifted in sensitivity towards the wavelengths of light that predominate under water (Fasick & Robinson 1998), evidence that cone-based vision continues to be of importance to at least some cetaceans. Moreover, only a single cetacean species, the Indian river dolphin, *P. gangetica*, exhibits any apparent reduction in visual anatomy that might be attributed to a lack of need for vision (Herald *et al.* 1969), and research continues to document the importance of vision for even the deepest-diving cetacean species (e.g. Frstrup & Harbison 2002). While several potential benefits of the loss of SWS cones are conceivable (see Peichl *et al.* (2001) for a discussion), we favour the hypothesis that the loss of this cone type is related to the high scattering coefficients of very short wavelengths of light ($\lambda < 450$ nm) in water (Kirk 1994, pp. 101–104). As noted by Lythgoe (1979), the reduction in sensitivity to these highly scattered wavelengths could serve to increase the visibility of broad-spectrum or relatively long-wavelength visual targets. Similar hypotheses have been put forth to explain trends in short-wavelength cone sensitivity observed in freshwater cottoid fishes (Bowmaker 1995). The associated loss of cone-based colour vision is presumably an inadvertent consequence of the loss of SWS cones, as it is even more difficult to imagine a scenario in which the ability to discriminate colour might be detrimental to visual functioning.

Regardless of the selective mechanism underlying the loss, it is clear from the data obtained here that the cetaceans examined lack SWS cone visual pigments. The absence of this visual-pigment type reduces the ability of

these marine mammals to detect stimuli in the short-wave-length region of the spectrum and renders them incapable of making conventional cone-based colour discriminations. Moreover, while the precise origin of the loss of this cone type is uncertain, it clearly occurred before the divergences of the mysticete and odontocete suborders. Consequently, by inheritance, all modern cetaceans are incapable of producing functional SWS cone visual pigments.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.