wall. We propose that the high speed of discharge is due to the release of energy stored in the stretched configuration of the collagen polymer of the capsule wall. How the ejection of the stylets is initiated during exocytosis and after fusion pore formation remains to be shown.

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Supplemental data

Supplemental data including Experimental Procedures are available at http://www.current-biology.com/cgi/content/full/16/9/R316/DC1/

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[1]Institute of Zoology, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany. [2]Department for Applied Physics, LM University Munich, 80799 München, Germany. [3]Department of Biology, Darmstadt University of Technology, Schnittpahnstrasse 10, 64287 Darmstadt, Germany. [4]Heidelberg Institute of Zoology, University of Heidelberg, 69120 Heidelberg, Germany. [5]E-mail: holstein@uni-hd.de

Molecular evidence for dim-light vision in the last common ancestor of the vertebrates

Davide Pisani[1,2]*, Samantha M. Mohun[2], Simon R. Harris[3], James O. McInerney[1] and Mark Wilkinson[2]

Animal vision is mediated through pigments belonging exclusively to the opsin family. These are members of the G-protein-coupled receptor family that bind retinal [1]. Based on function and phylogenetic relationship, vertebrate visual opsins can be clustered in five groups: Rhod photoreceptors (Rh1), Rh1-like (Rh2), Short Wave Sensitive (SWS1), SWS1-like (SWS2), and Long (LWS) or Medium (MWS) Wave Length Sensitive (LWS/MWS). Rh1 is used for seeing under dim light conditions (scotopic vision), while the others permit full colour (photopic) vision in bright light [2–5]. Opsins have diversified by a series of gene duplications, and the inferred order of these duplications indicates that photopic vision predated scotopic vision in vertebrates [2–5].

Assuming that the jawless vertebrates (Agnatha) are monophyletic [6], the broad distribution of opsins associated with photopic vision indicates that these — and thus the capacity for photopic vision — were present in the last common ancestor of all living vertebrates [2–5]. However, it is still unclear whether 'true' (i.e., Rh1-mediated [3]) dim-light vision predated the split between Agnatha and the jawed vertebrates (Gnathostomata), or whether it is an apomorphy of the Gnathostomata [2–5,7]. Solving this question has important palaeoecological implications and, assuming that orthologous opsins have inherited a common ancestral function, it depends on the correct classification (phylogeny based orthologous clustering) of the few agnathan Rh sequences available to date.

Based on a Neighbour Joining (NJ) analysis, Yokoyama [2] suggested that the two agnathan Rh sequences available at that time represented Rh1 opsins. This would imply that the vertebrate cenancestor was potentially capable of

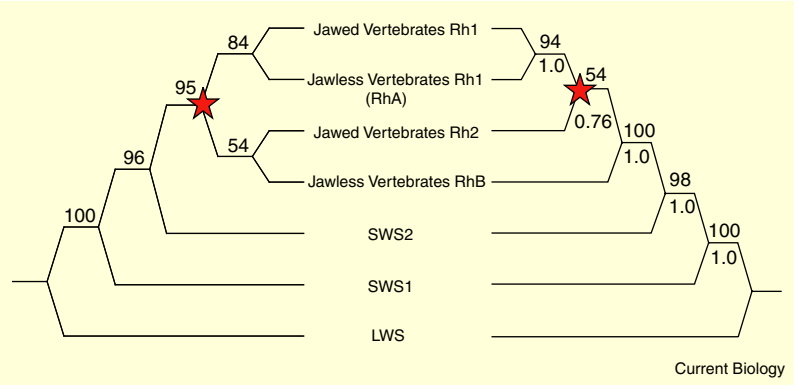

Figure 1. Phylogenetic relationships among vertebrate visual opsins.

The left tree shows a summary of the relationships obtained from the quartet puzzling and the minimum evolution analyses (Supplemental data). Numbers at the nodes represent quartet-puzzling support values. The right tree shows a summary of the relationships obtained in the ML analyses (PHYML, SPR-PHYML and SPR), in the Bayesian analysis, and in the equally and differentially weighted parsimony analyses (Supplemental data). Numbers at the nodes represent the bootstrap support values obtained in the PHYML analysis (above) and posterior probabilities (below). The red star represents the gene duplication resulting in the origin of Rh1.

true dim-light vision. Alternatively, Collin and co-workers [3–5,7] suggested that the vertebrate cenancestor did not possess an Rh1 gene and could not have been capable of true dim-light vision. This conclusion was based on the isolation and NJ analysis of LWS/MWS, SWS1, SWS2, and two Rh sequences from the southern lamprey *Geotria australis.* Collin *et al.* [3] included also the other available agnathan Rh sequences (i.e., a putative *Lampetra* and *Petromyzon* Rh1 sequences) in their analysis and recovered all four agnathan Rh sequences as the monophyletic sister group of the gnathostomatan Rh1 and Rh2. This implies that agnathan Rh opsins originated from a gene duplication predating that giving rise to Rh1 and Rh2, such that agnathans have neither an Rh1 nor an Rh2 gene [3–5,7]. They named the new agnathan-specific opsins they isolated in *G. australis* RhA and RhB, and reassigned the *Lampetra* and the *Petromyzon* Rh1 to RhA [3], thus concluding that the cenancestor did not possess the Rh1 gene and thus no true scotopic vision.

Here, we analyse the amino acid sequences of 47 representative vertebrate visual opsins (LWS, SWS1, SWS2, Rh1, Rh2, RhA, and RhB) using a variety of phylogenetic methods to test the alternative hypotheses that true dim-light vision evolved in the stem vertebrate lineage, or within the Gnathostomata. We analysed the data with minimum evolution, equally and differentially weighted maximum parsimony, Bayesian analysis and Maximum Likelihood while testing for long branch attraction artefacts using the method of Pisani [8] (see Supplemental Data available with this article online). Our analyses show that a clade containing RhA plus RhB is never recovered (Figure 1). Rather, quartet-puzzling Maximum Likelihood strongly supports the agnathan RhA being orthologous with the gnathostome Rh1 and, albeit less strongly, identifies the agnathan RhB as an Rh2 (Figure 1A). Standard Maximum Likelihood analyses and the Bayesian analysis provide very high support for the orthology of RhA and Rh1 (Figure 1B), but not for the orthology of RhB and Rh2. Minimum evolution supports the tree shown in Figure 1A, while the maximum parsimony analyses support that in Figure 1B. Pisani's method [8] for countering long branch attraction does not change the inferred relationships of RhA or RhB. Although the data do not allow us to choose between the alternative trees in Figure 1, the approximately unbiased test [9] unequivocally shows that these trees explain the data significantly better than the tree of Collin *et al.* [3] ($p = 0.0002$).

Based on these results, we can confidently conclude in accordance with [2] that the last common ancestor of the vertebrates possessed an Rh1 gene, which is thus much older then suggested in many recent studies and reviews [3–5,7]. The function of Rh1 in agnathans is not yet known, but assuming its function in the vertebrate cenancestor was not dramatically different from its scotopic function in most vertebrates, this implies that both photopic and scotopic vision evolved in the stem vertebrate lineage and must have been in place in the Cambrian by about 522–518 Ma [10,11].

Early vertebrate evolution probably took place in a brightly lit environment and thus the earliest stem vertebrates were probably diurnal and inhabited shallow waters. However, at some stage after photopic vision was already in place, scotopic vision also evolved in the stem vertebrate lineage, which implies that a behavioural or ecological shift — perhaps a move into deeper water or to nocturnality — occurred in an ancestral vertebrate. What drove this shift can only be conjectured, such as the emergence of large macrophagous predators [12], and is certainly a topic for further palaeobiological investigations.

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Supplemental data

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[1]Bioinformatics Laboratory, The National University of Ireland, Maynooth, Ireland. [2]Department of Zoology, The Natural History Museum. Cromwell Road, SW7 5BD, London, UK. [3]School of Biology and Psychology, Division of Biology, University of Newcastle-upon-Tyne, NE1 7RU, UK.
*E-mail: davide.pisani@nuim.ie