Nanopore Amplicon Sequencing Reveals Molecular Convergence and Local Adaptation of Rhodopsin in Great Lakes Salmonids

Katherine M. Eaton1,†, Moisés A. Bernal1,†, Nathan J.C. Backenstose1, Daniel L. Yule2, and Trevor J. Krabbenhoft 1,3,*

1Department of Biological Sciences, University at Buffalo, New York, USA
2U.S. Geological Survey, Great Lakes Science Center – Lake Superior Biological Station, Ashland, Wisconsin, USA
3RENEW Institute, University at Buffalo, New York, USA

*Corresponding author: E-mail: tkrabben@buffalo.edu.
Accepted: 3 November 2020
†Present address: Department of Biological Sciences, Auburn University, Auburn, Alabama, USA

Abstract

Local adaptation can drive diversification of closely related species across environmental gradients and promote convergence of distantly related taxa that experience similar conditions. We examined a potential case of adaptation to novel visual environments in a species flock (Great Lakes salmonids, genus *Coregonus*) using a new amplicon genotyping protocol on the Oxford Nanopore Flongle and MinION. We sequenced five visual opsin genes for individuals of *Coregonus artedi*, *Coregonus hoyi*, *Coregonus kiyi*, and *Coregonus zenithicus*. Comparisons revealed species-specific differences in a key spectral tuning amino acid in rhodopsin (Tyr261Phe substitution), suggesting local adaptation of *C. kiyi* to the blue-shifted depths of Lake Superior. Ancestral state reconstruction demonstrates that parallel evolution and “toggling” at this amino acid residue has occurred several times across the fish tree of life, resulting in identical changes to the visual systems of distantly related taxa across replicated environmental gradients. Our results suggest that ecological differences and local adaptation to distinct visual environments are strong drivers of both evolutionary parallelism and diversification.

Key words: genomics, genotyping, nanopore, long-read sequencing, parallel evolution, toggling.

Significance

Previous research has shown parallel evolution of vision genes across the fish tree of life. Two key questions are what role do they play in ecological diversification across light availability gradients and whether these changes can be reversible. This study demonstrates one vision gene in particular, *rhodopsin*, is involved in local adaptation across a depth gradient in Great Lakes salmonids. Additionally, we provide evidence that several species of salmonids have undergone parallel reversals to an ancestral rhodopsin allele that was last seen in this lineage over 175 Ma, showcasing an extreme example of evolutionary reversal over deep time.

Introduction

Local adaptation to novel environments can drive genetic and phenotypic differentiation among closely related organisms. Diversification may occur as populations become locally adapted to distinct conditions, leading to the evolution of divergent traits that are beneficial in each lineage’s preferred environment. Conversely, a trait may be sufficiently advantageous in a particular environment that multiple distantly related taxa converge upon it, sometimes due to the same mutation or amino acid substitution occurring independently.
Due to their importance in ecological interactions and their dynamic evolutionary history, the evolution of visual pigment genes (i.e., opsins) in marine and freshwater fishes has received considerable attention (Lin et al. 2017; reviewed in Rennison et al. 2012; Carleton et al. 2020). The vertebrate visual opsin system is divided into five subgroups—one rod opsin (rhodopsin) responsible for vision under low light conditions and four cone opsins (long-wave sensitive, short-wave sensitive 1, short-wave sensitive 2, and rhodopsin 2) responsible for color vision. These subgroups are based in part on differences in their peak absorbance spectra, with each group resulting from a series of gene duplications and subsequent amino acid substitutions, leading to small but measurable functional differentiation among the five opsin types (Okano et al. 1992; Yokoyama 2000). Opsin genes can shape the evolution of vision via several mechanisms. First, single nucleotide polymorphisms (SNPs) can cause nonsynonymous substitutions in key spectral tuning residues, in some cases driving adaptation to different light environments (Terai substitutions in key spectral tuning residues, in some cases nucleotide polymorphisms (SNPs) can cause nonsynonymous the evolution of vision via several mechanisms. First, single (Okano et al. 1992; Yokoyama 2000). Opsin genes can shape gene conversion for color vision. These subgroups are based in part on differences in their peak absorbance spectra, with each group resulting from a series of gene duplications and subsequent amino acid substitutions, leading to small but measurable functional differentiation among the five opsin types (Okano et al. 1992; Yokoyama 2000). Opsin genes can shape the evolution of vision via several mechanisms. First, single nucleotide polymorphisms (SNPs) can cause nonsynonymous substitutions in key spectral tuning residues, in some cases driving adaptation to different light environments (Terai et al. 2002; Marques et al. 2017). Alternatively, copy number variants (CNVs) may result from tandem duplications and/or whole genome duplications and undergo neo-functionalization to maximally absorb at a new wavelength (e.g., expansions of rod opsins in deep-sea fishes [Musilova et al. 2019] and cone opsins in shallow-water fishes [Weadick and Chang 2007]), although gene conversion may sometimes work to homogenize newly formed paralogs, inhibiting this process (Carleton et al. 2020). Vision can also be modified by variation in opsin expression patterns based on both ontogenetic and environmental cues (Shand et al. 2008; Hofmann et al. 2009).

The cisco species flock (genus Coregonus) of the Laurentian Great Lakes presents a well-suited opportunity to study local adaptation of visual opsins to novel light environments based on depth differences among species (Harrington et al. 2015). The four extant cisco species in Lake Superior show generally low levels of interspecific variation across the genome (Turgeon and Bernatchez 2003; Turgeon et al. 2016; Ackiss et al. 2020) despite considerable differences in depth preferences (Rosinski et al. 2020). Coregonus artedi is typically epilimnetic (10–80 m), whereas Coregonus hoyi and Coregonus zenithicus are both found at intermediate depths (40–160 m), and Coregonus kiyi can be found at depths of 80–200 m (Rosinski et al. 2020). Based on the observed ecological differentiation, we hypothesize that divergent selection may have acted to fine-tune opsins for maximum absorption of wavelengths of light that penetrate to each species’ preferred depth. Here we assess the evolution of five visual opsin genes in the Coregonus species flock to better understand mechanisms underlying their divergence across a depth gradient.

**Materials and Methods**

Oxford Nanopore sequencing is contributing to a rapidly expanding toolkit for DNA sequencing, owing to low up-front costs, enhanced ability to detect DNA or RNA base modifications, and read lengths limited only by the quality and quantity of input nucleic acids. Nanopore sequencing also allows for straightforward haplotyping, as whole molecules can be sequenced for each amplicon with no need for assembly. This approach has been successfully applied to microbial metatranscriptomics and pathogen identification (Shin et al. 2016; Moon et al. 2018; Rames and Macdonald 2018), as well as human genotyping (Cornelis et al. 2017, 2019). As flow cell quality and base-calling algorithms have improved, the accuracy and functionality of nanopore amplicon sequencing have rapidly improved. However, its application to SNP genotyping in nonhuman eukaryotes with large and complex genomes remains relatively unexplored. It is particularly relevant to understand whether accurate genotypes can be obtained, and, if so, what coverage depth is needed to do so.

In the present study, we sequenced amplicons of five teleost opsin genes in a total of 74 samples on the Oxford Nanopore Flongle device. In combination with the PCR Barcoding Expansion 1–12 (Oxford Nanopore Technologies), we sequenced and genotyped 12 individuals simultaneously on a single Flongle flow cell, following the pipeline shown in figure 1 (for a complete protocol, see Supplemental Protocol). This study is one of the first to demonstrate the accuracy and utility of amplicon sequencing with the Oxford Nanopore Flongle for SNP genotyping of eukaryotic samples.

A preliminary assembly of the de novo transcriptome of *C. artedi* (NCBI BioProject #PRJNA659559; Bernal et al. 2020) was used as a reference to extract gene sequences of long-wave sensitive (LWS), short-wave sensitive 1 (SWS1), short-wave sensitive 2 (SWS2), rhodopsin (RH1), and rhodopsin 2 (RH2), representing one gene from each teleost visual opsin subfamily. For each of the five genes of interest, a fragment ~700–2,100 bp in length was amplified for 18 samples of *C. artedi*, 19 *C. hoyi*, 21 *C. kiyi*, and 16 *C. zenithicus* (supplementary tables S1–S3, Supplementary Material online). All amplicons from a single individual were assigned a specific barcode and were pooled into a library containing amplicons from 12 samples, which were sequenced simultaneously on a single Flongle flow cell (fig. 1). This process was then repeated until amplicons from all samples were sequenced. Raw reads were deposited in SRA under NCBI BioProject...
Sample-specific barcodes were detected and trimmed using Guppy v3.2.4 (Oxford Nanopore Technologies) with the command `guppy_barcoder`, and reads from each sample were mapped with BWA v0.7.17 using the command `bwa mem`, with version one of the Coregonus sp. “balchen” genome assembly as a reference (De-Kayne et al. 2020; GCA_902810595.1). To verify the accuracy of nanopore amplicon genotyping, we performed a rarefaction analysis in which SNPs were called at various levels of coverage (i.e., maximum possible coverage; 2,000; 1,000; 500; 250; 100;
Although accuracy remained high at all sequencing depths with Sanger sequences of RH1, which is located in transmembrane helix six, facing away from the retinal binding pocket (fig. 2a and b, see also Baldwin 1993; Hunt et al. 1996). Neither residue 100 nor 255 are known to be key spectral tuning sites in rhodopsin (Yokoyama 2000), although Yokoyama and Jia (2020) recently reported that lle255Val may have an effect on spectral tuning of RH2. The three aforementioned SNPs possess the exact same FST and changes in genotype were completely consistent across all samples, suggesting that these sites are tightly linked.

The most strongly segregating SNP (FST = 0.88) occurred at amino acid residue 261 of rhodopsin, which is located in transmembrane helix six, facing the retinal binding pocket of the protein (fig. 2a and b, see also Baldwin 1993; Hunt et al. 1996; Yokoyama 2000). Coregonus artedi, C. hoyi, and C. zenithicus, inhabitants of a relatively shallow, broad-spectrum light environment, were primarily homozygous for tyrosine at this locus (fig. 3). Meanwhile, C. kiyi, which inhabits the blue-shifted deeper waters of Lake Superior, was completely homozygous for phenylalanine. The presence of phenylalanine at this site is known to cause an 8 nm blue-shift in the absorbance spectrum, likely due to the change in polarity associated with this substitution (i.e., polar to nonpolar Y261F) (Yokoyama et al. 1995). Genotypic associations at this site

Results and Discussion

On average, Flongle sequencing runs yielded 206.13 Mb (SD = 166.64 Mb; min–max: 26.84–471.50 Mb), with an average of 184,958 reads (± 154,877 reads; 23,468–435,138 reads), though yield varied based on flow cell quality (flow cells used were earlier versions with low number of active pores). The average sequence N50 was 1,117 bp (± 305 bp; 897–1,852 bp), with read length abundances peaking at the approximate lengths of our amplicons (fig. 1). After sequencing genes with low coverage following first-round sequencing, the average coverage was 3,199.58 across all five genes (± 4,804.24, ± 10.47–31,158.31; supplementary table S1, Supplementary Material online). Coverage varied slightly by species, but this is likely an artifact of stochastic differences in PCR efficiency and sequencing yield (supplementary table S4, Supplementary Material online). Amplicon reads mapped uniquely (i.e., one genomic region per amplicon) to the C. sp. “balchen” genome, confirming that each amplicon does indeed correspond to a sequence of a single gene. However, based on a BLAST search of our opsins against the C. sp. “balchen” genome, multiple functional copies of LWS, RH2, and SWS1 exist in the Coregonus sp. genome, providing another potential avenue for opsin evolution (supplementary table S5, Supplementary Material online). Additionally, it was determined that the copy of LWS analyzed here is partially pseudogenized and perhaps nonfunctional.

The SNP calls from nanopore data were then compared with Sanger sequences of RH1 for the same individuals. Although accuracy remained high at all sequencing depths (>90%), we found incongruencies in a small proportion of samples between 10× and 75×. Only when reaching 100× coverage were genotypes called with complete accuracy for all individuals, in relation to Sanger sequences. Considering that small errors can impact the results of analyses involving amplicons with few variant sites, we recommend a minimum per-amplicon coverage of 100× for future work.

We used a conservative genotyping approach, with the goal of assessing the coverage needed for accurate genotyping on a Flongle flow cell. Based on our findings, this approach could be used for higher throughput sequencing, which could involve more amplicons, more individuals, or a combination of both. Considering that we generated ~200 Mb of sequence data per run, the number of individuals and amplicons that can be sequenced simultaneously at 100× coverage can be estimated as follows:

200,000,000 bp = 100*A*N*NA,
locus vary consistently with depth (fig. 3), providing evidence that \textit{C. kiyi} is adapted to life in deep water after evolving from shallow-water ancestors. This hypothesis is further corroborated by phenotypic data, as \textit{C. kiyi} have significantly larger eye diameters (as a proportion of total head length) than \textit{C. artedi} (\(P < 0.001\)), \textit{C. zenithicus} (\(P < 0.001\)), and \textit{C. hoyi} (\(P < 0.001\); supplementary fig. S1, Supplementary Material online). The predictable variation of both genetic and morphological traits provides key evidence that local adaptation to depth accompanies diversification of Great Lakes ciscoes (supplementary fig. S2, Supplementary Material online).

The shift between the two aforementioned amino acids at rhodopsin residue 261 was recently analyzed in a deep phylogenetic context. Hill et al. (2019) showed that fish lineages that have undergone a habitat change from blue-shifted marine waters to red-shifted brackish or freshwater have independently converged on the red-shift-associated 261Tyr phenotype over 20 times across the fish tree of life, including in the channel catfish (\textit{Ictalurus punctatus}), rainbow trout (\textit{Oncorhynchus mykiss}), Kessler’s sculpin (\textit{Leocottus kesslerii}), and European flounder (\textit{Platichthys flesus}), among others, and can occur in both directions (i.e., Phe\textsubscript{261}Tyr or Tyr\textsubscript{261}Phe; fig. 4). The Salmoniformes and Esociformes in particular appear to have undergone a high degree of parallel reversal at this site, with multiple taxa (\textit{Oncorhynchus} spp., \textit{Brachymystax lenok}, \textit{C. kiyi}, and \textit{Dallia pectoralis}) undergoing independent parallel reversals to the deep ancestral blue-shifted allele (fig. 4). These findings indicate that rhodopsin residue 261 may be able to toggle between these two amino acids depending on what is advantageous in a particular light environment, even across incredibly long time scales.

**Conclusions**

Our results indicate that local adaptation to distinct visual environments is associated with genetic and morphological differentiation among the closely related ciscoes of Lake Superior. The identification of several high F\textsubscript{ST} SNPs in rhodopsin, including Phe\textsubscript{261}Tyr, is particularly relevant, as the shifts between these two amino acids at residue 261 are identical to those observed across similar light availability gradients in shallow waters. This suggests a common evolutionary response to visual adaptation among these lineages, which may be subject to similar selective pressures in different lake environments.
phylogenetically distant fishes (Hill et al. 2019). Adaptation to similar light conditions therefore seems to lead to phenotypic convergence due to parallel single-nucleotide changes at this site. Additionally, the discovery of independent reversals to the ancient ancestral state in C. kiyi (and other lineages), provides evidence of genetic toggling, whereby organisms transition bidirectionally between different alleles in response to environmental pressures. This result is striking because the genetic background is presumably very different across these taxa after more than \( \frac{1}{24} \) Myr of divergence. In this case, the conserved nature of rhodopsin protein function likely potentiates evolutionary reversals over deep time periods. The observation of amino acid toggling in Great Lakes Coregonus species and across the fish tree of life is at the extreme end of the time scale of parallel evolution and evolutionary reversals, given the generally held prediction that the likelihood of evolutionary reversal diminishes as time progresses (Storz 2016; Blount et al. 2018).

Long-read amplicon sequencing using the Oxford Nanopore Flongle is highly amenable to genotyping complex eukaryotes. The methodology described here is simple and reliable, and offers the promise of rapid, low-cost genotyping in nonmodel organisms. This protocol has many potential applications—nanopore amplicon genotyping could be used for CRISPR validation, screening for inherited genetic disorders, and eukaryotic eDNA metabarcoding, among others.

Supplementary Material
Supplementary data are available at Genome Biology and Evolution online.

Acknowledgments
We thank the US Geological Survey Research Vessel Kiyi Captain Joe Walters, First Mate Keith Peterson, and Engineer Charles Carrier, as well as Lake Superior Biological Station staff Mark Vinson and Lori Evrard for assistance with sample collections. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government. All sampling and handling of fish were carried out in accordance with guidelines for the care and use of fishes by the American Fisheries Society (Jenkins...
et al. 2014). Jessie Pelosi provided critical assistance with data analysis. Christopher Loretz, Kelly Harrington, Mary Alice Coffroth, Daniel MacGuigan, Tianying Lan, Wendylee Stott, Amanda Ackiss, Andrew Muir, Thomas Dowling, Hannah Waterman, Victor Albert, Vincent Lynch, and Omer Gokcumen provided valuable assistance or feedback on the study. Jessica Poulin coordinated the University at Buffalo Honors Program in Biological Sciences that facilitated this research. Finally, we wish to thank the editors and anonymous reviewers for providing thoughtful, constructive, and timely feedback, particularly in the midst of a global pandemic. This study was supported by the Great Lakes Fishery Commission (Award #2018_KRA_44073 to T.J.K.), the University at Buffalo Department of Biological Sciences (Philip G. Miles Fellowship to K.M.E.), and the University at Buffalo Honors College (Award to K.M.E.).

Data Availability

Raw sequence reads generated on the Oxford Nanopore Flongle were deposited in SRA, under the BioProject #PRJNA664981. BAM alignments of these sequences to the Coregonus sp. balchen genome V1 were deposited in Dryad (doi:10.5061/dryad.crjdfn32s), along with the VCF file used to call SNPs. Raw Sanger sequencing files were also deposited in the aforementioned Dryad repository.
Literature Cited

Ackiss AS, Larson WA, Stott W. 2020. Genotyping-by-sequencing illuminates high levels of divergence among sympatric forms of coregonines in the Laurentian Great Lakes. Evol Appl. 13(5):1037–1054.

Baldwin JM. 1993. The probable arrangement of the helices in G protein-coupled receptors. EMBO J. 12(4):1693–1703.

Bernal MA, et al. 2020. Transcriptomic divergence predicts morphological and ecological variation underlying an adaptive radiation. BioRxiv.

Blount ZD, Lenski RE, Losos JB. 2018. Contingency and determinism in evolution: replaying life’s tape. Science 362(6415):eaam5979.

Carleton KL, Escobar-Camacho D, Stieb SM, Cortesi F, Marshall NJ. 2020. Seeing the rainbow: mechanisms underlying spectral sensitivity in teleost fishes. J Exp Biol. 223(8):ebj193334.

Chang J, Rabosky DL, Smith SA, Alfaro ME. 2019. An R package and online resource for macroevolutionary studies using the ray-finned fish tree of life. Methods Ecol Evol. 10(7):1118–1124.

Cornelis S, et al. 2019. Forensic tri-allelic SNP genotyping using nanopore sequencing. Forensic Sci Int Genet. 38:204–210.

Cornelis S, Gansenmans Y, Deleye L, Deforce D, Van Nieuwerburgh F. 2017. Forensic SNP genotyping using Nanopore MinION sequencing. Sci Rep. 7(1):41579.

De-Kayne R, Zoller S, Feulner PGD. 2020. A de novo chromosome-level genome assembly of Coregonus sp. “Balchen”: one representative of the Swiss Arctic whitefish radiation. Mol Ecol Resour. 20(4):1093–1109.

Delport W, Scheffler K, Seoighe C. 2008. Frequent toggling between alternative amino acids is driven by selection in HIV-1. PLoS Pathog. 4(12):e1000242.

Futuyma D, Kirkpatrick M. 2017. Evolution. 4th ed. Sunderland (MA): Sinauer.

Harrington KA, Habib TR, Mensinger AF. 2015. Visual sensitivity of deep-water fishes in Lake Superior. PLoS One 10(2):e0116173.

Hill J, et al. 2019. Recurrent convergent evolution at amino acid residue 261 in fish rhodopsin. Proc Natl Acad Sci USA. 116(37):18473–18478.

Hofmann CM, et al. 2009. The eyes have it: regulatory and structural changes both underlie cichlid visual pigment diversity. PLoS Biol. 7(12):e1000266.

Hunt DM, Fitzgibbon J, Slobodyanyuk SJ, Bowmaker JK. 1996. Spectral tuning and molecular evolution of rod visual pigments in the species flock of cottoid fish in Lake Baikal. Vis Res. 36(9):1217–1224.

Jenkins JA, et al. 2014. Guidelines for the use of fishes in research—revised and expanded. 2014. Fisheries 39(9):415–416.

Kelley LA, Mezulis S, Yates CM, Wiss MN, Sternberg MJE. 2015. The Phyre2 web portal for protein modeling, prediction, and analysis. Nat Protoc. 10(6):845–858.

Lin JJ, Wang F-Y, Li W-H, Wang T-Y. 2017. The rises and falls of opsin genes in 59 ray-finned fish genomes and their implications for environmental adaptation. Sci Rep. 7(1):15568.

Marques DA, et al. 2017. Convergent evolution of SWS2 opsin facilitates adaptive radiation of three-eyed stickleback into different light environments. PLoS Biol. 15(4):e2001627.

Moon J, et al. 2018. Diagnosis of Haemophilus influenzae pneumonia by nanopore 16S amplicon sequencing of sputum. Emerg Infect Dis. 24(10):1944–1946.

Musilova Z, et al. 2019. Vision using multiple distinct rod opsins in deep-sea fishes. Science 364(6440):588–592.

Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T. 1992. Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc Natl Acad Sci USA. 89(13):5932–5936.

Pettersen EF, et al. 2004. UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem. 25(13):1605–1612.

Porubsky D, et al. 2020. Recurrent inversion toggling and great ape genome evolution. Nat Genet. 52(8):849–858.

Rabosky DL, et al. 2018. An inverse latitudinal gradient in speciation rate for marine fishes. Nature 559(7714):392–395.

Rames E, MacDonald J. 2018. Evaluation of MinION nanopore sequencing for rapid enterovirus genotyping. Virus Res. 252:8–12.

Rennison DJ, Owens GL, Taylor JS. 2012. Opsin gene duplication and divergence in ray-finned fish. Mol Phylogenet Evol. 62(3):986–1008.

Romero-Herrera AE, Lehmann H, Joysey KA, Friday AE. 1978. On the evolution of myoglobin. Philos Trans R Soc Lond B Biol Sci. 283(995):61–163.

Rosinski CL, Vinson MR, Yule DL. 2020. Niche partitioning among native ciscoes and nonnative rainbow smelt in Lake Superior. Trans Am Fish Soc. 149(2):184–203.

Shand J, et al. 2008. The influence of ontogeny and light environment on the expression of visual pigment opsin in the retina of the black bream, Acanthopagrus butcheri. J Exp Biol. 211(9):1495–1503.

Shin J, et al. 2016. Analysis of the mouse gut microbiome using full-length 16S rRNA amplicon sequencing. Sci Rep. 6(1):29681.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9):1312–1313.

Stewart CB, Schilling JW, Wilson AC. 1987. Adaptive evolution in the stomach lysozymes of forage fermenters. Nature 330(6416):401–404.

Storz JF. 2016. Causes of molecular convergence and parallelism in protein evolution. Nat Rev Genet. 17(4):239–250.

Tera Y, Mayer WE, Klein J, Tichy H, Okada N. 2002. The effect of selection on a long wavelength-sensitive (LWS) opsin gene of Lake Victoria cichlid fishes. Proc Natl Acad Sci USA. 99(24):15501–15506.

Torjeen J, Bernatchez L. 2003. Reticulate evolution and phenotypic diversity in North American ciscoes, Coregonus spp. (Teleostei: Salmonidae): implications for the conservation of an evolutionary legacy. Conserv Genet. 4(1):67–81.

Torjeen J, et al. 2016. Morphological and genetic variation in Cisco (Coregonus artedii) and Shortjaw Cisco (C. zenithicus): multiple origins of Shortjaw Cisco in inland lakes require a lake-specific conservation approach. Conserv Genet. 17(1):45–56.

Weadick CJ, Chang BSW. 2007. Long-wavelength sensitive visual pigments of the guppy (Poecilia reticulata): six opsins expressed in a single individual. BMC Evol Biol. 7(Suppl. 1):S11.

Yokoyama R, Knox BE, Yokoyama S. 1995. Rhodopsin from the fish, Astyanax: Role of tyrosine 261 in the red shift. Invest Ophthalmol Vis Sci. 36(5):939–945.

Yokoyama S. 2000. Molecular evolution of vertebrate visual pigments. Prog Retin Eye Res. 19(4):385–419.

Yokoyama S, Jia H. 2020. Origin and adaptation of green-sensitive (RH2) pigments in vertebrates. FEBS Open Bio. 10(5):873–882.

Yu G, Smith DK, Zhu H, Guan Y, Lam TTY. 2017. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol. 8(1):28–36.

Zhang J, Kumar S. 1997. Detection of convergent and parallel evolution at the amino acid sequence level. Mol Biol Evol. 14(5):527–536.

Associate editor: Bonnie Fraser