

Evolution of Non-visual Opsin Genes Across the Frog Tree of Life



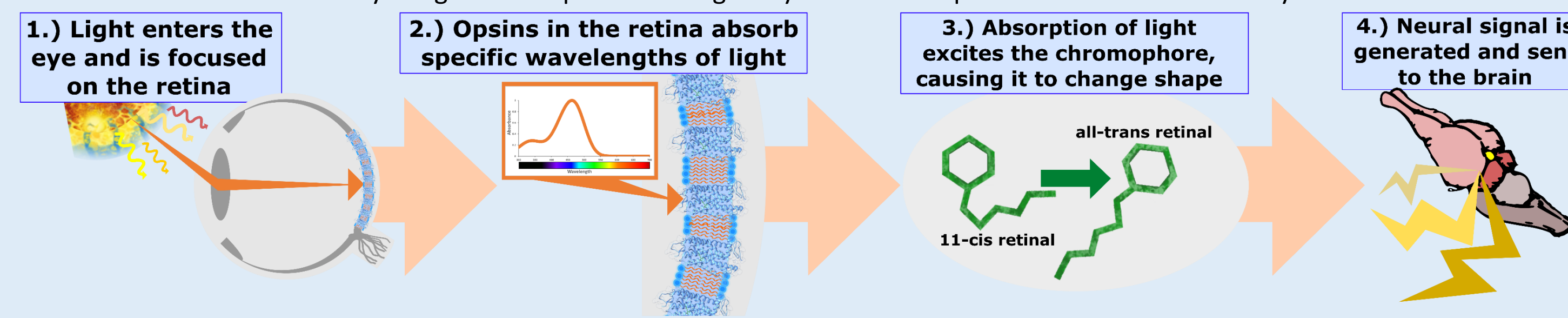
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Opsins and Light Detection

What are opsins?

Opsins are transmembrane proteins which encapsulate chromophores. When the opsin absorbs light, the chromophore changes shape, resulting in a neural signal. Changes in the amino acid composition of opsins may impact their sensitivity to light. This "spectral tuning" may allow for adaptive evolution to new lifestyles.



Intro to Non-visual Opsins

The function & evolution of non-visual opsins is not well understood in frogs

Visual vs non-visual opsins	Non-visual opsin genes found in frogs	Where are these genes expressed?	What light are they sensitive to?
Visual Opsins -Formation of visual images Non-Visual Opsins -Calibration of Circadian Rhythm -Melatonin release -Pupil response	1. NEUR1 (Neurospain 1) 2. NEUR2 (Neurospain 2) 3. NEUR3 (Neurospain 3) 4. NEUR4 (Neurospain 4) 5. NEUR5 (Neurospain 5) 6. NEUR6 (Neurospain 6) 7. OPN4m (Opsin-like melanopsin) 8. OPN4x (Opsin-like melanopsin) 9. PAR (Paratopospain) 10. PARA (Paratopospain) 11. PIN (Pinopsin) 12. RGR (Retinal G-protein coupled receptor) 13. RRH (Rhodopsin) 14. TMT1 (Tritan Multiple Tissue 1) 15. TMT2 (Tritan Multiple Tissue 2) 16. TMT3 (Tritan Multiple Tissue 3) 17. VAOP (Vertebrate Opsin Ancestor)	Eyes Pineal Complex (In yellow) Skin	Some information is available regarding the spectral sensitivities of non-visual opsins in vertebrates

Objectives

1. Identify which non-visual opsin genes are expressed in frog eyes
2. Compare selective pressure between non-visual opsin genes and test for positive selection
3. Test for potential adaptive evolution by comparing selection between discreet lifestyle classes within each gene

Methods

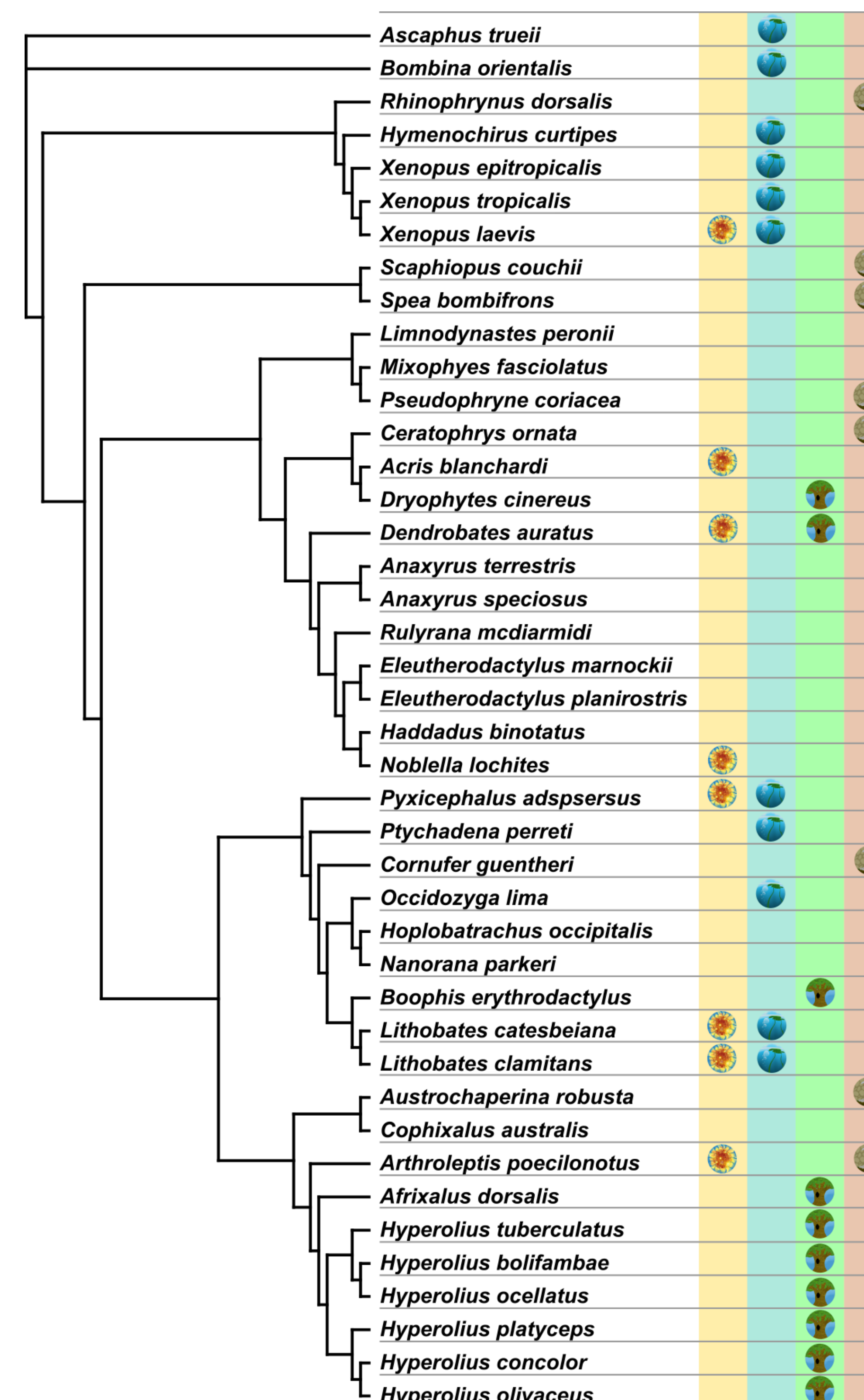
1. RNA extracted & sequenced from 42 frog species
2. Assembled & aligned non-visual opsin genes for each species using *Xenopus* & *Nanorana* references
3. Generated trees for all recovered genes (NEUR2, NEUR5, PAR, PARA, & PIN not recovered)
4. Inferred selective pressure (dN/dS) acting on each gene using PAML codon models
 P.A.M.L. = Phylogenetic Analysis by Maximum Likelihood
 dN/dS = (Nonsynonymous substitution) / (Synonymous substitution)
 RNA Codon → Amino Acid
 (substitution) → Aspartic acid (different amino acids) vs (substitution) → Asparagine (same amino acids)
5. Tested for variation in selection between discreet classes using PAML clade models
 Nocturnal vs. Diurnal
 $dN/dS \neq dN/dS$
 Significant disparities in selective pressure could indicate adaptive evolution

Species Sampling



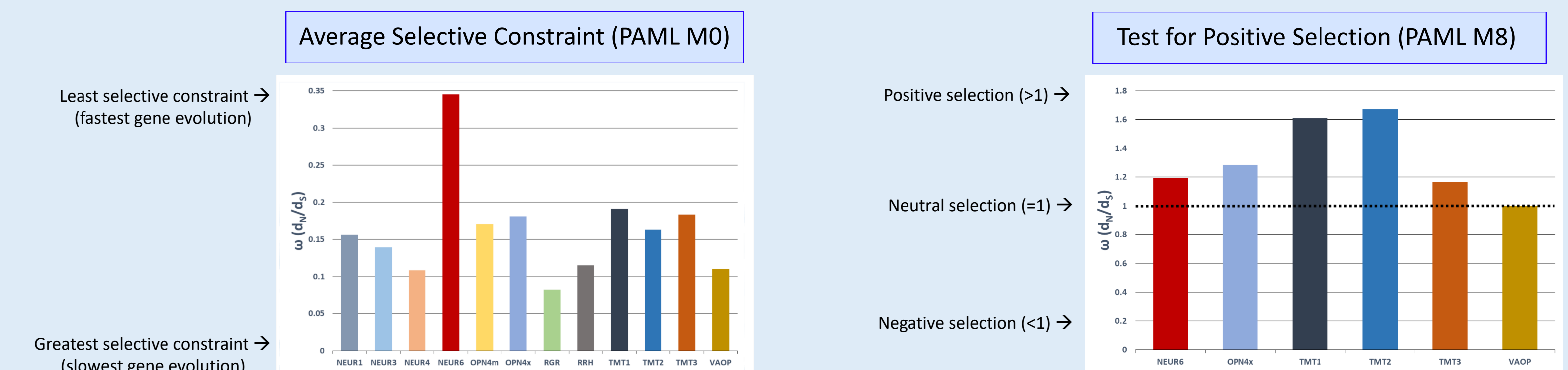
Discreet Lifestyle Classes

DIURNAL	YES	NO	SCANSORIAL	YES	NO
AQUATIC/ SEMIAQUATIC	YES	NO	SECRETIVE/ FOSSORIAL	YES	NO

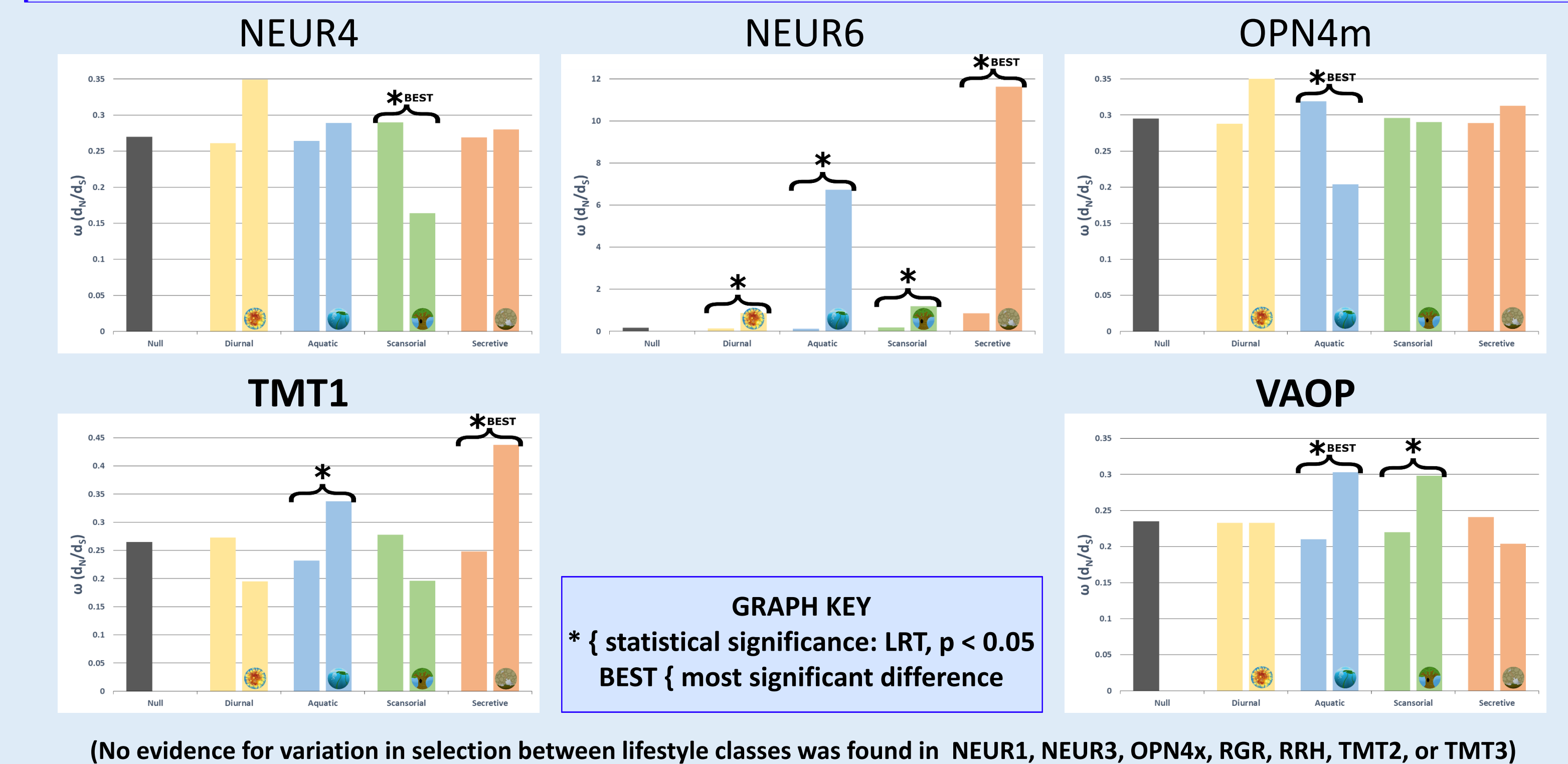


Selection Analysis

$dN/dS > 1$: positive selection { Higher proportion of non-synonymous substitutions Suggests adaptive evolution
 $dN/dS = 1$: neutral selection
 $dN/dS < 1$: negative selection { Lower proportion of non-synonymous substitutions Suggests strong selective constraint Most functional genes are subject to selective constraint



Comparing Selection Between Lifestyle Classes (PAML Clade Model Analyses)



Summary

- 12 of the 17 non-visual opsin genes were consistently recovered across frog species
- Selective constraint was similar across non-visual opsins with the exception of NEUR6, which displayed elevated dN/dS
- Positive selection in 5 genes suggests potential adaptive evolution
- Environmental light variations associated with lifestyle appear to have influenced the evolution of 5 non-visual opsins and may reflect functional adaption in these genes

Acknowledgements

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