

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



John Boyette^{1,2}, Ryan Schott¹, Rayna Bell¹

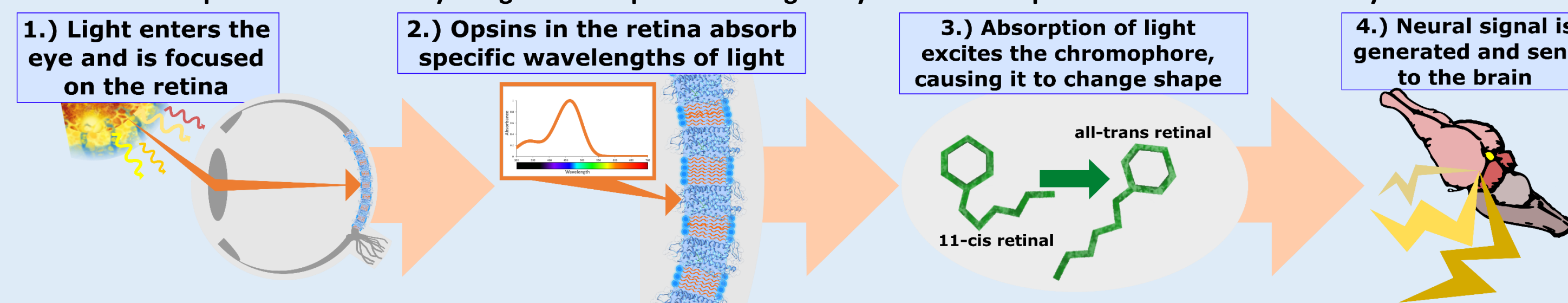
¹Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution
²Department of Biology, Berry College



Opsins & 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 -Calibration of Circadian Rhythm -Melatonin release -Pupil response | 1. NEUR1 (Neurospine 1) 2. NEUR2 (Neurospine 2) 3. NEUR3 (Neurospine 3) 4. NEUR4 (Neurospine 4) 5. NEUR5 (Neurospine 5) 6. NEUR6 (Neurospine 6) 7. OPN4m (Opsin-like melanopsin) 8. OPN4x (Opsin-like melanopsin) 9. PAR (Paraptopsin) 10. PARA (Paraptopsin) 11. PIN (Pinopsin) 12. RGR (Retinal G-protein coupled Receptor) 13. RRH (Rhodopsin) 14. TMT1 (Tetrazin Multiple Tissue 1) 15. TMT2 (Tetrazin Multiple Tissue 2) 16. TMT3 (Tetrazin 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 (d_N/d_S) acting on each gene using PAML codon models
5. Tested for variation in selection between discreet classes using PAML clade models

P.A.M.L. = Phylogenetic Analysis by Maximum Likelihood

d_N/d_S (Nonsynonymous substitution) vs d_N/d_S (Synonymous substitution)
 RNA Codon → Amino Acid (substitution) → Asparagine (different amino acids) vs Asparagine (same amino acids)

Nocturnal vs. Diurnal

$d_N/d_S \neq d_N/d_S$

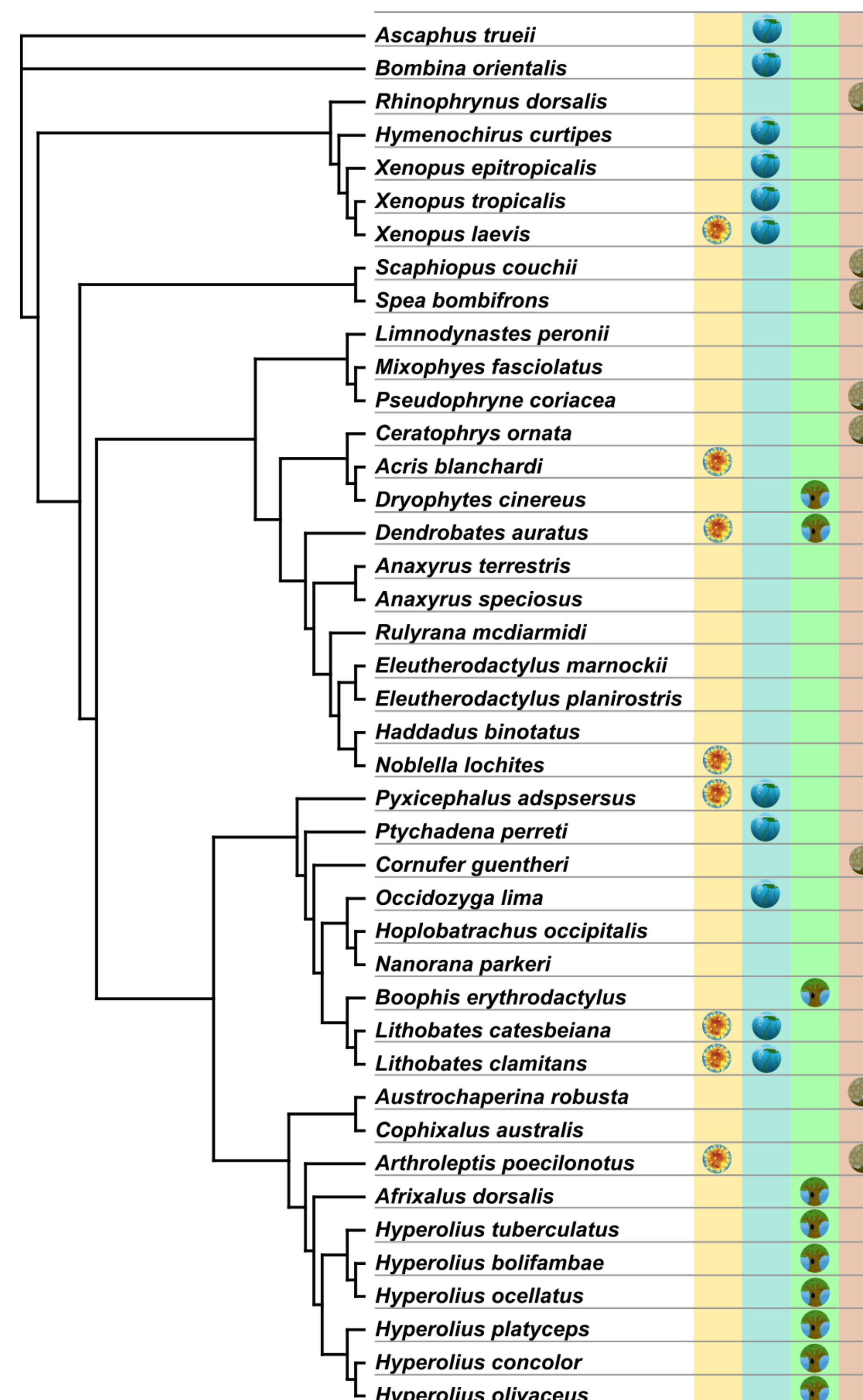
Significant disparities in selective pressure could indicate adaptive evolution

Species Sampling



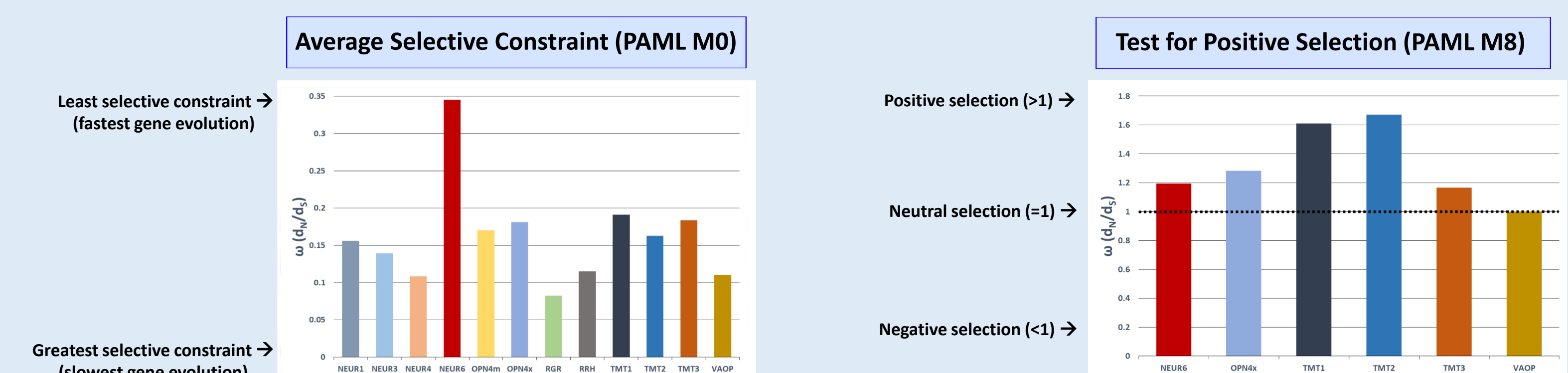
Discreet Lifestyle Classes

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

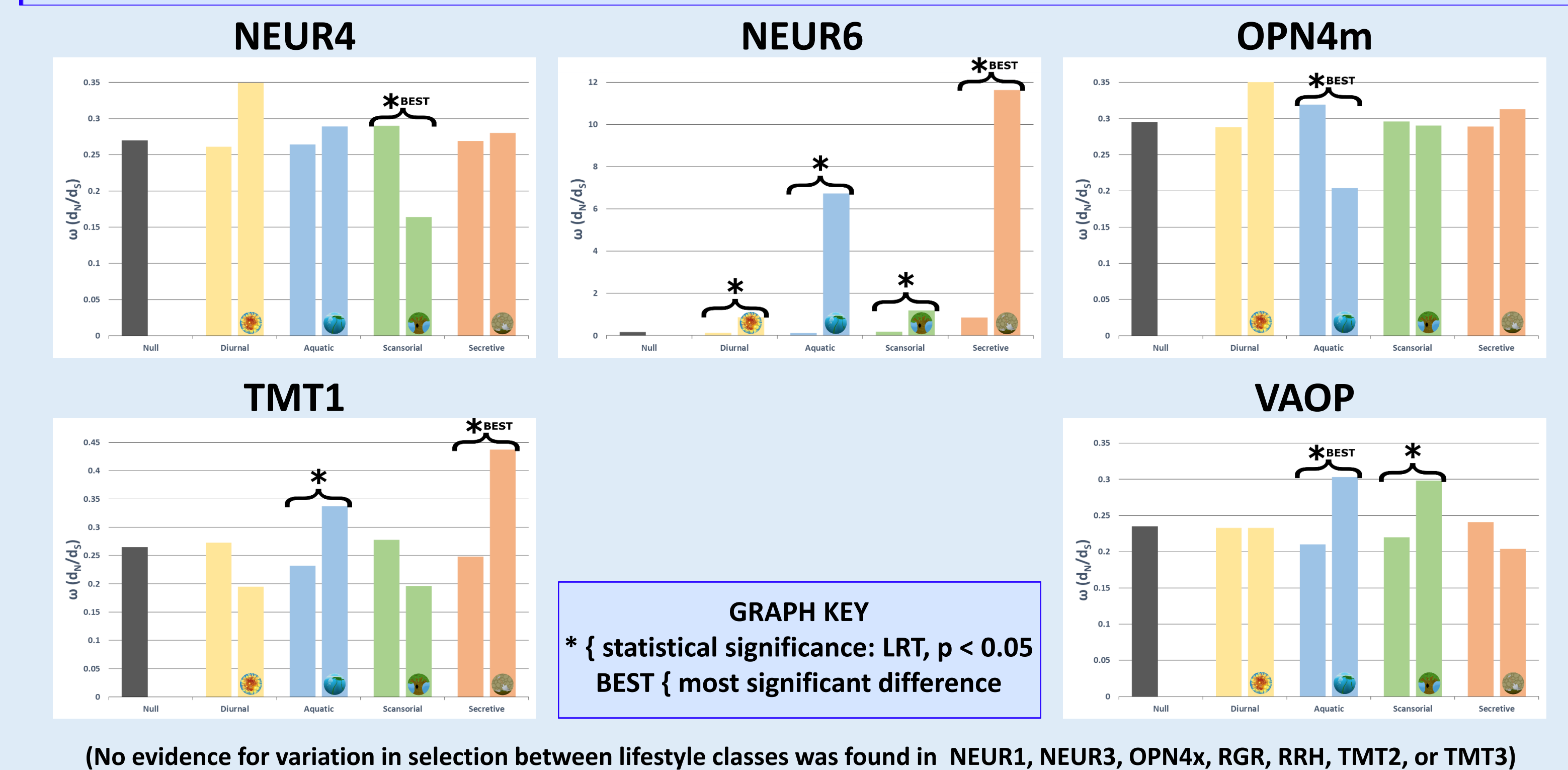


Selection Analyses

$d_N/d_S > 1$: positive selection { Higher proportion of non-synonymous substitutions Suggests adaptive evolution
 $d_N/d_S = 1$: neutral selection
 $d_N/d_S < 1$: 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 d_N/d_S
- 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

We thank our collaborators Matthew Fujita, Jeffrey Streicher, David Gower, and Kate Thomas. We also thank Klaus-Peter Koepfli and Austin Mudd for access to their unpublished *Atelopus zeteki* genome. Special thanks to NHRE program administrator Gene Hunt and program administrator Virginia Power. This project was funded by NSF DEB-NERC 1655751.