Molecular Evolution of Phototransduction Genes During Major Life History Transitions in Frogs

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Introduction

As one of the primary senses, vision plays an important role in an animal's life, including finding food, avoiding predators, and seeking mates. How the eye works to achieve these functions depends on the lifestyle of the animal, such as whether it is nocturnal or diurnal, and whether it lives underground, in water, or on land. This project investigates the evolution of vision genes in frogs with different lifestyles.

In frogs, the eye contains two primary kinds of photoreceptor cells with characteristic photopigments (composed of opsins and chromophores):

- Cones that express one of several pigment classes (SWS1, SWS2 or LWS) and typically confer bright-light (photopic) and color vision
- "Red" rods, which express rhodopsin 1 (RH1) based pigments are responsible for low-light (scotopic) sensitivity, and novel "green" rod photoreceptors that express a cone visual pigment (SWS2)

In other vertebrates, gene duplications, amino acid substitutions and expression variation all contribute to the evolution of photoreceptor pigments and we expect these same mechanisms shape the evolution of rods and cones across the frog tree of life.

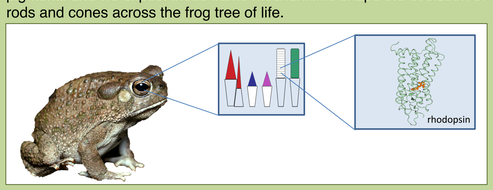
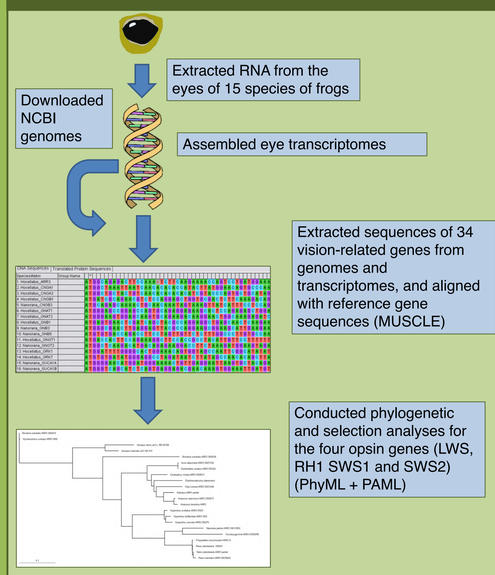


Figure 1. Frog retinas are composed of rod and cone photoreceptor cells with characteristic photopigments. For instance, "red" rods express rhodopsin.

Objectives

1. Recover vision genes from eye transcriptomes and genomes across multiple families of frogs with different lifestyles
2. Quantify gene duplication and selection in vision genes to determine whether phenotypically similar adaptive solutions have shared or unique molecular underpinnings.

Materials & Methods



Results

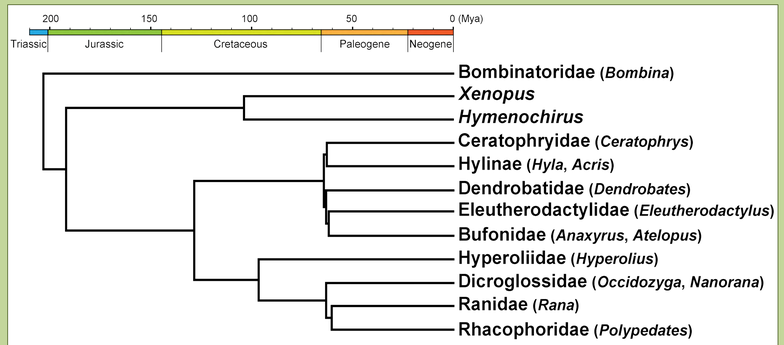


Figure 2. Phylogenetic relationships of frog species sampled for this study

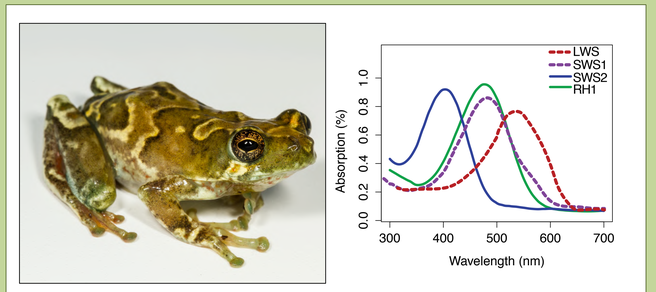
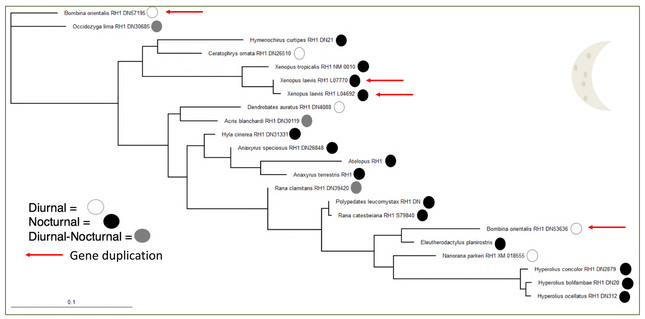
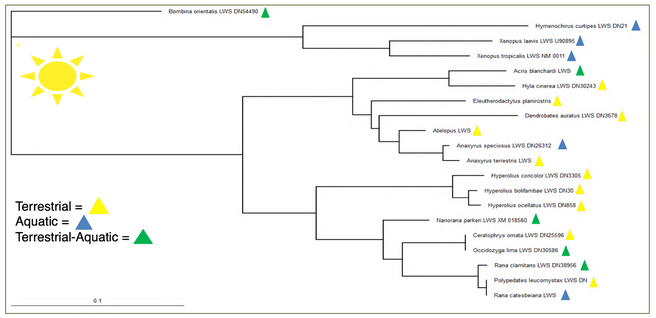


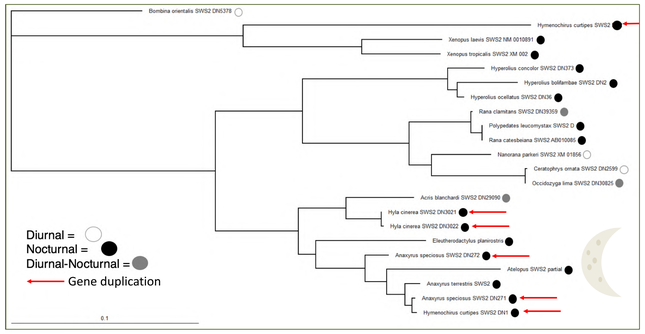
Figure 3. Normalized absorbance for rods and cones of a nocturnal, terrestrial (arboreal) reed frog, *Hyperolius tuberculatus* (Bell & Loew, unpubl.) Photo credit A. Lopez



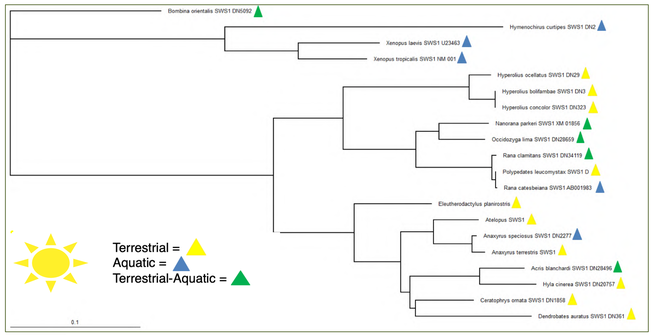
The RH1 opsin gene is expressed as the protein rhodopsin, which is sensitive in low-light environments. We expect to see variation in RH1 between species that differ in activity period.



The LWS (Long wave sensitive) opsin gene is expressed in red cones. Light in freshwater aquatic habitats is more red-shifted than in terrestrial habitats, therefore we expect to see variation in LWS between terrestrial and aquatic species.



The SWS2 (Short wave sensitive 2) opsin gene is expressed in both green rods and blue cones. Because it is expressed in the novel "green" rods, we expect to see variation in SWS2 between species that differ in activity period.



The SWS1 (Short wave senstive 1) opsin gene is expressed in cones. Because it is expressed in cones (associated with bright-light vision) we expect to see variation in SWS1 between terrestrial and aquatic species.

Figure 4. Gene trees for each opsin gene with corresponding activity period (RH1, SWS2) or habitat (LWS, SWS1) traits for each species.

Results

Tree	Model	lnL	Parameters					Null	df	P
LWS										
	M7	-6300.33	p:	0.14603	q:	1.08003		n/a		
	M8a	-6296.15	p:	0.193	q:	2.283		n/a		
			p1:	0.034	w:	1.000				
	M8	-6293.42	p:	0.163	q:	1.397	M7	2		0.0010
			p1:	0.006	w:	3.263	M8a	1		0.0194
RH1										
	M7	-5648.70	p:	0.11522	q:	0.93271		n/a		
	M8a	-5647.02	p:	0.130	q:	1.398		n/a		
			p1:	0.023	w:	1.000				
	M8	-5644.05	p:	0.125	q:	1.161	M7	2		0.0095
			p1:	0.006	w:	2.619	M8a	1		0.0149

The random sites analysis shows that the dN/dS value ω is significantly greater than 1, indicating positive selection acting on both LWS and RH1 genes.

Model	AIC	Parameters				Null	df	P
SWS1 - Aquatic vs. Terrestrial	13991.41	site	0	1	2	M2a_rel	1	0.0102
		proportion	0.665	0.031	0.304			
		branch 0	0.015	1.000	0.241			
		branch 1	0.015	1.000	0.382			
SWS1 - Diurnal vs. Nocturnal	13997.79	site	0	1	2	M2a_rel	1	0.6518
		proportion	0.683	0.029	0.288			
		branch 0	0.017	1.000	0.298			
		branch 1	0.017	1.000	0.326			
SWS2 - Aquatic vs. Terrestrial	-6094.38	site	0	1	2	M2a_rel	1	0.6183
		proportion	0.273	0.027	0.700			
		branch 0	0.270	1.000	0.014			
		branch 1	0.270	1.000	0.010			
SWS2 - Diurnal vs. Nocturnal	-6094.38	site	0	1	2	M2a_rel	1	0.6825
		proportion	0.689	0.029	0.282			
		branch 0	0.011	1.000	0.267			
		branch 1	0.011	1.000	0.239			

Although there was no significant evidence for positive selection in the SWS1/SWS2 genes, we wanted to test if there might still be a differnce in in selective patterns based on the species' ecology. The Clade Model C analysis reveals significant divergent selection occurring between aquatic and terrestrial species in the SWS1 gene, while no significant divergent selection was noted in the SWS2 gene. In order to account for semi-aquatic species, we ran the analysis with the semi-aquatics grouped first with terrestrial species, and then with aquatic species. The same process was used for the diurnal-nocturnal species. According to the results, there was no significant difference between the two versions of the test.

Conclusions

- Gene duplication
- Positive selection in RH1 and LWS
- Divergent selection in SWS1
- Differences in selection may be related to adaptation to different lifestyles (diurnal/nocturnal, terrestrial/aquatic)

References

PAML:
Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol* 24:1586-1591.

Clade models:
Bielawski JP, Yang Z. 2004. A maximum likelihood method for detecting functional divergence at individual codon sites, with application to gene family evolution. *J Mol Evol* 59:121-132.

Weadick CJ, Chang BSW. 2012. An improved likelihood ratio test for detecting site-specific functional divergence among clades of protein-coding genes. *Mol Biol Evol* 29:1297-1300.

PhyML:
Guindon S, Dufayard J, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Syst Biol* 59:307-321.

Anisimova M, Gascuel O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst Biol* 55:539-552.

MUSCLE:
Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797.

Acknowledgements

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