# Testing for molecular convergence and selection on gecko opsin genes

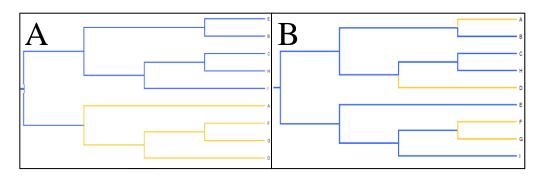
By Natalie Allen With faculty mentor Dr. Tony Gamble

## **Introduction and Objectives**

Though geckos are ancestrally nocturnal (active at night), diurnality (active during the day) has independently evolved multiple times (1, 2, 3). To adapt to changes in light conditions, diurnal geckos may have experienced changes in visual rods and cones or their opsin proteins, which initiate phototransduction at specific light wavelengths (4). Geckos have long-wavelength (LWS), shortwavelength (SWS1), and mid-wavelength (RH2) sensitive opsins (4, 5). I previously found evidence of molecular convergence in the LWS and SWS1 opsins. Here, I gather additional sequence data and examine all three gecko opsins for 1. further evidence of convergence and 2. evidence of differing rates of evolution throughout the gecko lineage, which may indicate selection. I expect to see convergence on diurnal geckos (Fig. 1 (A)), and if so, evidence of selection as well. Alternatively, I may see no evidence of convergence (Fig. 1 (B)) in RH2 and no differing evolutionary rates in any opsins.

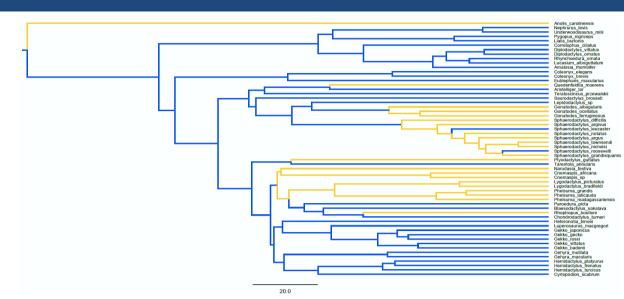
#### **Methods**

- Sequences for all three opsins were gathered from whole genomes, eye transcriptomes, and Sanger Sequenced cDNA
- To obtain additional sequences, Sanger Sequencing was done with cDNA obtained from gecko eye mRNA
- o Gene identifies were verified using BLAST
- Geneious software was used to align sequences and create gene trees from amino acid alignments
- To test for convergence, amino acid gene trees were compared to the species tree (pruned from (1))
- To test for differing rates of evolution, gene trees were used to run molecular clock tests for each opsin in MEGA (6)



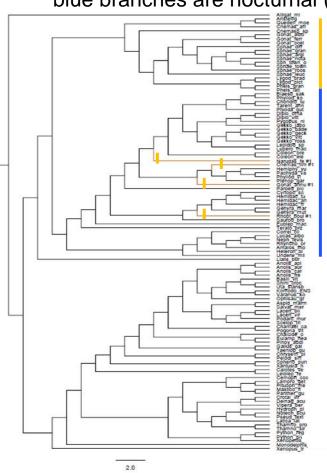
**Figure 1.** Amino acid tree examples where image (A) depicts convergence, while (B) depicts lack of convergence.

#### Results



**Figure 2.** Accepted species tree where yellow branches are diurnal and blue branches are nocturnal (Pruned from Gamble et al. 2015).

Mostly



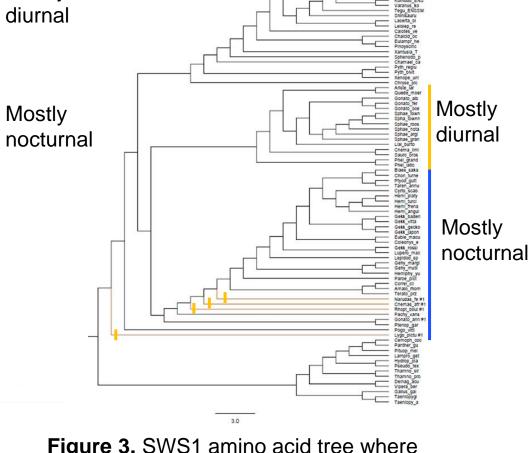


Figure 3. LWS amino acid tree where diurnal geckos are colored yellow, with yellow bands, when they show up in the mostly nocturnal clade

Figure 3. SWS1 amino acid tree where diurnal geckos are colored yellow, with yellow bands, when they show up in the mostly nocturnal clade

	rtr2_raeniopygia
	Anolis_apletophallus_RH2
	Anolis_auratus_RH2
	Anolis_frenatus_RH2
	RH2_Anolis
17	
	Basiliscus_vittatus_RH2
	Sceloporus tristichus RH2
	Uta_stansburlana_DQ100324.1_RH2
	Lacerta blineata RH2
$\neg$	Lacerta viridis RH2
	Varanus_komodoensis_RH2
$\overline{}$	RH2_Chrysemys
	RH2_Pelodiscus
	Calotes_versicolor_KU645229.1_RH2
	Leiolepis reevesii KU645227 RH2
	Eulamprus heatwolel RH2
	RH2_Ophisaurus
<u></u>	RH2_Chamaeleo
	RH2_Pogona
	RH2_Shinisaurus
	Aristelliger_lar_RH2
_	Quedenfelta_morens_RH2
	Lygodactylus_bradfieldi_RH2 reversed
-	Lygodactylus_picturatus_RH2
	Phelsuma madagascariensis RH2 2
	RH2 Pheisuma laticauda
Marie Control of the	Rhoptropus_boultoni_RH2_rev
	Cnemaspsl_limi_KU645213.1_Rh2
	Phyliodactylus_victus_KU645216.1_Rh2
	RH2_Gehyra_mutliata
	Gehyra_marginata_TG2908_RH2
	Coleonyx_brevis_RH2
	Coleonyx_elegans_RH2
	RH2_Eublepharis
	Paroedura_picta_RH2
	Gekko_badeni_TG738F_RH2
10 B 00 B	Gekko_gecko_M92035_RH2_2
	Gekko_vittatus_TG0010_RH2
	Gekko_rossi_CDS9209_RH2
+	RH2_Gekko
_	Lepidodactylus_sp_RH2
	Luperosaurus macgregori CDS9273 RH2
	Pachydactylus_vansomic_KU645215.1_RH2
	Cyrtopodion_scaper_RH2
	RH2_Hemidactylus_turcicus
	Hemidactylus_angulatus_RH2
	Hemidactylus_frenatus_RH2
200	Gonatodes_annularis_KU645217.1_Rh2
	Gonatodes_ferrugineus_RH2
	Gonatodes_ocellatus_RH2
	Sphaerodactylus_notatus_TG3847_RH2
	Sphaerodactylus roosevelti TG4163_RH2
	Sphaerodactylus_townsendi_RH2
_	Sphaerodactylus_townsendl_RH2_new
	Sphaerodactylus_argivus_SVN997_RH2
	Saurodactylus_brosseti_RH2
	Hemiphyliodactylus_yunnanensis_KU645203_RH2
	Ptenopus garrulous_KU645214.1_Rh2
	RH2_Correlophus
-	Amaiosia_mombifer_CCM6779_RH2
	Underwoodisaurus_milli_TG1795_RH2
	RH2_Lialis
	Cnemaspis_africana_RH2
	Cnemaspis sp RH2
	Teratoscincus_przewalskii_RH2

Figure 3. RH2 amino acid tree where diurnal geckos are colored yellow with yellow bands

	LWS	SWS1	RH2
lnL with	-6734.582	-9587.592	-5986.475
clock			
lnL without	-6156.258	-9279.396	-5367.134
clock			
Result at 5%	Null	Null	Null
significance	rejected	rejected	rejected
P-value	4.580E-202	1.038E-099	9.137E-231

Figure 6. Molecular clock test was performed in MEGA (6) by comparing the ML value for the given topology with and without the molecular clock constraints under Jones-Taylor-Thornton (1992) model (7). The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level for all three opsins.

### Conclusion

With more taxa added to the LWS and SWS1 datasets, the previous result of one mostly diurnal gecko clade and one mostly nocturnal clade is seen again. In both opsins, results are more similar to Fig. 1 (A) than Fig. 1 (B), providing evidence for molecular convergence in diurnal geckos. However, the RH2 amino acid tree shows results more similar to Fig. 1 (B) than Fig. 1 (A), which does not provide evidence of molecular convergence. It is possible that molecular convergence does occur on this opsin but is not visible in the tree due to the smaller taxon sampling of RH2.

The molecular clock tests rejected the null hypothesis of equal evolutionary rates throughout geckos in all three opsins. This indicates that there are different rates of molecular change throughout the tree, which may provide evidence for selection on the opsins of diurnal geckos. Further selection tests are needed to confirm the specific branches experiencing selection.

The combination of molecular convergence and differing evolutionary rates supports the hypothesis that diurnal geckos have undergone spectral tuning of their opsins to the daytime light environment (8). I hope to do further tests of selection and ancestral state reconstruction to uncover the evolutionary history of gecko opsins and determine how molecular evolution has contributed to the visual adaptations of diurnal geckos.

## Acknowledgements

Marquette University Honors Program; The Gamble Lab: Dr. Tony Gamble, Gabby Charbonneau, Blake Gamble, Shannon Keating, Aaron Griffing, Kathryn Sullivan, Amber Petty

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