Daily activity patterns influence retinal morphology, signatures of selection, and spectral tuning of opsin genes in colubrid snakes.

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Additional file 1 with Tables S1-S7 and Figures S1-S2.

Primer	Onsin	Annealing	Nucleotide Sequence							
i inner	Opsin	Temperature								
SquaLWSFw	LWS	59.5	5' ATGACAGAGGCCTGGAATGT 3'							
SquaLWSRv	LWS	60.0	5' CCTTGCTGGCATTCTCTTCT 3'							
LWS DipsFw	LWS	62.0	5' CTTCGTCACCATCAAGTACAAGAAGT 3'							
LWS DipsRv	LWS	62.0	5' GTCTTCTGAGAGCTCAGGTCAGACT 3'							
LWS DegFw	LWS	62.4	5' GRYCCTTTTGAAGGYCCAAAYTAYC 3'							
LWS DegRv	LWS	62.8	5' GTTGTAAATGGTKGCGCTTTTTG 3'							
LWS TdorFw	LWS	61.6	5' TTTACTTACACCAACAGCAACAATACC 3'							
LWS TdorRv	LWS	61.5	5' TTCATGAAGACATAGATAATTGGGTTG 3'							
LWS LpoeFw	LWS	62.5	5' GTGTATTTGCTTACACCAATAGCAACA 3'							
LWS LpoeRv	LWS	63.4	5' AAAATGTATATGGTCCCCAGCAGAC 3'							
SquaRH1Fw	RH1	59.9	5' GCATTGTTCGGAGTCCATTT 3'							
SquaRH1Rv	RH1	62.5	5' TTCTGCTGGCACCAAGACAG 3'							
RH1SmikFw	RH1	65.8	5' TATTTGGCAGATCCATGGAAATATTCTG 3'							
RH1SmikRv	RH1	66.1	5' GGGGAAACCTTACTTGTGGAGACTGTAG 3'							
RH1OxyFw	RH1	64.5	5' GTCACCATCCAACACAAGAAACTCA 3'							
RH1OxyRv	RH1	64.0	5' AAATCACTGGGTTGTAGATTGAAGAGC 3'							
RH1Tdor1Fw	RH1	60.0	5' CACCACTACCATGTACACTTCCAT 3'							
RH1Tdor1Rv	RH1	60.0	5' GTCATAAAGACAGGGCCAAAGT 3'							
RH1Tdor2Fw	RH1	60.0	5' CAAGAAACTCAGAACACCTCTGAA 3'							
RH1Tdor2Rv	RH1	60.0	5' CGGAACTGTTTGTTCATTACGATA 3'							
SquaSWS1Fw	SWS1	55.6	5' CCTCTTTGAAAACATCTCCTC 3'							
SquaSWS1Rv	SWS1	59.8	5' GTCCTCCTGTCAAGTCAGCC 3'							
SWS1ArtfFw	SWS1	64.0	5' GACGGTCCCCAGTATCACATTG 3'							
SWS1ArtfRv	SWS1	64.7	5' CTTGACAGGAGGACACTGAGGAAAC 3'							
SWS1 TdorFw	SWS1	63.7	5' CTTTCCACTTCCAAACCATCTTCAT 3'							
SWS1 TdorRv	SWS1	63.4	5' TTCTGAGAAGTCACATCAGAGTCGT 3'							
SWS1 Tdor2Fw	SWS1	64.8	5' ATCCTCTTCGTCACCATCAAGTACAAG 3'							
SWS1 Tdor2Rw	SWS1	64.1	5' ATGAAGCAGTAGATGATGGGGTTGT 3'							
SWS1 DipsFw	SWS1	62.0	5' TACCACCAGGGAGAGTGTATTTGTGTA 3'							
SWS1 DipsRv	SWS1	62.0	5' ACTTTCTTGCCAAAGAGCTGCATTAT 3'							

Table S1. Primers designed and used in this study, to amplify the opsin genes *LWS*, *RH1* and *SWS1*, expressed in retinas of Colubridae snakes.

Comon name	Species	GenBank acesison number									
	Species	RH1	RH2	SWS1	SWS2	LWS/MWS					
Salamander	Ambystoma tigrinum	Q90245	-	-	AF038946	-					
Worm lizard	Amphisbaena alba	KR336729	-	KR336720	-	KR336705					
Worm lizard	Amphisbaena infraorbitale	KR336730	-	KR336719	-	KR336704					
Lizard	Anolis carolinensis	NM001291387	AF134189	AH007736	-	P41592					
Lizard	Bachia flavescens	KR336731	-	KR336715	-	KR336703					
Cow	Bos taurus	NM001014890	-	NP776992	-	AAG49893					
Goldfish	Carassius auratus	L11863	-	Q90309	-	L11867					
Pigeon	Columba livia	-	AH007731	AH007798	-	-					
Zebrafish	Danio rerio	NP571287	Q9W6A6	Q9W6A9	BAC24133	NM001002443					
Chicken	Gallus gallus	P22328	P28683	P28684	P28682	P22329					
Lizard	Gekko gekko	-	-	P35357	-	P35358					
Lamprey	Geotria australis	AAR14682	AAR14683	AY366495	AAR14681	AY366491					
Gorilla	Gorilla gorilla	XM004036292	-	XM004046176	-	-					
Human	Homo sapien	NP00530	-	P03999	-	NM020061.5					
Coelacanth	Latimeria chalumnae	-	AF131258	-	-	-					
Lizard	Melanoseps occidentalis	KR336743	-	KR336718		KR336713					
Mouse	Mus musculus	NP663358	-	P51491	-	O35599					
Lungfish	Neoceratodus forsteri	EF526295	EF526296	EF526298	-	EF526297					
Platypus	Ornithorhynchus anatinus	-	-	-	ABN43075	-					
Day Gecko	Phelsuma madagascariensis	-	AF074044	AAD45183	-	AAD25917					
Rat	Rattus norvegicus	NM033441	-	NM031015	-	NM053548					
Fish	Scleropages formosus	XM018762271	-	XM018755948	-	XM018725556					
Canary	Serinus canaria	XM009099359	-	AY227178	-	XM009100678					
Boar	Sus scrofa	NM214221	-	NM214090	-	NM001011506					
Zebra finch	Taeniopygia guttata	AF222329	NM00107669	AF222331	NM001076697	AF222333					
Lizard	Takydromus sexlineatus	KR336727	-	KR336722	-	KR336707					
Lizard	Uta stansburiana	DQ100323	DQ100324	DQ100325	DQ100326	DQ129869					
Frog	Xenopus laevis	AAC42232	-	P51473	AAO38746	U90895					
Frog	Xenopus tropicalis	NM00109733	-	NM001126076	-	NM001102861					

Table S2. Species used as outgroups for phylogenetic analysis and respective GenBank accession numbers.

Family	Subfamily	Sneries	GeneBank Acession Number						
	Sublatility	Species	LWS	RH1	SWS1				
		Ahaetulla nasuta	KX237798	KX237852	KX23790				
		Arizona elegans	KU323986	KU324006	KU32399				
		Bogertophis subocularis	KX237822	KX237844	KX23792				
		Boiga ceylonensis	KX237819	-	-				
		Boiga forsteni	KX237818	KX237867	-				
		Chironius carinatus	KX237795	KX237846	KX23791				
		Chironius bicarinatus	MG544928	MG544945	MG5449				
		Chironius fuscus	KX237794	KX237845	KX23791				
		Chrysopelea ornata	KX237799	KX237851	KX23790				
		Dasypeltis scabra	KX237821	KX237856	KX23790				
		Elaphe climacophora	KX237817	KX237845	KX2379				
		Hemorrhois hippocrepis	KX237911	KX237835	KX2379				
	Colubrinae	Lampropeltis californiae	KX237825	KX237858	KX2379				
	colubrinac	Lampropeltis conjornac	KX237825	KX237857	KX23792				
			KX237820	KY237875	KY23734				
		Macroprotodon bravis	KX237020	1(1257075	KX23731				
		Ophaodrys gastiyus	KA237813 F0772744	-	KAZ3730				
		Orthrianhis tanniurus	KAZ37737	KA237633	KAZ373.				
		Danthorophic auttatus	KX237010	KA237002	KAZ379.				
		Puntieropins guildius	KXZ378Z4	KA237803	KAZ379.				
		Phyliornynchus decurtatus	KU323985	-	KU3239				
			KX237823	KX237854	KX2379				
		Pseustes poecilonotus	KR336/11	KR336/41	KR3367				
		Spalerosophis diadema	KX237814	KX237853	KX2379				
		Spilotes pullatus	MG544940	MG544957	MG5449				
		Telescopus fallax	KU323984	KU324005	KU3239				
		Atractus flammigerus	KR336712	KR336740	KR33672				
Colubridae		Atractus badius	KX237809	KX237842	KX23790				
		Atractus reticulatus	MG544927	MG544944	MG5449				
		Dipsas indica	KX237813	KX237849	KX23790				
		Dipsas catesbyi	KX237812	KX237848	KX23790				
		Dipsas petersi	MG544929	MG544946	MG5449				
		Echinanthera cephalostriata	MG544930	MG544947	MG5449				
		Echinanthera undulata	MG544931	MG544948	MG5449				
		Erythrolamprus aesculapii	MG544932	MG544949	MG5449				
		Erythrolamprus miliaris	MG544933	MG544950	MG5449				
		Erythrolamprus poecilogyrus	MG544934	MG544951	MG5449				
		Erythrolamprus reginae	KX237800	KX237855	KX23789				
		Helicops angulatus	KX237806	KX237836	KX23789				
		Helicops modestus	MG544935	MG544952	MG5449				
	Dipsadinae	Heterodon nasicus	KX237793	KX237850	KX23789				
		Hypsialena iani	KU323988	KU324007	KU3239				
		Imantodes lentiferus	KX237807	KX237841	KX2379(				
		l entodeira annulata	KX237808	KX237840	KX2379(				
		Oxyrhonus quibei	MG544936	MG544953	MG5449				
		Oxyrhonus melanogenys	KX237804	KX237838	KX23780				
		Philodryas natagoniensis	MG544937	MG544954	MG5449				
		Pseudobog coronata	KAJJ244221	KXJ27027	KXJJJ443				
		Sibon nebulatus	NAZ3/0U3	NAZ3/03/	KAZ3/8				
		Sibunomorphus mikanii	NCE44030	NAZJ/843	MCEAAO				
		Sibynomorphus Mikanii Sibunomorphus nouuriadi	IVIG344938		IVIG5449				
		Subyriornorphus neuWiedi	IVIG544939	IVIG544956	IVIG5449				
		i aeniopnailus persimilis	IVIG544941	IVIG544958	IVIG5449				
		i namnoaynaestes hypoconia	WIG544942	MG544959	MG5449				
		Tomodon dorsatus	MG544943	MG544960	MG5449				
		Xenopholis scalaris	KX237810	KX237834	KX23789				
enopeltidae		Xenopeltis unicolor	FJ497235	FJ49723	FJ49723				
Pythonidae		Python regius	FJ4977238	FJ497236	FJ49772				

Table S3. Colubridae species used for phylogenetic and evolutionary analysis and respective GenBank accession numbers. In bold, species sequenced in this study.

Onsins	Model	Inl	D	Parameter Estimates
Opsilis	Branch-Site Models		Г	
	Null Model	-11 370 8	150	w = 1 00
	Model A (diurnal)	-11 3/17 9	152	w = 1.00 w = 1.00 $(y_2 = 0.05 (p_2 = 0.798) (y_3 = 1.0 (p_3 = 0.18); (y_2 = 34.3 (p_2 + p_3 = 0.03))$
	Model A (nocturnal)	-11 320 3	152	$(u_0 = 0.05 (p_0 = 0.750), w_1 = 1.0 (p_1 = 0.10), w_2 = 34.5 (p_2 + p_3 = 0.05)$
	Site Models	11,520.5	152	$\omega_0 = 0.05 \ (p_0 = 0.77), \ \omega_1 = 1.0 \ (p_1 = 0.17), \ \omega_2 = 14.0 \ (p_2 + p_3 = 0.00)$
IWS	Model 0	-4 598 5	101	$\omega_0 = 0.49$
LVVS	Model 3	-4 235 5	101	$\omega_0 = 0.03 (n_0 = 0.82) (\omega_1 = 1.8 (n_1 = 0.14); (\omega_2 = 6.08 (n_2 = 0.04))$
	Model 1a	-4.316.4	102	$\omega_0 = 0.014 (p_0 = 0.798), \omega_1 = 1.0 (p_1 = 0.202)$
	Model 2a	-4.238.3	104	$\omega_0 = 0.017$ ( $p_0 = 0.78$ ), $\omega_1 = 1.0$ ( $p_1 = 0.13$ ); $\omega_2 = 4.19$ ( $p_2 + p_3 = 0.08$ )
	Model 7	-4.324.8	102	p = 0.0158, q = 0.0987
	Model 8	-4,238.7	104	$p_0 = 0.9095$ , $p = 0.038$ , $q = 0.24$ ( $p_1 = 0.090$ , $\omega = 3.989$ )
	Branch-Site Models	,		
	Null Model	-10,943.6	146	ω = 1.00
	Model A (diurnal)	-10,939.4	148	$\omega_0 = 0.055 (p_0 = 0.86), \omega_1 = 1.0 (p_1 = 0.14); \omega_2 = 4.36 (p_2 + p_3 = 0.007)$
	Model A (nocturnal)	-10,918.9	148	$\omega_0 = 0.057 (p_0 = 0.86), \omega_1 = 1.0 (p_1 = 0.12); \omega_2 = 11.4 (p_2 + p_3 = 0.02)$
	Site Models			
RH1	Model 0	-4,692.8	97	$\omega_0 = 0.288$
	Model 3	-4,479.1	101	$\omega_0 = 0.002 \ (p_0 = 0.67), \ \omega_1 = 0.43 \ (p_1 = 0.23); \ \omega_2 = 2.02 \ (p_2 = 0.10)$
	Model 1a	-4,497.7	98	$\omega_0 = 0.022$ (p <sub>0</sub> = 0.77), $\omega_1 = 1.0$ (p <sub>1</sub> = 0.23)
	Model 2a	-4,483.4	100	$\omega_0 = 0.024$ (p <sub>0</sub> = 0.77), $\omega_1 = 1.0$ (p <sub>1</sub> = 0.18); $\omega_2 = 2.7$ (p <sub>2</sub> + p <sub>3</sub> = 0.048)
	Model 7	-4,496.8	98	p = 0.018, q = 0.059
	Model 8	-4,479.5	100	p <sub>0</sub> = 0.926, p = 0.098, q = 0.555 (p <sub>1</sub> = 0.074, ω = 2.26)
	Branch-Site Models			
	Null Model	-14,248.6	154	ω = 1.00
	Model A (diurnal)	-14,225.3	156	$\omega_0 = 0.05$ (p <sub>0</sub> = 0.84), $\omega_1 = 1.0$ (p <sub>1</sub> = 0.077); $\omega_2 = 1.23$ (p <sub>2</sub> + p <sub>3</sub> = 0.078)
	Model A (nocturnal)	-14,237.9	156	$\omega_0 = 0.05$ (p <sub>0</sub> = 0.87), $\omega_1 = 1.0$ (p <sub>1</sub> = 0.08); $\omega_2 = 1.7$ (p <sub>2</sub> + p <sub>3</sub> = 0.046)
	Site Models			
SWS1	Model 0	-5,784.0	99	$\omega_0 = 0.1$
	Model 3	-5,565.2	103	$\omega_0 = 0.00 \ (p_0 = 0.64), \ \omega_1 = 0.17 \ (p_1 = 0.25); \ \omega_2 = 0.73 \ (p_2 = 0.11)$
	Model 1a	-5.618.6	100	$\omega_0 = 0.039 (p_0 = 0.86), \omega_1 = 1.0 (p_1 = 0.14)$
	Model 2a	-5.618.6	102	$(\mu_0 = 0.039 \ (p_0 = 0.86), \ (\mu_1 = 1.0 \ (p_1 = 0.1); \ (\mu_2 = 1.0 \ (p_2 + p_3 = 0.039))$
	Model 7	-5.568 3	100	p = 0.13, q = 0.899
	Model 8	-5,568.3	102	$p_0 = 0.998$ , $p = 0.13$ , $q = 0.92$ ( $p_1 = 0.002$ , $\omega = 1.04$ )

Table S4. Summary of phylogenetic	evolutionary	branch-site	and	site	models	paramete	rs for
LWS, RH1 and SWS1 opsin genes.							

p, number of parameters;  $p_0$ ,  $p_1$ ,  $p_2$ , proportion of sites in different omega classes; l, likelihood.

Table S5. Known amino acid spectral tuning sites of the LWS opsin gene and predicted peak
absorbance ( $\lambda_{max}$ ) [1]. Sites numbered based on the bovine rhodopsin sequence. All $\lambda_{max}$ values
are estimates predicted based on amino acid sequences. Predominant daily activity patterns
stablished based on [2, 3].

Species	164	181	261	269	292	$\lambda_{max}$
Primarily Nocturnal						
Atractus reticulatus	А	Н	Y	А	А	~537
Dipsas petersi	А	н	Y	А	А	~537
Sibynomorphus mikanii	А	н	Y	Α	Α	~537
Sibynomorphus neuwiedi	А	н	Y	А	А	~537
Oxyrhopus guibei	А	н	Y	Т	А	~553
Primarily Diurnal						
Chironius bicarinatus	S	н	Y	Т	А	~560
Echinanthera cephalostriata	S	н	Y	Т	А	~560
Echinantera undulata	S	н	Y	Т	А	~560
Erythrolamprus aesculapii	S	н	Y	Т	А	~560
Erythrolamprus miliaris	S	н	Y	Т	А	~560
Erythrolamprus poecilogyrus	А	н	Y	т	А	~553
Helicops modestus	S	н	Y	т	А	~560
Philodryas patagoniensis	S	н	Y	т	А	~560
Spilotes pullatus	S	Н	Y	т	А	~560
Taeniophallus persimilis	S	Н	Y	т	А	~560
Thamnodynastes hypoconia	А	Н	F	т	А	~543
Tomodon dorsatus	А	н	F	т	А	~545

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Species	83	96	102	122	183	194	195	253	261	289	292	299	317	$\lambda_{\text{max}}$
Primarily Nocturnal														
Atractus reticulatus	Ν	Y	Y	Е	М	Р	Т	М	F	Т	Α	Α	М	~493
Dipsas petersi	D	Y	Y	Е	М	Р	S	М	F	т	Α	S	М	~500
Sibynomorphus mikanii	D	Y	Y	Е	М	Р	S	М	F	Т	Α	S	М	~500
Sibynomorphus neuwiedi	D	Y	Y	Е	М	Р	S	М	F	Т	Α	S	М	~500
Oxyrhopus guibei	D	Y	Y	Е	М	Р	S	М	F	т	Α	S	М	~500
Primarily Diurnal														
Chironius bicarinatus	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Echinantera cephalostriata	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Echinantera undulata	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Erythrolamprus aesculapii	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Erythrolamprus miliaris	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Erythrolamprus poecilogyrus	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Helicops modestus	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Philodryas patagoniensis	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Spilotes pullatus	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Taeniophallus persimilis	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Thamnodynates hypoconia	Ν	Y	Y	Е	М	Р	Т	М	F	Т	S	Α	М	~483
Tomodon dorsatus	Ν	Y	Y	Е	М	Р	т	М	F	т	S	Α	М	~483

Table S6. Known amino acid spectral tuning sites of the RH1 rhodopsin gene and predicted peak absorbance ( $\lambda_{max}$ ) [4, 5, 6]. Sites numbered based on the bovine rhodopsin sequence. All  $\lambda_{max}$  values are estimates predicted based on amino acid sequences. Predominant daily activity patterns stablished based on [2, 3].

Table S7. Known amino acid spectral tuning sites of the SWS1 [7] and predicted peak absorbance ( $\lambda_{max}$ ). Site numbered based on bovine rhodopsin. Asterisk indicates amino acids with stronger effects on spectral tuning [8, 9, 10]. All  $\lambda_{max}$  values are estimates predicted based on amino acid sequences (for a review see [11]). Predominant daily activity patterns stablished based on [2, 3].

Species	46	49	52	86*	90	93*	97	113*	114	116	118	265	$\lambda_{max}$
Primarily Nocturnal													
Atractus reticulatus	L	F	Т	F	А	Т	S	Е	А	L	т	Y	UV
Dipsas petersi	L	F	Т	F	А	Т	S	Е	А	L	т	Y	UV
Sibynomorphus mikanii	L	F	Т	F	А	Т	S	Е	А	L	т	Y	UV
Sibynomorphus neuwiedi	L	F	Т	F	Α	Т	S	Е	А	L	т	Y	UV
Oxyrhopus guibei	L	F	т	F	А	V	S	Е	А	L	Т	Y	UV
Primarily Diurnal													
Chironius bicarinatus	L	F	Т	V	А	V	S	Е	А	L	т	Y	?
Echinantera cephalostriata	L	F	Т	F	А	V	S	Е	А	L	т	Y	UV
Echinantera undulata	L	F	т	F	А	V	S	Е	А	L	т	Y	UV
Erythrolamprus aesculapii	L	F	Т	F	А	Т	S	Е	А	L	т	Y	UV
Erythrolamprus miliaris	L	F	Т	F	А	Т	S	Е	А	L	т	Y	UV
Erythrolamprus poecilogyrus	L	F	Т	F	Α	Т	S	Е	Α	L	т	Y	UV
Helicops modestus	L	F	Т	F/V	А	T/V	S	Е	А	L	т	Y	UV/?
Philodryas patagoniensis	L	F	Т	F	Α	Т	S	Е	Α	L	т	Y	UV
Spilotes pullatus	L	F	Т	F	Α	V	S	Е	Α	L	т	Y	UV
Taeniophallus persimilis	L	F	Т	F	Α	V	S	Е	Α	L	Т	Y	UV
Thamnodynates hypoconia	L	F	Т	F	Α	Т	S	Е	Α	L	Т	Y	UV
Tomodon dorsatus	L	F	Т	F	Α	Т	S	Е	А	L	Т	Y	UV



Figure S1. Species phylogenetic tree used for Codeml analysis.



Figure S2. Chromatograms of the same region of the *SWS1* opsin gene of *Helicops modestus*, amplified with different primer pairs. The black boxes indicate the heterozygous site, with codons TTC and GTC, responsible for the transduction of the amino acids phenylalanine and valine at residue 86.

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