

SUPPLEMENTAL INFORMATION

**CONSERVATION GENETICS OF THE IMPERILED STRIPED WHIPSNAKE
IN WASHINGTON, USA**

***DAVID S. PILLIOD, LISA A. HALLOCK, MARK P. MILLER, THOMAS D. MULLINS,
AND SUSAN M. HAIG***

The following material is provided by the authors and was not subjected to peer review or editing by *Herpetological Conservation and Biology*.

TABLE S1. Results of tests for Hardy-Weinberg Equilibrium (HWE) at each microsatellite locus for Striped Whipsnakes (*Masticophis taeniatus*) in the state of Washington, USA, from shed skins collected between 2006–2014. The left set of tables include all samples with duplicate genotypes included, whereas the right set of tables show estimates with duplicate genotypes removed from the dataset. The top set of tables include all data in the dataset with both populations combined whereas the middle (Site one) and lower (Site two) tables show each site individually. Statistical significance indicates that the population is not in HWE, either because they have excess homozygotes or excess heterozygotes.

Duplicates included						Duplicates removed					
Data combined						Data combined					
Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value	Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value
A114	171	7	0.6550	0.5013	0.0000	A114	143	7	0.6294	0.5059	0.0000
B107	179	8	0.2961	0.2823	0.0005	B107	151	8	0.3179	0.3023	0.0007
D11	174	5	0.5287	0.7306	0.0000	D11	146	5	0.5206	0.7283	0.0000
A109	178	9	0.5618	0.6317	0.0300	A109	150	9	0.5733	0.6431	0.0609
B105	172	8	0.4302	0.4265	0.0065	B105	144	8	0.4375	0.4444	0.0159
Site one						Site one					
Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value	Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value
A114	18	3	0.8333	0.5381	0.0144	A114	14	3	0.7857	0.5476	0.1010
B107	18	2	0.3889	0.3222	1.0000	B107	14	2	0.5000	0.3889	0.5135
D11	18	4	0.6111	0.6873	0.0322	D11	14	4	0.6429	0.7116	0.0488
A109	18	4	0.6667	0.5730	0.2950	A109	14	4	0.7143	0.6111	0.3476
B105	17	3	0.1765	0.1693	1.0000	B105	13	3	0.2308	0.2185	1.0000
Site two						Site two					
Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value	Locus	Sample Size	Number of alleles	Observed heterozygosity	Expected heterozygosity	P-value
A114	153	6	0.6340	0.4970	0.0000	A114	129	6	0.6124	0.5022	0.0000
B107	161	8	0.2857	0.2774	0.0012	B107	137	8	0.2993	0.2917	0.0012
D11	156	5	0.5192	0.7207	0.0000	D11	132	5	0.5076	0.7222	0.0000
A109	160	8	0.5500	0.6319	0.0345	A109	136	8	0.5588	0.6428	0.0632
B105	155	8	0.4581	0.4496	0.0091	B105	131	8	0.4580	0.4632	0.0182

TABLE S2. Primers used to amplify and sequence four genes for this investigation of Striped Whipsnakes (*Masticophis taeniatus*), including two nuclear genes (Cmos, NT3) and two mitochondrial genes (cytB, COI).

Gene	Primer	Primer sequence	Reference
cytB	L14919	5'-AACCACCGTTGTTATTCAACT-3'	Burbrink et al. 2000
cytB	H16064	5'-CTTTGGTTTACAAGAACAATGCTTA-3'	Burbrink et al. 2000
Cmos	S77	5'-CATGGACTGGGATCAGTTATG-3'	Lawson et al. 2005
Cmos	S78	5'-CCTTGGGTGTGATTTTCTCACCT-3'	Lawson et al. 2005
COI	LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. 1994
COI	HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	Folmer et al. 1994
NT3	NT3F-B	5'- TATTTCTGGCTTTTCTCTGGTGG-3'	This study
NT3	NT3R-B	5'-GCGTTTCATAAAAAATATTGTTTGA-3'	This study

TABLE S3. Multilocus genotypes of Striped Whipsnake (*Masticophis taeniatus*) samples

(extracted from sheds) that possessed identical genetic profiles. All sheds were collected in the state of Washington from 2006–2014. Microsatellite markers (A114, B107, D11, A109, B105) were developed by Richmond et al. (2016). Numbers in the matrix represent microsatellite allele sizes in base pairs.

Sample IDs	A114		B107		D11		A109		B105	
WS_1, 54	281	285	137	143	193	201	119	119	191	197
WS_103, 125	281	285	143	143	185	185	115	119	191	191
WS_108, 161	281	285	143	143	197	197	115	115	191	191
WS_112, 162	281	285	143	143	185	197	115	119	191	197
WS_118, 169	281	281	143	143	197	197	115	119	191	197
WS_129, 146	281	285	143	143	185	189	115	119	191	191
WS_132, 144	281	281	143	143	185	197	115	119	191	191
WS_136, 148	281	281	143	143	197	197	101	119	191	197
WS_147, 149	281	285	143	143	193	193	115	119	191	191
WS_153, 168, 247	281	285	143	143	185	185	115	115	191	191
WS_159, 138	281	285	143	143	189	189	115	119	191	191
WS_18, 47, 109	281	285	137	143	193	197	115	115	191	194
WS_21, 34	281	281	143	143	193	197	115	115	191	194
WS_234, 239	283	293	143	143	183	189	101	105	173	191
WS_250, 253, 258	281	281	143	143	185	193	101	109	173	173
WS_28, 78, 80, 179	281	285	143	143	189	197	119	119	191	197
WS_3, 11	281	285	143	143	197	201	119	119	191	197
WS_33, 44	281	285	143	143	185	197	115	115	191	191
WS_39, 150, 151	281	285	143	143	185	197	115	119	191	191
WS_45, 48, 175	281	285	143	143	185	189	119	119	191	191
WS_52, 53, 63	281	281	140	143	193	193	115	115	191	191
WS_56, 59, 114	281	285	143	143	197	197	115	119	191	191
WS_62, 104	281	285	137	143	185	185	101	115	191	197
WS_99, 120	281	285	143	143	189	197	101	119	191	191

TABLE S4. Pairwise estimates of F_{ST} , a measure of genetic differentiation, for all pairwise combination of Striped Whipsnake (*Masticophis taeniatus*) samples collected in five western U.S. states. The matrices on the left show values where duplicate multilocus genotypes are included and the matrices on the right show values where duplicate genotypes were removed. Values in bold font identify significant values after sequential Bonferroni corrections. UPGMA dendrograms in Figure 4 provide graphical representations of these differentiation matrices.

Microsatellites: duplicates included						Microsatellites: duplicates removed					
	WA	OR	ID	NV	UT		WA	OR	ID	NV	UT
WA	-					WA	-				
OR	0.126	-				OR	0.120	-			
ID	0.307	0.112	-			ID	0.304	0.115	-		
NV	0.239	0.098	0.068	-		NV	0.230	0.098	0.066	-	
UT	0.284	0.113	0.066	0.014	-	UT	0.275	0.113	0.063	0.014	-
Cmos						NT3					
	WA	OR	ID	NV	UT		WA	OR	ID	NV	UT
WA	-					WA	-				
OR	-0.089	-				OR	-0.058	-			
ID	0.682	0.501	-			ID	0.421	0.473	-		
NV	0.392	0.113	0.120	-		NV	0.431	0.415	0.013	-	
UT	0.203	-0.024	0.314	0.026	-	UT	0.426	0.476	-0.008	-0.026	-
COI						cytB					
	WA	OR	ID	NV	UT		WA	OR	ID	NV	UT
WA	-					WA	-				
OR	0.167	-				OR	0.517	-			
ID	0.006	0.136	-			ID	0.567	0.382	-		
NV	0.415	0.210	0.325	-		NV	0.161	0.255	0.276	-	
UT	0.137	0.104	0.093	0.209	-	UT	0.136	0.178	0.317	0.084	-

TABLE S5. Results of Mantel tests for the significance of the correlations between inter-individual genetic and geographic distances for each Striped Whipsnake (*Masticophis taeniatus*) data set in this study.

Data set	<i>r</i>	p-value
Microsatellites – duplicates included	0.67	< 0.001
Microsatellites – duplicates removed	0.68	< 0.001
Cmos	0.22	< 0.001
COI	0.30	< 0.001
cytB	0.06	0.174
NT3	0.42	< 0.001

TABLE S6. Estimated effective population size (N_e) and parametric confidence limits for two populations of Striped Whipsnakes (*Masticophis taeniatus*) in Washington using five microsatellite loci. The allele frequency cutoff discards "rare" alleles, which can affect analysis outcomes. In this case, analyses were performed where alleles with frequencies less than 0.05, 0.02, and 0.01 were discarded along with estimates that had no discard cutoff.

Data Source	Site	Allele Frequency Cutoff			
		0.05	0.02	0.01	0
Duplicates	Site one	12.4 (2.1 - ∞)	20.8 (2.5 - ∞)	20.8 (2.5 - ∞)	20.8 (2.5 - ∞)
Included	Site two	68.9 (33.5 - 181.7)	70.7 (37.7 - 159.1)	85.7 (48.1 - 185.0)	73.2 (50.4 - 112.6)
Duplicates	Site one	54.8 (2.5 - ∞)	∞ (3.2 - ∞)	∞ (3.2 - ∞)	∞ (3.2 - ∞)
Removed	Site two	134.0 (47.6 - ∞)	124.1 (56.0 - 668.4)	38.9 (26 - 60)	96.7 (60.5 - 180.3)

FIGURE S1. Years between detections for 22 cases of duplicate multilocus genotypes revealed by microsatellite analysis of shed skins from Striped Whipsnakes (*Masticophis taeniatus*) in Washington between 2006 and 2014. Sixteen snakes were found at the same site over multiple years (Residents) and six snakes were found at different locations over multiple years (Dispersers). Shed skins were removed from the sites as they were found, hence most “recaptures” occurred in the 0- and 1-y apart categories.

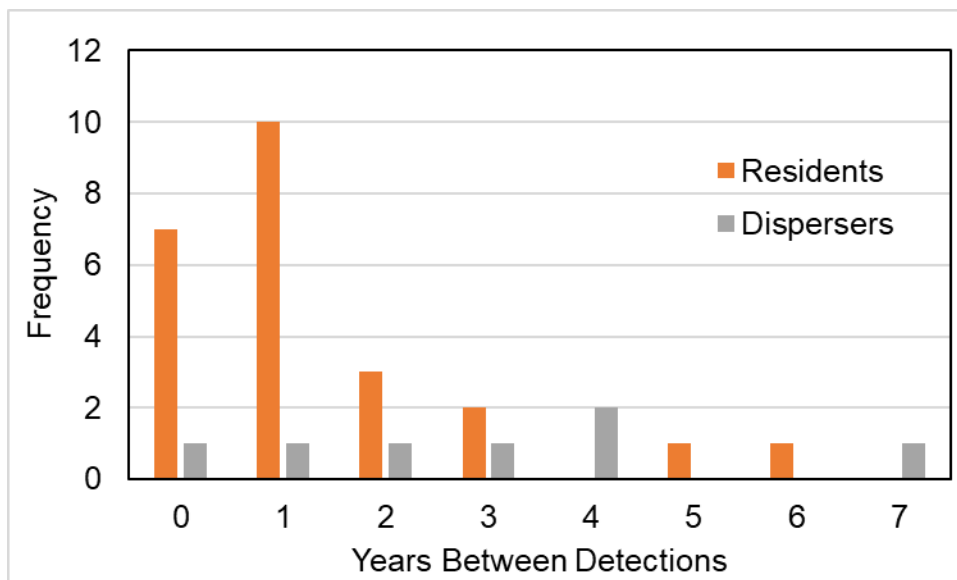


FIGURE S2. Dot distribution maps of Striped Whipsnake (*Masticophis taeniatus*) observations from three sources: A) Parker (1982); B) Camper (1996); C) iNaturalist (unpubl. data). The iNaturalist records were obtained as research grade observations on 20 January 2020.

