Amphibia-Reptilia

Short Note

Genetic diversity and gene flow decline with elevation in the Near Eastern fire salamander (*Salamandra infraimmaculata*) at Mount Hermon, Golan Heights

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*This contribution is dedicated to Leon Blaustein who passed away on June 23rd, 2020 to remember him as an expert on Israeli fire salamanders.

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Supplementary material

Sequencing of the mitochondrial D-loop

The PCR was conducted in 1µl genomic DNA and 11.5 µl Master Mix (buffer, dNTPs, Taq polymerase, H_2O) under the following program (96°C for 2 min, 38 cycles of 94°C for 20 sec, 60°C for 50 sec, 72°C for 3 min, then 72°C for 10 min, hold at 10°C).

Table S1. Additional samples used in the mitochondrial D-loop haplotype network.

Region	Site Name	Lat	Long	No. individuals
Mt. Carmel	El Balad	32°71'94.0""	35°07'16.1"	10
	Ein Alon	32°72'69.2""	35°02'28.4"	9
	Nahal Galim	32°75'07.8""	35°01'78.6"	10
	Secher	32°73'43.3""	35°03'11.1"	10
	Tsumaka	32°67'17.4""	35°03'74.4"	2
Galilee	Ein Kamon	32°91'06.9""	35°35'04.5"	9
	Haraschim	32°95'48.9""	35°33'42.7"	8
	Nahal Amud	32°91'69.5""	35°46'79.1"	9
	Kaukav	32°82'38.8""	35°25'62.9"	8
	Tsalmon	32°88'63.2""	35°38'85.5"	10
	Dovev	33°05'01.6""	35°41'51.9"	10
	Ein Humema	33°00'81.9""	35°39'52.8"	10
	Navoreia	33°00'05.8""	35°50'94.0'	9
	Sarach	33°06'79.0""	35°31'61.2"	10

Mt. Hermon	Tel Dan	33°24'81.0""	35°65'08.5"	7

Microsatellite analysis

PCR protocol

The PCR was conducted in 1µl primer mix (2µM primer concentration), 5µl Multiplex PCR Master-Mix by QIAGEN®, 3µl H_2O and 1µl DNA. We set the PCR program as follows: 95°C for 15 min, 30 cycles of 94°C for 30 sec, 60°C for 90 sec, 72°C for 60 sec, then 60°C for 30 min and cool down to 8°C. The microsatellites were genotyped by labelling the primers with fluorescent dyes (6-FAM™, HEX™, NED™). A dye size standard (0.1µl GeneScan™ 500 Rox™ by Applied Biosystems™) and a buffer (10µl Hi-Di™ Formamide by Applied Biosystems™) were added to 1 µl of the diluted PCR products (15µl H_2O). After denaturation (96°C for 5 min, 10 min on ice), samples were run for fragment analysis. The multiplex approach enabled us to amplify all loci within four PCR reactions.

Hardy-Weinberg Equilibrium

Deviations from Hardy-Weinberg-Equilibrium after Bonferroni were detected in TD at loci SST-C3 and SST-F10, in NC at locus E11 and in NP at loci E11, B11, SST-C3 and F10.

Table S2. Exact test for deviations from Hardy-Weinberg-Equilibrium using a Markov chain. Microsatellite loci of four *S. infraimmaculata* populations are tested. Results are presented for the number of tested genotypes per locus, the observed (H_o) and expected heterozygosity (H_e),

the probability of deviation (P-value) with standard deviation (± SD) and the steps of the chain. Significant P-values (P < 0.004) are depicted in bold font type.

Population 1 (Banias)						
Locus	#Genotypes	H_o	H_e	P-value	± SD	Steps
Sal E8	7	0.71429	0.78022	0.34709	0.00164	100172
SST-A6-II	7	0.71429	0.71429	1	0	100172
Sal E11	7	0.42857	0.78022	0.00599	0.00023	100172
SST-A6-I	7	1	0.82418	0.16314	0.00109	100172
SST-B11	7	0.42857	0.53846	1	0	100172
Sal E6	7	0.85714	0.81319	0.85942	0.00097	100172
SST-C3	7	0.42857	0.49451	1	0	100172
SST-C2	7	0.71429	0.6044	1	0	100172
Sal 3	7	0.71429	0.83516	0.39165	0.00121	100172
Sal E11	7	0.28571	0.26374	1	0	100172
SST-F10	7	0.57143	0.72527	0.18597	0.001	100172
Population 2 (Tel Dan)						
Sal E8	25	0.4	0.42531	0.19706	0.00131	100172
SST-A6-II	25	0.6	0.6302	1	0	100172
Sal E11	25	0.52	0.49388	1	0	100172
SST-A6-I	23	0.69565	0.743	0.1694	0.00133	100172
SST-B11	25	0.48	0.48245	1	0	100172
Sal E6	25	0.72	0.65306	0.93782	0.00078	100172
SST-C3	23	0.34783	0.63865	**0.00113**	0.00009	100172
SST-C2	25	0.72	0.65224	0.64542	0.00149	100172
Sal 3	23	0.52174	0.52947	0.06637	0.00049	100172
Sal E11	24	0.5	0.43351	0.72424	0.00132	100172
SST-F10	24	0.41667	0.61259	**0.00222**	0.00014	100172
Population 3 (Nimrod Castle)						
Sal E8	21	0.7619	0.55401	0.03815	0.00063	100172
SST-A6-II	21	0.47619	0.61672	0.11882	0.00098	100172
Sal E11	20	0.3	0.53462	**0.00086**	0.00008	100172
SST-A6-I	20	0.9	0.75897	0.01922	0.00038	100172
SST-B11	21	0.38095	0.48316	0.35398	0.00144	100172
Sal E6	21	0.71429	0.58653	0.65395	0.00115	100172
SST-C3	21	0.52381	0.52962	0.36958	0.00128	100172
SST-C2	21	0.52381	0.47038	0.66286	0.00146	100172
Sal 3	18	0.72222	0.69365	0.29387	0.00127	100172
Sal E11	18	0.05556	0.05556	1	0	100172
SST-F10	20	0.3	0.67821	0	0	100172

Population 4 (Nimrod Pool)						
Sal E8	46	0.69565	0.67941	0.289000	0.00132	100172
SST-A6-II	46	0.17391	0.16054	1	0	100172
Sal E11	46	0.3913	0.43263	**0.00036**	0.00006	100172
SST-A6-I	43	0.16279	0.15294	1	0	100172
SST-B11	46	0.32609	0.52484	**0.00665**	0.00025	100172
Sal E6	45	0.37778	0.3588	0.407	0.00111	100172
SST-C3	45	0.31111	0.48015	**0.00036**	0.00006	100172
SST-C2	46	0.06522	0.06426	1	0	100172
Sal 3	45	0.6	0.56754	0.92954	0.00075	100172
Sal E11	44	0.54545	0.46813	0.33185	0.00146	100172
SST-F10	44	0.61364	0.74739	**0.00376**	0.00016	100172

Isolation by elevation

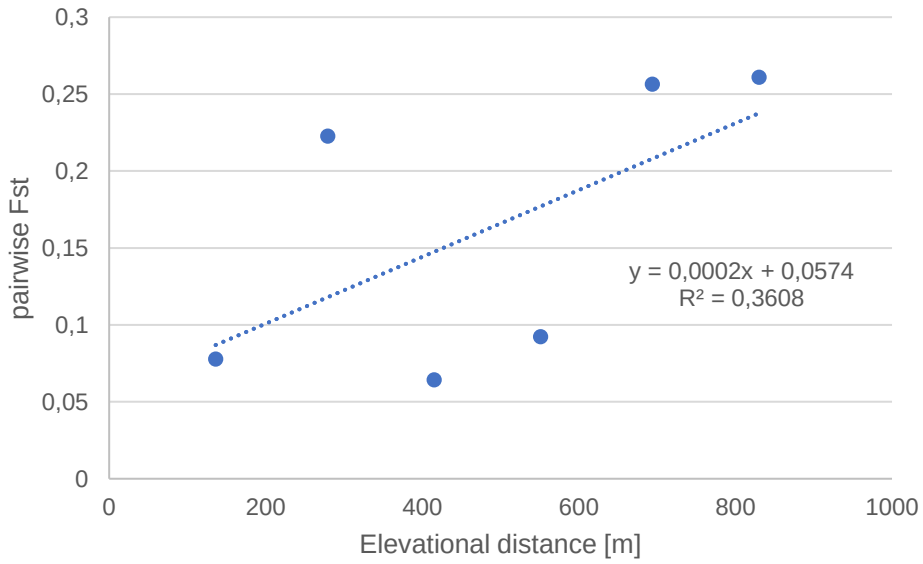

Figure S1. Correlation between pairwise genetic distance (F_{st}) and pairwise elevational distance between *S. infraimmaculata* populations at Mt. Hermon.

STRUCTURE HARVESTER

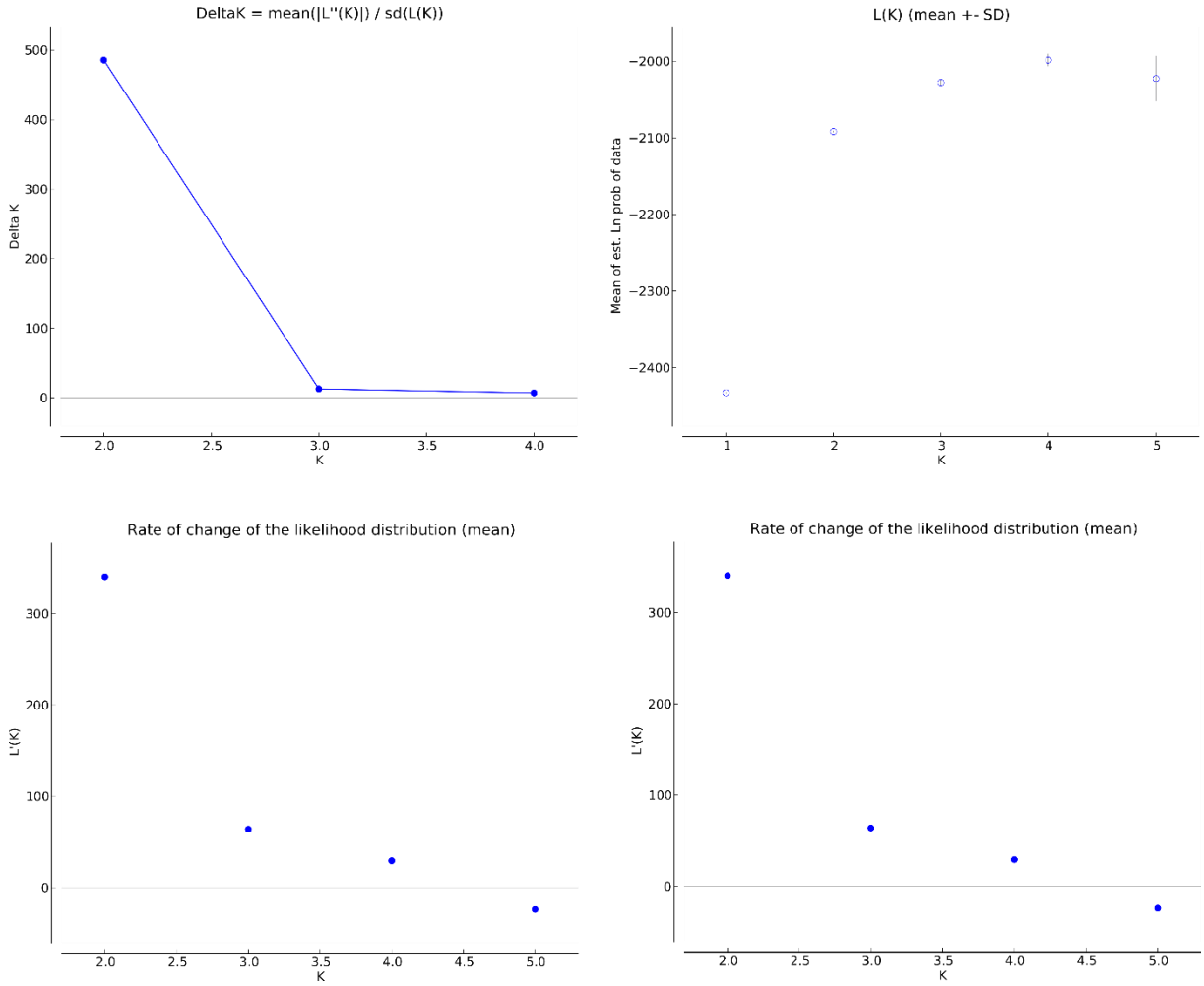

Figure S2. STRUCTURE HARVESTER output.

AMOVA

Table S3. Results of the hierarchical analysis of molecular variance (AMOVA) testing two strata (population, cluster groups) for the sampled Near Eastern fire salamanders. 1000 permutations, pairwise differences.

1 Group (TD, Ba, NC, NP)				
source of variation	**D.F.**	Sum of Squares	Variance components	Percentage of variation
Among populations	3	64.033	0.43295 Va	17.86
Among individuals within populations	95	208.007	0.19831 Vb	8.18
Within individuals	99	177.500	1.79293 Vc	73.96
Total	197	449.540	2.42419	
2 Groups (Group 1 = TD, Ba, NC; Group 2 = NP)				
Among groups	1	72.270	0.51072 Va	14.93
Among populations within groups	2	23.337	0.27938 Vb	8.17
Among individuals within populations	95	260.221	0.10948 Vc	3.20
Within individuals	99	249.500	2.52020 Vd	73.69
Total	197	605.328	3.41978	
3 Groups (Group 1 = TD; Group 2 = Ba, NC; Group 3 = NP)				

Among groups	2	88.268	0.45994 Va	13.90
Among populations within groups	1	7.339	0.21905 Vb	6.62
Among individuals within populations	95	260.221	0.10948 Vc	3.31
Within individuals	99	249.500	2.52020 Vd	76.17
Total	197	605.328	3.30868	
3 Groups (Group 1 = TD, Ba; Group 2 = NC; Group 3 = NP)				
Among groups	2	86.752	0.40017 Va	12.09
Among populations within groups	1	8.855	0.27956 Vb	8.45
Among individuals within populations	95	260.221	0.10948 Vc	3.31
Within individuals	99	249.500	2.52020 Vd	76.15
Total	197	605.328	3.30942	

STRUCTURE results when K = 5

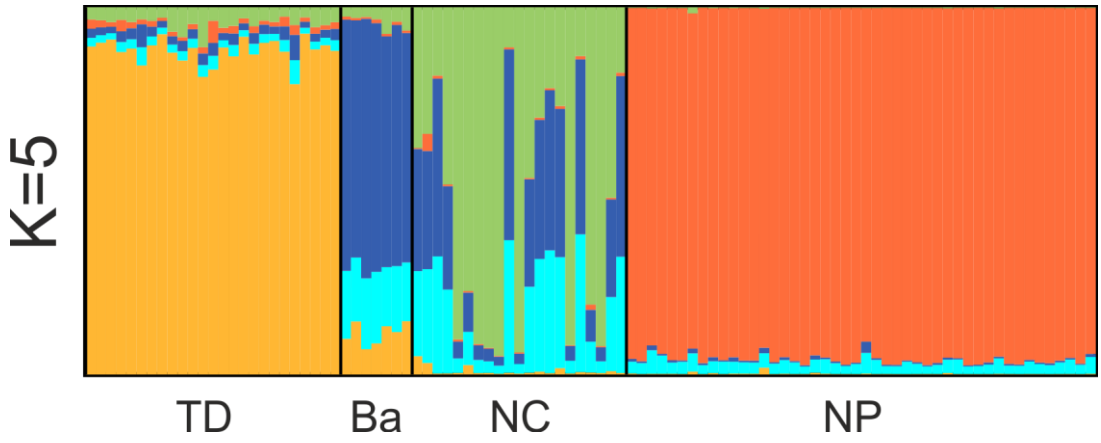

Figure S3. Population structure analysis of four *S. infraimmaculata* sites (Tel Dan, Banias, Nimrod Castle, Nimrod Pool) located at Mt. Hermon, Israel. Genetic structure assuming K = 5 as inferred by STRUCTURE. Bar plot depicts individual proportions of assignment to each color-coded cluster.