



The role of landscape and history on the genetic structure of peripheral populations of the Near Eastern fire salamander, *Salamandra infraimmaculata*, in Northern Israel

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Abstract

Genetic studies on core versus peripheral populations have yielded many patterns. This diversity in genetic patterns may reflect diversity in the meaning of “peripheral populations” as defined by geography, gene flow patterns, historical effects, and ecological conditions. Populations at the lower latitude periphery of a species’ range are of particular concern because they may be at increased risk for extinction due to global climate change. In this work we aim to understand the impact of landscape and ecological factors on different geographical types of peripheral populations with respect to levels of genetic diversity and patterns of local population differentiation. We examined three geographical types of peripheral populations of the endangered salamander, *Salamandra infraimmaculata*, in Northern Israel, in the southernmost periphery of the genus *Salamandra*, by analyzing the variability in 15 microsatellite loci from 32 sites. Our results showed that: (1) genetic diversity decreases towards the geographical periphery of the species’ range; (2) genetic diversity in geographically disjunct peripheral areas is low compared to the core or peripheral populations that are contiguous to the core and most likely affected by a founder effect; (3) ecologically marginal conditions enhance population subdivision. The patterns we found lead to the conclusion that genetic diversity is influenced by a combination of geographical, historical, and ecological factors. These complex patterns should be addressed when prioritizing areas for conservation.

Keywords Endangered salamander · Genetic diversity · Gene flow · Ecology · Peripheral populations · Conservation

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Introduction

The contrast between core (central) populations of a species versus peripheral (marginal) populations has attracted the attention of evolutionary biologists ever since Darwin, but particularly since the 1950’s (Pironon et al. 2017). The most straightforward manner of classifying core and peripheral populations is geographically. In the classification given in Gaston (2003), following Gorodkov (1986), the geography of permanent populations of a species fall into four categories: (1) a zone of continuous distribution, but with the possibility of lacuna (areas where the species is absent but surrounded by an otherwise continuous distribution), (2) the limit of the zone of continuous distribution (an edge or periphery), (3) a zone of disjunct distribution in which populations can be found that are geographically separated from each other and from the continuous distribution area, and (4) the limit of the zone of disjunct distribution. Not all species display all four types of these geographical range

features, but one that does is the fire salamander, *Salamanca inframaculata* (Fig. 1). The zone of continuous distribution is found in the higher elevations along the eastern Mediterranean region (Fig. 1a), with the southern part of the continuous distribution extending into the Galilee region of Northern Israel (Fig. 1b) (Bogaerts et al. 2013; Steinfartz et al. 2000). The Galilee is subdivided geologically into the Lower and Upper Galilee. The Upper Galilee is located at a higher elevation than the Lower Galilee and has a more mesic and cooler climate—and thereby also denser vegetation cover—than the Lower Galilee. The limit of continuous distribution is the edge of the lower Galilee (Fig. 1b). There is then a zone of disjunct distribution, with many populations found on Mount Carmel that is geographically separated from the Galilee by a low-elevation valley (Fig. 1b). The Mount Carmel populations represent the southernmost limit for this species, and indeed the entire genus *Salamanca*, so Mount Carmel also represents the limit of disjunct distribution (Blank et al. 2013).

A geographical classification of a species' range is of heuristic value, but it is more useful, particularly for conservation planning of endangered species such as *S. inframaculata*, to determine what limits the geographic range and positions of the borders (Gaston 2003). First, there could be abiotic and/or biotic factors that prevent further spread, such as physical barriers (e.g., seas, rivers,

mountains, and valleys), climatic factors, absence of essential resources, and the impact of other species. Another complication that has become increasingly important in this era of climate change is the low-latitude edges of a species range that may becoming less optimal. Hampe and Petit (2005) reviewed studies from the fossil record, phylogeography and ecology, and concluded that these low-latitude peripheral populations are disproportionately important for the survival and evolution of biota, yet these are the very populations that remain understudied despite having the highest chances for local extinction under climate change (Cahill et al. 2013; Chen et al. 2011). Second, there can be historical factors (Duncan et al. 2015). For example, suppose past climatic conditions changed, resulting in a contraction of the species range but leaving isolated populations in favorable habitat islands in the previous range to create a zone of disjunct distribution. On the other hand, suppose a zone of disjunct distribution is created by past colonization events of habitat islands through founders derived from the zone of continuous distribution. Many of these historical events leave genetic signatures such that inferences about the past can be made from current genetic surveys, as has been shown in other salamanders (Templeton et al. 1995). Third, genetic mechanisms may be operating directly to limit the range. For example, suppose the populations at the border are small in variance effective

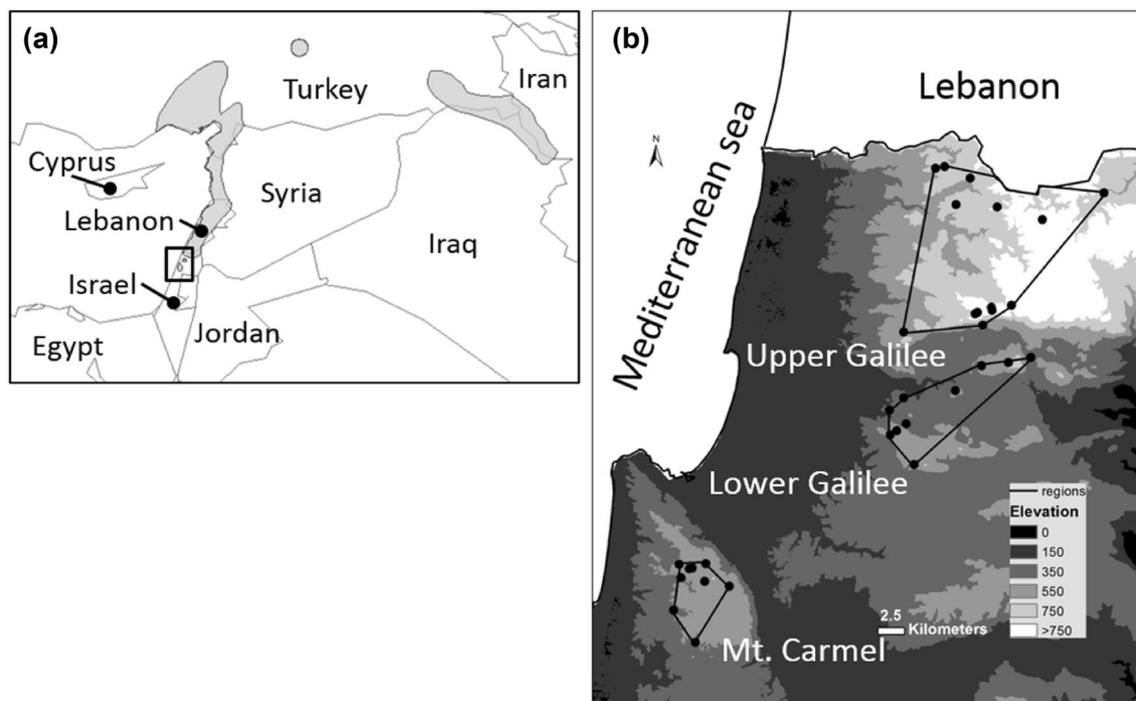


Fig. 1 **a** *Salamanca inframaculata* distribution range according to the IUCN (IUCN 2018). Black frame denotes the study area. **b** The three studied regions: Mount Carmel, the Lower Galilee, and the

Upper Galilee. Black points represent the 32 breeding sites that were sampled (see Table 1 for their names and coordinates)

size and have little to no genetic variation, thereby limiting the ability of these populations to adapt to local conditions (Carson 1955). Alternatively, suppose there is much gene flow from the core to the periphery that can also impede local adaptation (Kawecki 2008). Hence, patterns of genetic variation and gene flow/population subdivision can play important roles in understanding the nature of the periphery of a species' range (for reviews, see: Brusard 1984; Eckert et al. 2008; Hoffmann and Blows 1994; Kawecki 2008; Nevo 1998; Pironon et al. 2017; Vucetich and Waite 2003). These considerations indicate the need to take an interdisciplinary approach that integrates genetics, ecology, history, and geography to understand the multifaceted nature of species' borders (Holt and Keitt 2005).

The purpose of this paper is to perform such an integrative analysis on the southernmost part of the species' range of the endangered salamander *S. infraimmaculata*. A previous genetic survey revealed significant genetic differentiation between the Mount Carmel and the Lower Galilee populations and lower genetic diversity in Mount Carmel (Blank et al. 2013). Blank et al. (2013) argued that this pattern indicates that the non-contiguous Mount Carmel populations represent an isolated peripheral region that had experienced bottleneck and/or founder effects in its recent demographic history. This earlier survey only included Mount Carmel, the Lower Galilee and the southern edge of the Upper Galilee region. To understand better the potential diversity of peripheral populations with respect to genetic diversity, gene flow patterns, and recent evolutionary history, a more complete genetic sampling across the entire core–periphery gradient would be needed, and this was a major goal of the current study. A better understanding of the edge of the species' range also requires an ecological assessment of the factors that explain the species' distribution in a geographic context, as well as how gene flow patterns relate to landscape and other environmental features. We therefore analyze how the genetic structure of *S. infraimmaculata* populations is influenced by geographical, ecological, and landscape factors at the southernmost edge of its global distribution. We then test the impact of landscape and ecological factors on different geographical types of peripheral populations with respect to levels of genetic diversity and patterns of local population differentiation. Specifically, we test three hypotheses commonly made in the core-peripheral population literature by analyzing the variability in 15 microsatellite loci from 32 sites: (1) genetic diversity will decrease towards the geographical periphery of a species' range; (2) genetic diversity in geographically disjunct peripheral areas will be low compared to the core or peripheral populations that are contiguous to the core; and (3) ecologically marginal conditions tend to enhance population subdivision. By addressing these hypotheses, we will be able to elucidate the relative roles ecological,

evolutionary and historical factors have in shaping genetic diversity within and among these populations.

Materials and methods

Sample collection and DNA extraction

We sampled salamanders in three regions: the Upper Galilee, the Lower Galilee, and Mount Carmel (Fig. 1b). We collected genetic samples from 680 fire salamanders (mostly adults with some postmetamorphic juveniles) from 32 breeding sites (Table 1, Fig. 1b). Mount Carmel is a disjunct peripheral region, the Lower Galilee is largely a contiguous peripheral area, and the Upper Galilee is continuous with the core area that extends through Lebanon, Syria and Turkey (Fig. 1a).

Tissue samples for molecular analysis were collected by capturing adults (larvae in two cases; see Table 1) during rainy nights and cutting a small tip of the tail (2–3 mm) with a sterile scalpel, placing it in an eppendorf tube with 99% ethanol, and then storing at -20°C until further processing. Tail-tip tissue in salamander larvae was found to have only little effect on fitness (Segev et al. 2015; Blaustein et al. 2017). Other genetic samples were collected early in the morning from fresh road kills of salamander adults in eight sites in the Upper Galilee. Our goal was to collect samples from at least 20 individuals per site, but lower numbers were obtained for many of the sites due to their small population sizes (Table 1).

Each sampled adult individual was photographed in order to identify dorsal spot patterns to ensure that the same individuals were not sampled on different sampling nights (Blank et al. 2013; Segev et al. 2010; Warburg 2011). Genomic DNA was extracted using QIAamp DNA minikit (Qiagen) with the following modifications: protocol-devised RNA free option and incubation with proteinase K.

Microsatellite genotyping

Allelic variation in nuclear markers was assessed using 15 microsatellite loci using primers described earlier (Sal E2, Sal E5, Sal E6, Sal E7, Sal E8, Sal E11, Sal E12, Sal E14, Sal 3, Sal 23, SST-A6-I, SST-A6-II, SSTC3, SST-E11 and SST-G6: (Hendrix et al. 2010; Steinfartz et al. 2004). Each forward primer was labeled with a fluorescent dye (HEX, FAM, or TET) for visualization of PCR products. PCRs were carried out using the Qiagen Multiplex PCR Kit (Qiagen). The annealing temperatures for each primer pair were optimized using gradient PCR. PCR products were visualized with a MegaBACE 1000 automated sequencer (Amersham Biosciences) and the microsatellite allele sizes were determined with the ET-ROX 400 size

Table 1 The 32 study sites and basic information on the sample sizes (N) and genetic variability in 15 microsatellite loci at each site; A allelic richness; H_O observed heterozygosity; H_E expected heterozygosity

Region and site	Longitude	Latitude	N	A	H_O	H_E
Upper Galilee (13 sites)						
Even Menachem	33.247°N	35.287°E	20 [•]	3.46	0.63	0.64
Shomera	33.077°N	35.278°E	6 [•]	3.13	0.51	0.56
Shrach	33.069°N	35.313°E	8 [•]	3.07	0.6	0.6
Dishon	33.055°N	35.447°E	32 [•]	3.53	0.62	0.65
Pasuta	33.046°N	35.298°E	16 [•]	3.38	0.6	0.65
Elkosh	33.043°N	35.34°E	18 [•]	3.75	0.69	0.68
Sasa	33.032°N	35.385°E	19 [•]	3.54	0.63	0.65
Ein Sala	32.96°N	35.354°E	15	3.09	0.59	0.58
Kser	32.937°N	35.246°E	11 ^{•••}	2.86	0.47	0.52
Halutz	32.953°N	35.312°E	23	3.39	0.61	0.62
Harashim	32.956°N	35.332°E	26	3.51	0.59	0.64
Harashim South	32.954°N	35.333°E	16	3.32	0.57	0.61
Kshatot	32.952°N	35.318°E	10	3.47	0.55	0.66
Lower Galilee (ten sites)						
Zalmon	32.915°N	35.373°E	10 ^{••}	3.31	0.56	0.62
Ein Camon	32.91°N	35.349°E	35	3.01	0.51	0.6
Michmanim	32.907°N	35.322°E	15	2.65	0.55	0.49
Yaad	32.881°N	35.246°E	21	3.55	0.63	0.64
Eshhar	32.887°N	35.296°E	30	3.05	0.57	0.59
Segev	32.869°N	35.229°E	12	3.33	0.6	0.62
Atzmon	32.857°N	35.247°E	17	3.16	0.52	0.58
Manof pool	32.849°N	35.232°E	30	3.17	0.59	0.59
Manof	32.848°N	35.231°E	11	2.77	0.52	0.51
Kaukab	32.823°N	35.255°E	31	2.85	0.52	0.53
Mount Carmel (nine sites)						
Ein El Balad	32.719°N	35.07°E	33	1.95	0.33	0.3
Ein Nesher	32.738°N	35.047°E	36	1.8	0.32	0.3
Ein Chik	32.723°N	35.046°E	55	1.96	0.29	0.31
Damun	32.734°N	35.033°E	19	1.99	0.33	0.34
Secher	32.734°N	35.03°E	34	1.85	0.27	0.28
Pine Club	32.738°N	35.02°E	18	1.79	0.24	0.28
Ein Alon	32.726°N	35.022°E	27	1.97	0.29	0.32
Bustan Stream	32.698°N	35.014°E	7	2.2	0.33	0.36
Sumak	32.671°N	35.036°E	19	1.99	0.35	0.34

Samples were taken from adults only except for two sites noted below, [•]=road kills, ^{••}=Larvae only, ^{•••}=Larvae and adults

standard (Amersham Biosciences). Alleles were scored using visual inspection and manual corrections of alleles with MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004). Microsatellite genotypes were checked for the presence of null alleles, stutter products, or allelic dropout using MICRO-CHECKER. Linkage disequilibrium and deviations from Hardy–Weinberg equilibrium were investigated using GENEPOP on the web (Rousset 2008).

Data analyses

Quantifying genetic diversity

To interpret patterns in genetic diversity between regions, we calculated the average values of allelic richness, number of unique alleles, and observed and expected

heterozygosity for each of the regions. We used a randomization test to evaluate the differences in observed and expected heterozygosity, inbreeding index within local populations (F_{is}), and a measure of between population differentiation (F_{st}) between each pair of regions (999 permutations, implemented in FSTAT).

Analysis of population structure

We used the program STRUCTURE to cluster the individuals into a finite number of populations based solely on genetic data. STRUCTURE requires the number of populations to be specified a priori, and we used the delta K method of Evanno et al. (2005), a widely used method for determining K , the number of populations.

It is worth stressing, however, that such clustering method has to be used cautiously because it is based on various model assumptions (e.g. Hardy–Weinberg equilibrium) and it is sensitive to both sampling scheme and size. The objective of inferring the number of population clusters (K) is not based on a rigorous statistically method and thus may sometimes generate unrealistic results (Kalinowski 2011). Moreover, as will be shown, our results indicate an isolation by distance pattern in one of our regions. Perez et al. (2018) found that STRUCTURE outputs are extremely affected by isolation by distance, mostly through the detection of artificial and misleading genetic clusters. Thus, in practice, it is strongly recommend using at least two independent clustering methods.

We used principal component analysis (PCA) as a second population structure inference method (adeget v2.1.1 R package; Jombart (2008)). This multivariate descriptive method is not dependent on any model assumption (e.g. Hardy–Weinberg equilibrium or linkage disequilibrium).

And lastly, we used the program NetStruct (Greenbaum et al. 2016) to investigate population structure solely from genetic data and with no a priori number of clusters. NetStruct is a network-based method for population structure inference, in which inter-individual genetic similarity networks are constructed, and dense subnetworks (also called “communities” in network theory) are searched for. The dense subnetworks represent groups of genetically similar individuals, and are interpreted as subpopulations. The genetic similarity networks can be pruned systematically to remove weak edges below an edge-pruning threshold, and to detect population structure at different hierarchical level. For each hierarchical level, the detected genetic signal can be tested for significance using permutation tests.

The significant clusters found by NetStruct reflect only genetic similarity among individuals and are not necessarily geographic regions, particularly when gene flow and admixture occur. Accordingly, more than one genetic cluster may be found at a single geographic site, and a single genetic

cluster may be found at multiple geographic sites. When this occurs, we test the null hypothesis that the NetStruct clusters are homogeneously distributed geographically by constructing a G by C table, where G is the number of geographic sites, C is the number of genetic clusters, and the elements are the number of individuals at geographic site g that are also members of genetic cluster. We then test the null hypothesis of geographic homogeneity in this G by C table by an exact permutation test with 10,000 random permutations to determine the p value under the null hypothesis as well as a 99% confidence interval for the p-value with the program StatExact (Cytel Studio, Cambridge, MA, v 9.0). A rejection of the null hypothesis indicates that assignment of individuals to clusters in the region is biased, and gene flow within the region is not panmictic.

Another indicator of population structure is isolation by distance. To test this possibility, we determined whether pairwise $F_{st}/(1 - F_{st})$ [as calculated by Arlequin (Schneider et al. 2000) between subpopulations correlated with the Euclidian distance (calculated in ArcGIS (ESRI, Redlands, CA)] using Mantel’s test (999 permutations) implemented in PASSaGE (Rosenberg and Anderson 2011).

Characterization of geographic and environmental variation

We quantified the altitude (obtained from Hall et al. 2013), average precipitation, and average annual day and night temperatures (data obtained from the Israeli Meteorological Service) at each of the 32 sites. We also quantified the differences in these environmental factors between Mount Carmel, the Lower Galilee, and the Upper Galilee (Fig. 1b). We used radiometric and geometric corrected LANDSAT8 satellite imagery data (Roy et al. 2014) for producing Normalized Difference Vegetation Index data (NDVI) (Levin et al. 2011; Tucker 1979). NDVI was computed for two different seasons—winter (February 2014) and summer (July 2014) in order to differentiate between evergreen vegetation and annual vegetation. The continuous NDVI values from both seasons was classify into several discrete categories of Mediterranean flora. The output classes were adjusted to the accepted vegetation cover type names after field validations in four locations along the climatic gradient of the Mediterranean ecosystem. The names of the vegetation cover classes were given according to the Israeli guide for Mediterranean vegetation mapping (Leshner and Ramon 2013).

Maximum entropy modeling

We used data on 97 salamander breeding sites to examine the landscape and environmental characteristics that can explain the distribution of these salamanders in the three regions. We learned of these 97 potential breeding sites

based on previous surveys done in the area (Blank and Blaustein 2012, 2014, Sinai and Oron unpublished data) and interviews with Nature and Park Authority rangers. For these 97 sites, we employed maximum entropy distribution (Maxent) modeling to infer the suitable areas for *S. infraimmaculata*. Maxent, unlike other distributional modeling techniques, uses only presence and background data instead of presence-absence data (Elith et al. 2011; Hernandez et al. 2008; Navarro-Cerrillo et al. 2011). Maxent predicts the probability distribution across all the cells in the study area. We implemented Maxent using version 3.3.3e of the software developed by Phillips et al. (2006). Recommended default values were used for the convergence threshold (10^5) and maximum number of iterations (500). Model performance was evaluated using ‘Area under the curve’ (AUC with a range from 0.0 to 1.0; Swets 1988).

We considered 10 environmental variables as potential predictor variables of *S. infraimmaculata* distribution in the Maxent analysis: Elevation (meters asl), Northness (degrees), Eastness (degrees), Slope (degrees), Soil type (categorical), Land cover including vegetation type (categorical), Precipitation (mm), Distance to nearest road (meters), Distance to nearest built area (meters), and mean daily temperature in January ($^{\circ}\text{C}$) (the mid-point of the active breeding season). Previous studies on salamander distributions have indicated the importance of elevation and slope (Blank and Blaustein 2012, 2014; Blank et al. 2013; Bogaerts et al. 2013; Kershenbaum et al. 2014), precipitation (Haan et al. 2007; Semlitsch and Anderson 2016), temperature (Goldberg et al. 2011; Peleg 2009), and land-cover (Hocking et al. 2013; Manenti et al. 2009; O’Donnell et al. 2014; Pisa et al. 2015; Sepulveda & Lowe 2009). Aspect (Northness and Eastness) is expected to affect the overall radiation reaching the ground. Solar radiation is a direct ecological factor affecting habitat conditions, such as water temperature and soil and hydroperiod of the ponds. Soil was previously found to be an important environmental variable explaining the distribution of *S. infraimmaculata* (Blank and Blaustein 2012). Quickly drained soils limit the time length that water is available for breeding (Hardy 1945). Roads could affect amphibians for three main reasons. First, roads pose mortality risk for individuals crossing the roads (Fahrig and Rytwinski 2009; Garriga et al. 2012, T. Oron, personal communication), and indeed many of our samples came from road kills. Second, avoidance of roads restricts dispersal and migration (Ray et al. 2002). Third, pollution from road runoff was identified as a threat to aquatic habitats (Dorchin and Shanas 2010; Harless et al. 2011). Segev et al. (2010) found a positive correlation between built areas and *S. infraimmaculata* population size but suggested that this was because human settlements tended to be established close to springs.

Given the Maxent model based on 97 sites that cover more uniformly the distribution of these salamanders within Israel (Fig. 7), we assigned Maxent scores (Dubey et al. 2013) to the 32 salamander breeding sites surveyed genetically. Such scores are a measure of local habitat suitability for the species.

Results

MICRO-CHECKER analyses revealed no evidence of null alleles or scoring issues across loci. Only three of 105 pairwise loci Fisher exact probability tests of deviation from genotypic equilibrium were significant at $P < 0.05$. Significant linkage disequilibrium was found at only 5.86% of loci combinations at the 32 sites.

Genetic diversity and population structure

There were 18 alleles unique to the Upper Galilee, only one to the Lower Galilee, and none unique to Mount Carmel. In the Upper and Lower Galilee, the average allelic richness and the observed and expected heterozygosity were significantly higher than Mount Carmel (Table 2). Although the two Galilee regions were not statistically different from one another in genetic diversity measures (Table 2), the F_{st} estimated among the Lower Galilee sites was greater than zero and exceeded that estimated for the Upper Galilee and the Mount Carmel regions, both of which had F_{st} estimates not significantly different from zero (Table 2). We observed moderate decreases in allelic richness and observed heterozygosity when moving from the Upper Galilee to the Lower Galilee, and sharp decreases in these parameters in

Table 2 Summary of genetic diversity from the major sampling regions

Region	Upper Galilee	Lower Galilee	Mount Carmel
Number of sites	13	10	9
Number of individuals	232	212	248
Unique alleles	18	1	0
Observed heterozygosity	0.597 ^b	0.559 ^b	0.302 ^a
Expected heterozygosity	0.621 ^b	0.582 ^b	0.309 ^a
F_{is}	0.040 ^a	0.039 ^a	0.022 ^a
F_{st}	0.073 ^a	0.108 ^a	0.064 ^a

Significance is based on permutation tests (999 permutations). Different superscripted letters signify statistically significant differences

^{a,b}Represent significant differences between regions; p value < 0.05 . Areas sharing a superscript are not significantly different from each other

Fig. 2 A regression analyses of Allelic richness and observed heterozygosity as a function of latitude ($^{\circ}$ N) in different sampling regions: Mount Carmel sites (circles), Lower Galilee sites (triangles), Upper Galilee sites (squares)

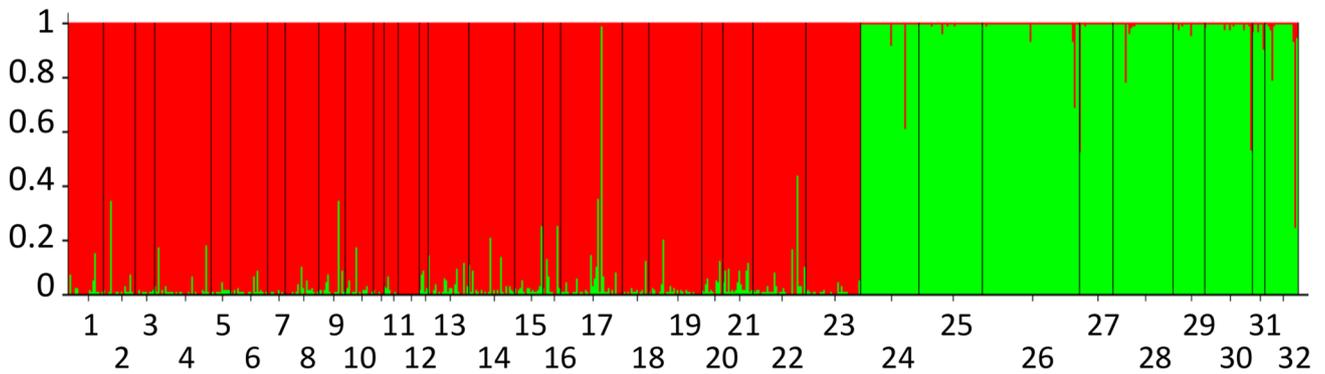
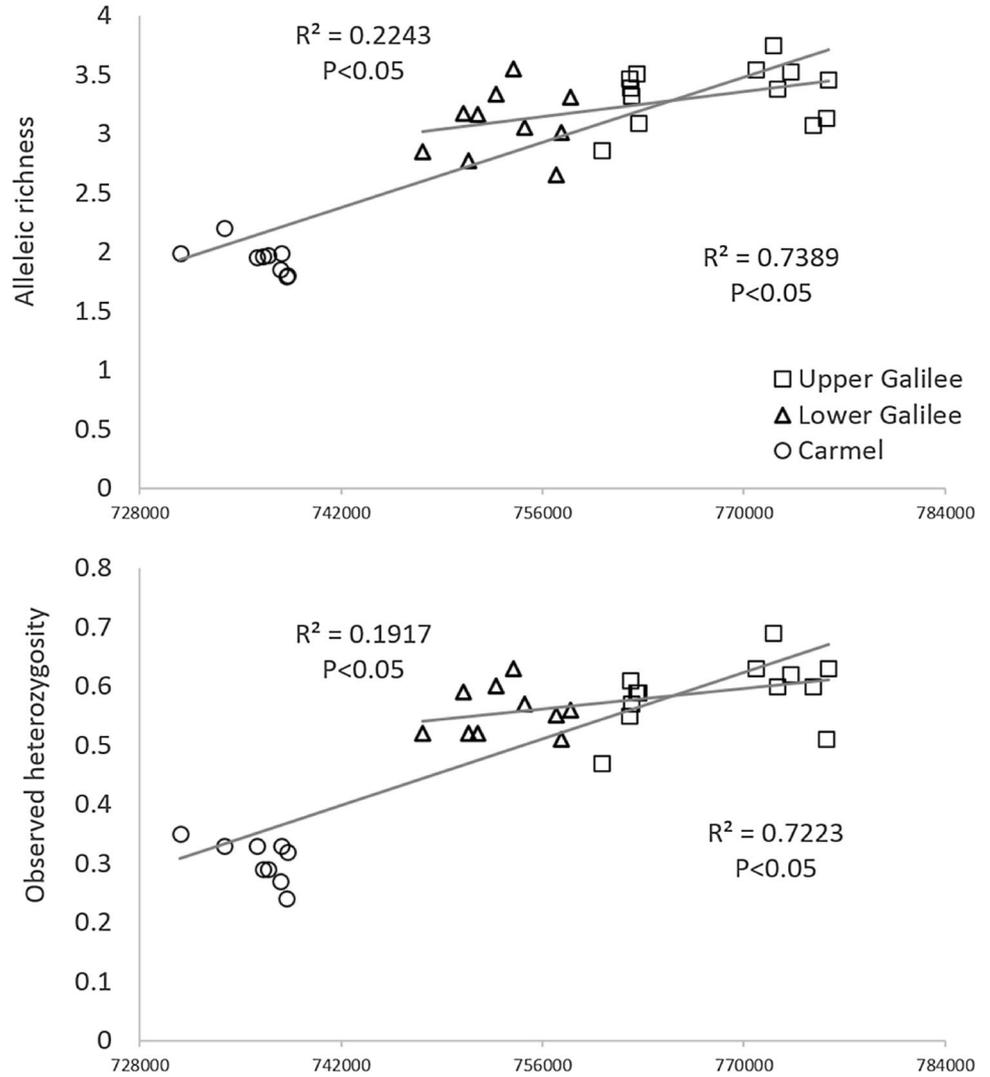


Fig. 3 Genetic clustering in the study area obtained with STRUCTURE with $K=2$, the optimal K under the delta K method. Identical colors identify populations with a homogeneous genetic composition,

while different colors represent genetically differentiated populations. The red color is associated with individuals sampled from the Galilee, and green from Mount Carmel

the Mount Carmel region (Tables 1, 2). Allelic richness and observed heterozygosity declined significantly with decreasing latitude when the regression included all three multi-site

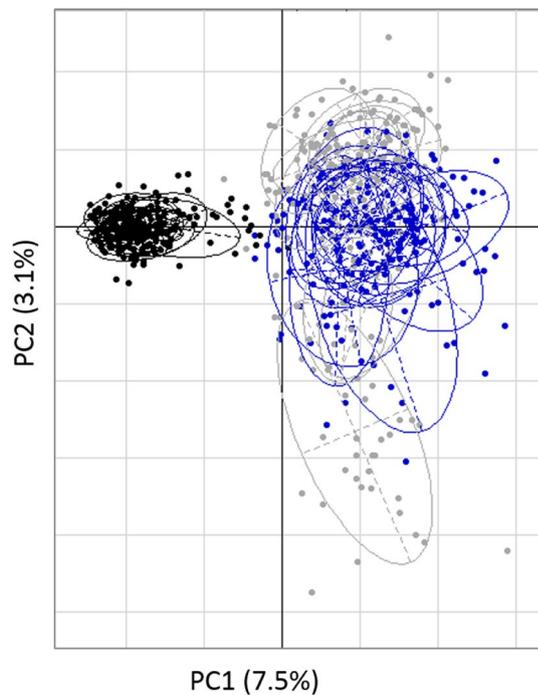


Fig. 4 Results of the PCA analysis. First and second axes are presented. The dots shows individual salamanders. Ovals represent 95% inertia ellipses. Blue- Upper Galilee; Gray- Lower Galilee; Black- Mount Carmel

regions, but also when it was restricted just to the sites in the Galilee (Fig. 2).

STRUCTURE analyses revealed that the optimal K using the delta K criterion was two. Most individuals fell in one of the two clusters that corresponded geographically to the Mount Carmel region and the Galilee sites, with few admixed individuals between these two geographic clusters (Fig. 3). Like STRUCTURE, the first two PCA axes clearly divided the Galilee region from the Mount Carmel region (Fig. 4).

NetStruct provided further insight into population structure. At the lowest edge-pruning threshold (coarse-scale structure), two significant clusters emerged- the Mount Carmel populations and the Galilee populations (Fig. 5a). Hence, this analysis captured the same subdivision as the STRUCTURE analysis, but now with added information that these two clusters are statistically significant. Indeed, not a single random permutation out of 1000 equaled or exceeded the observed modularity for these two clusters, indicating a strong degree of genetic differentiation between these two geographic areas. Because the allele frequencies were so different between these two clusters, we decided to separate them for the subsequent analyses because these large allele frequency differences would dominate the weights assigned to the allele sharing similarity measures within each cluster. No additional significant clusters were found within Mount Carmel for any edge-pruning threshold (Fig. 5b, c), indicating a high degree of genetic homogeneity among individuals within this geographic region. However, in the Galilee, at an edge-pruning threshold of 0.12, three significant genetic

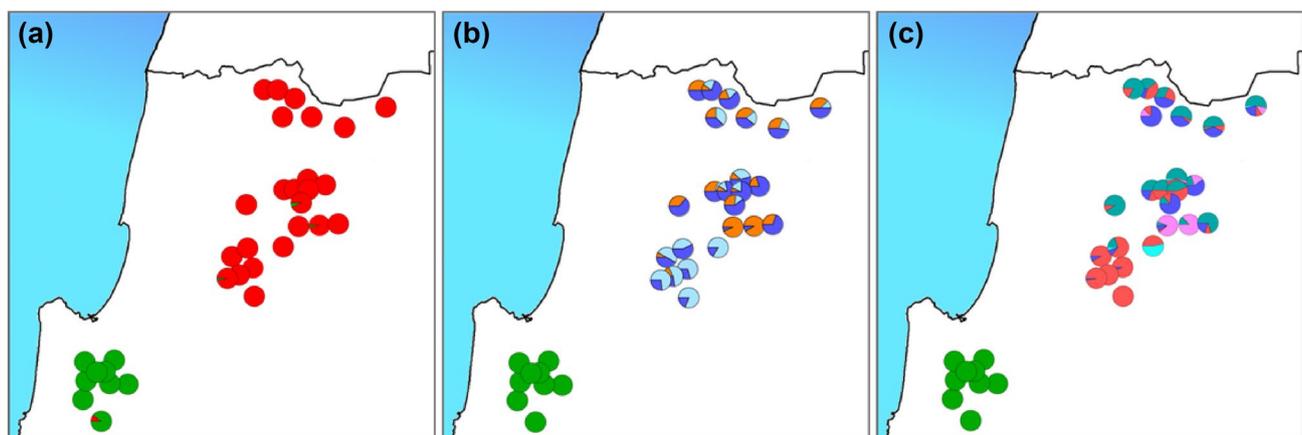


Fig. 5 Genetic clustering in the study at three hierarchical levels obtained with NetStruct. Different colors represent different genetic clusters. At each sampling site, the distribution of assignments of individuals to clusters is shown. **a** The highest hierarchical level, obtained by analyzing the network of all individuals without edge pruning. Two statistically significant ($p < 0.001$) clusters were detected at this level. **b** The second hierarchical level, obtained by analyzing the network constructed only for individuals in the Galilee (both upper and lower), with edges representing genetic-similarity below 0.12 pruned. Three significant clusters ($p < 0.001$) were detected at this level, and the Carmel was designated as an additional cluster since analysis of the Carmel network did not reveal any discernible sub-structuring. **c** The third hierarchical level, obtained by analyzing the Galilee network, with edge weights below 0.22 pruned. Five significant clusters ($p < 0.001$) were detected at this level, and Mount Carmel was assigned as an additional cluster

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Table 3 Results of exact permutation tests of the null hypothesis of geographic homogeneity for several regions with respect to the geographic distribution of the genetic clusters found by NetStruct in the Galilee

Threshold	Regions	Exact p	99% confidence interval
0.12	Galilee	0.0000	0.0000–0.0005
0.12	Upper Galilee	0.1639	0.1544–0.1734
0.12	Lower Galilee	0.0000	0.0000–0.0005
0.12	Upper Galilee plus Zalmon	0.1547	0.1454–0.1640
0.22	Galilee	0.0000	0.0000–0.0005
0.22	Upper Galilee	0.0000	0.0000–0.0005
0.22	Lower Galilee	0.0000	0.0000–0.0005
0.22	Upper Galilee Sites Halutz, Harashim, Harashim South, and Kshaton, plus Zalmon	0.1223	0.1139–0.1307

clusters emerged, as indicated by the three colors in Fig. 5b. All three genetic clusters were found both in the Upper and Lower Galilee, and Table 3 presents the results of testing the null hypothesis of geographic homogeneity in the distribution of these clusters. The null hypothesis of geographic homogeneity was strongly rejected for the Galilee as a whole, and equally strongly for just the Lower Galilee sites (Table 3). However, note that in the Upper Galilee, the null hypothesis of geographic homogeneity is not rejected (Table 3). Many individuals from the Lower Galilee site of Zalmon, clustered with individuals from the Upper Galilee sites near tributaries of an Upper Galilee stream that descends to the valley between the Upper and Lower Galilee close to Zalmon. Thus, we also tested the null hypothesis that Zalmon plus the Upper Galilee sites are homogeneous and found that the hypothesis of geographic homogeneity among these sites was not rejected (Table 3).

The next significant change in NetStruct clustering occurs at edge-pruning threshold of 0.22, with the Galilee populations now consisting of five significant clusters (Fig. 5c). Table 3 shows that the null hypothesis of geographic homogeneity is still strongly rejected both for the Galilee as a whole, as well as for the Lower Galilee. However, the null hypothesis of geographic homogeneity is now strongly rejected for the Upper Galilee sites as well (Table 3). As can be seen from Table 3, the null hypothesis of geographic homogeneity is accepted for Zalmon and these four Upper Galilee sites. This pattern of geographic homogeneity indicates that this stream from the Upper Galilee is likely a dispersal corridor that genetically connects the Lower Galilee to the Upper Galilee.

Because the results given above indicate restricted gene flow among the three geographic regions in our study, we tested for isolation by distance separately using Mantel test within each of these three regions. The pairwise standardized F_{st} among subpopulations correlated positively with Euclidian distance within the Lower Galilee ($r=0.42$, $p<0.05$) and Mt. Carmel regions ($r=0.43$, $p<0.05$), but there was no significant correlation in the Upper Galilee ($r=0.16$, $p=0.29$), as shown in Fig. 6 (see Appendix for full pairwise tables).

The Mantel test for all the populations together resulted with significant correlation ($r=0.72$, $p<0.05$).

Environmental variation

We examined the differences in environmental variables between the three major regions. We found that the Lower Galilee had the lowest average elevations and annual precipitation, but the highest average temperatures (Fig. 7). All regions differed from each other in all three response variables (elevation, precipitation, and temperature).

Table 4 shows the differences in vegetation cover between the three regions. All three regions had similar percentages of their area affected by human development. The Lower Galilee had a greater proportion of forested areas than the other two regions, whereas the Upper Galilee had less medium-dense maquis, but much more dense maquis and woodland than the Carmel or Lower Galilee.

Habitat suitability

The results of the Maxent modelling are shown in Fig. 8. The AUC for the replicate runs was 0.857, indicating a high level of accuracy for the Maxent predictions. Generally, most of the Lower Galilee is represented with low suitability values (<0.4), while the Upper Galilee and Mount Carmel regions were more suitable (Fig. 8). Four variables collectively contributed 86% to this optimal Maxent model: soil (36.1%), precipitation (24.1%), temperature (14.3%) and altitude (11.7%). As can be seen from Fig. 7, the last three of these variables differ considerably in the three geographic areas that are in our survey.

There was a significant linear increase of allelic richness with increasing Maxent suitability scores in the Upper Galilee, but not in Lower Galilee or Mount Carmel (Fig. 9). On Mount Carmel, the Maxent scores were generally higher than those in the Lower Galilee, but the allelic richness was consistently lower in Mount Carmel as compared to the Lower Galilee (Fig. 9).

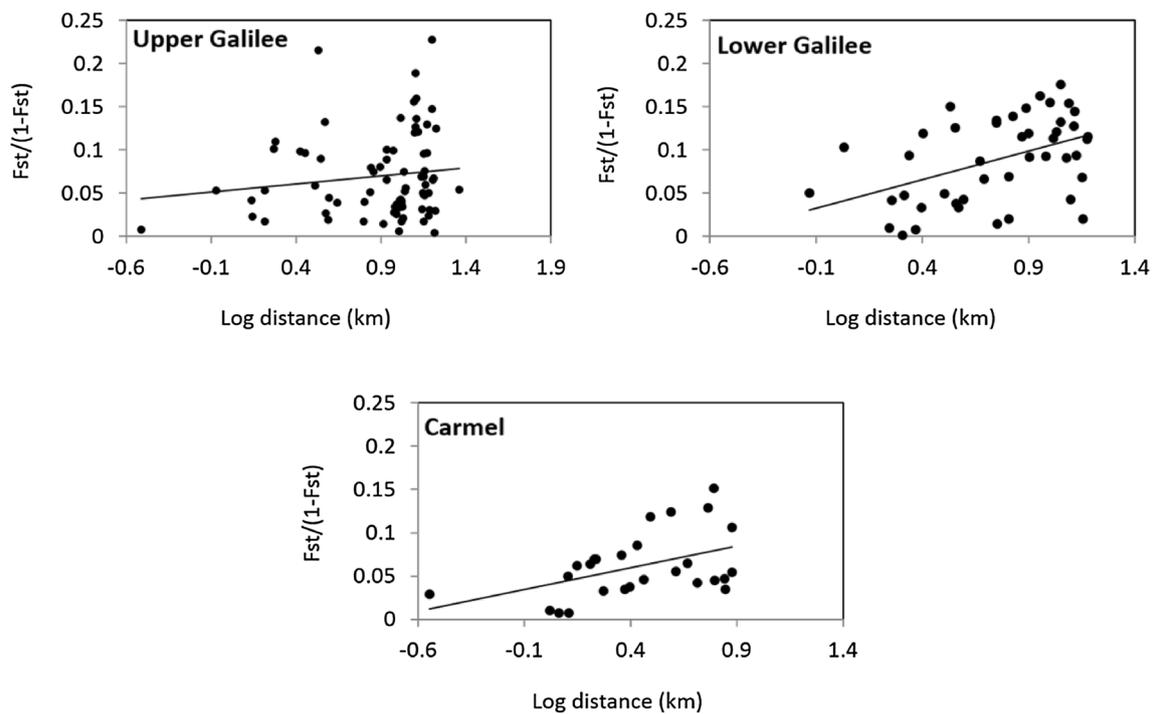


Fig. 6 Isolation by distance within the three major geographic regions. The Mantel test was not significant for the Upper Galilee (top panel), but was significant for the Lower Galilee (middle panel) and Carmel (lower panel)

Discussion

We set out to test three hypotheses: (1) that genetic diversity will decrease towards the geographical periphery of a species' range; (2) that genetic diversity in geographically disjunct peripheral areas (Mount Carmel) will be low compared to the core (Upper Galilee) or peripheral populations that are contiguous to the core (Lower Galilee); and (3) that ecologically marginal conditions tend to enhance population subdivision. The results gave support for all these hypotheses.

Genetic diversity will decrease towards the geographical periphery of a species' range

Going from the Upper Galilee to the Lower Galilee defines an increasingly peripheral geographical gradient and a decreasing latitude gradient. Our results clearly show that this gradient is associated with declining genetic diversity as measured by allelic richness, observed and expected heterozygosity, and number of unique alleles (Table 2, Fig. 2). Allelic richness and the number of unique alleles are particularly sensitive indicators of how well the balance of gene flow versus local genetic drift can maintain genetic diversity in a species' gene pool (Greenbaum et al. 2014). Allelic richness showed a significant decline across this entire gradient and also across the latitudinal gradient confined just to the

contiguous core-periphery in the Galilee (Fig. 2). The number of unique alleles shows an even more dramatic pattern, with 18 alleles unique to the Upper Galilee, and only one in the Lower Galilee. The low frequency of unique alleles in the Lower Galilee population indicates a significant decrease in gene flow, an increase in local genetic drift in traversing this core-peripheral gradient, both the entire gradient and just the contiguous portion in the Galilee (Fig. 2), and/or historical founder or bottleneck effects during colonizations of peripheral areas. Overall, this pattern supports the hypothesis of decreased genetic diversity at the periphery.

Genetic diversity in geographically disjunct peripheral areas will be low compared to the core or peripheral populations that are contiguous to the core

Figure 2 suggest that the low measures of genetic diversity found in Mount Carmel are not simply an extrapolation of the trends seen in the contiguous Galilee regions, but rather represent a more extreme drop in genetic diversity. The STRUCTURE, PCA and NetStruct analyses also indicated that the Mount Carmel populations are genetically homogeneous and highly differentiated from the Galilean populations. Allelic diversity was consistently lower in Mount Carmel than in the Galilee, and no unique alleles were found in Mount Carmel. All these patterns are consistent with a

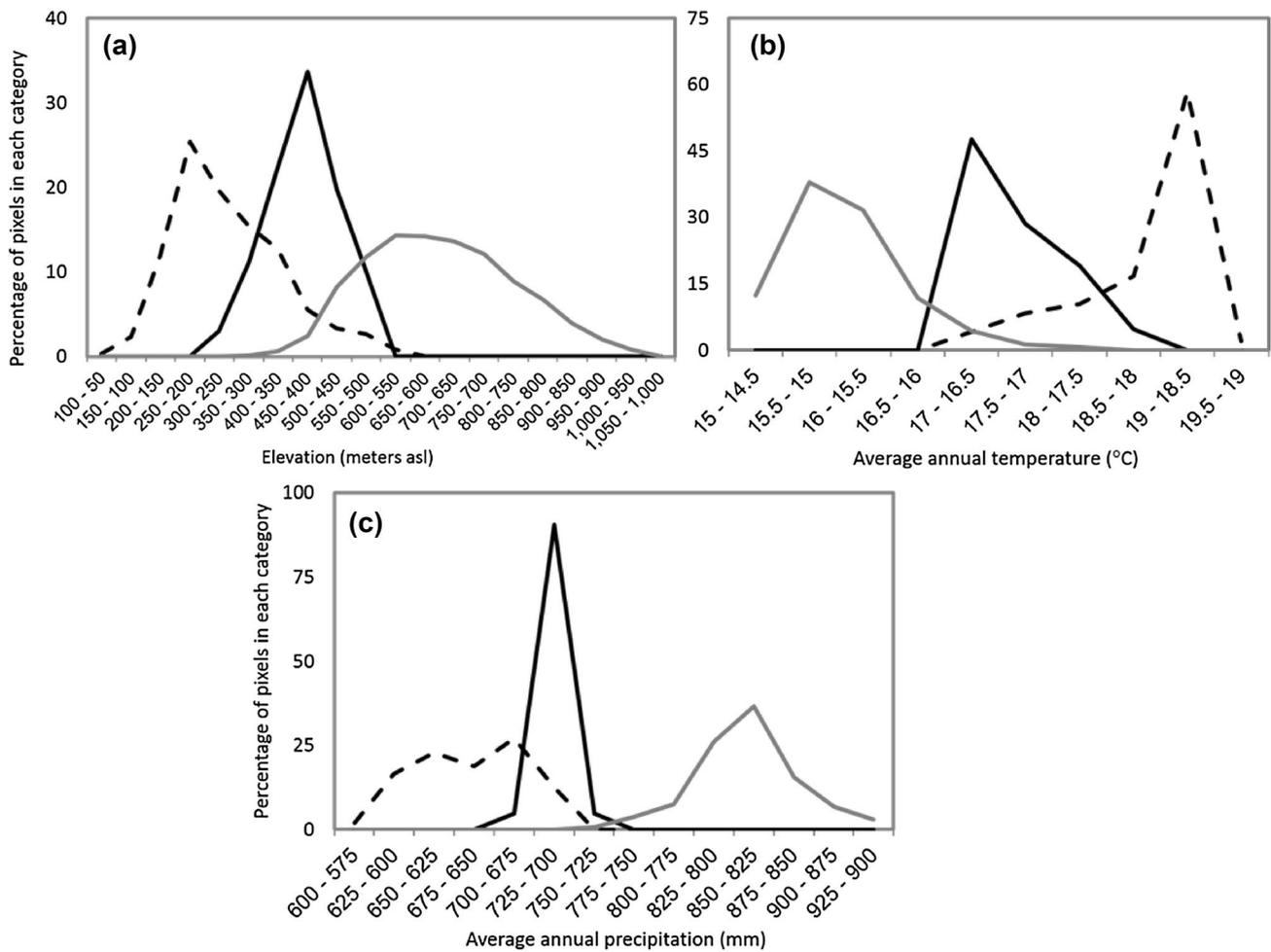


Fig. 7 The distributions of **a** elevation, **b** annual average temperature and average annual precipitation **c** in the three regions. The black lines indicate the distributions on Mount Carmel, the dashed lines the distributions in the Lower Galilee, and the gray lines in the Upper Galilee

Table 4 The percentages of the vegetation types found in the Upper Galilee, Mount Carmel, and Lower Galilee

Vegetation type	Upper Galilee	Mount Carmel	Lower Galilee
Herbaceous areas	1.3	1.7	4.3
Dwarf-shrub garrigue	0.2	0.2	0.4
Dense and medium maquis	18.2	22.3	14.4
Medium-dense maquis	9.5	19.8	18.4
Dense maquis and woodland	25.9	8.1	3.7
Forest	4.4	7.7	15.4
Other (agriculture, built, roads...)	40.5	40.2	43.4

Quantified from the vegetation cover map described in the Methods section

recent colonization event associated with a strong founder effect (Blank et al. 2013). Another possibility is that the continuous range of the species has been regressing towards the north, stranding the Mt. Carmel populations on a habitat island. Stranding alone would not explain the extreme drop in genetic diversity observed in the Mt. Carmel populations unless coupled with extremely small population size that

persisted for many generations on Mt. Carmel. We do not have estimates for the total population size on Mt. Carmel, but it is possible to collect several hundreds of individuals in just a small portion of Mt. Carmel (Bar-David et al. 2007). Moreover, our MaxEnt analysis indicates that Mt. Carmel represents an optimal habitat. These observations suggest that small population size for many generations on Mt.

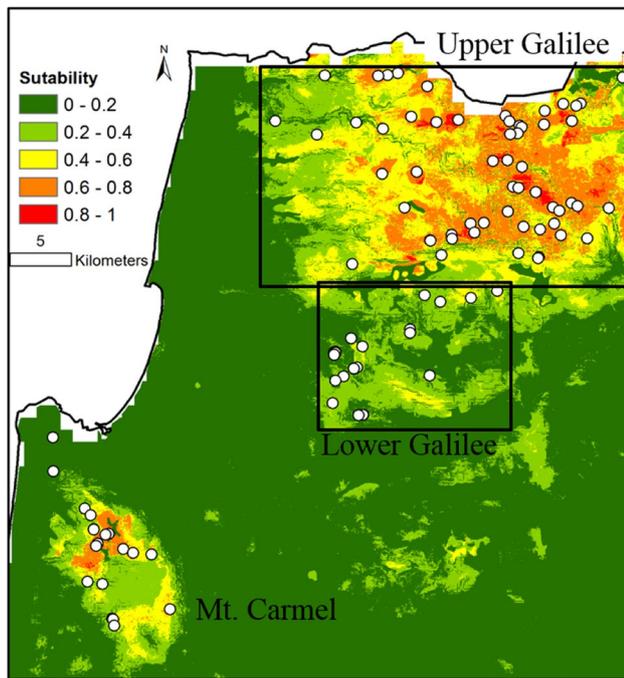


Fig. 8 Maxent habitat suitability scores over the three major regions sampled. Mount Carmel is shown in the lower left-hand corner, the Upper Galilee in the upper right-hand corner, and the Lower Galilee just south of the Upper Galilee. White circles mark the 97 water bodies known to serve for breeding

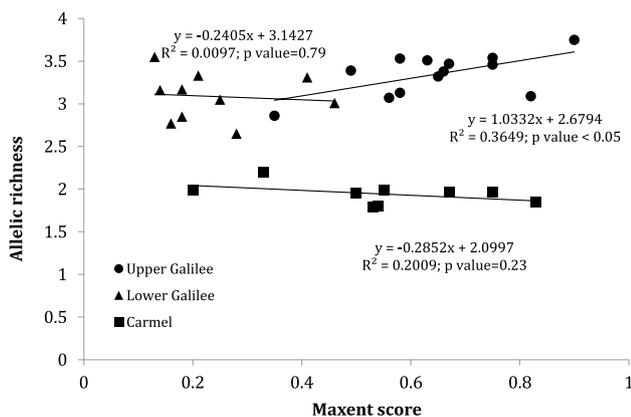


Fig. 9 Correlation of allelic richness against the Maxent model score. The allelic richness was scored in the 32 salamander breeding sites, but correlations were performed separately for breeding sites in the Upper Galilee, the Lower Galilee, and Mount Carmel

Carmel is unlikely. The MaxEnt analysis also demonstrates that this species only inhabits the higher elevation areas, which makes it unlikely that a continuous population ever existed between the Lower Galilee and Mt. Carmel that are separated by a low and wide valley. An isolation by resistance analysis also indicated that low elevations represent a

significant and strong dispersal barrier (Kershenbaum et al. 2014). These results and observations favor a colonization event of Mt. Carmel with few founders rather than Mt. Carmel being a stranded habitat island after regression of a continuously distributed population.

This genetic pattern of low diversity and great homogeneity over all of Mount Carmel cannot be explained by this disjunct population living in an ecologically marginal environment for the species. The environmental conditions on Mount Carmel are more similar to those in the Upper Galilee than the Lower Galilee is to the Upper Galilee. Figure 8 reveals that the ecological suitability of Mount Carmel is high and similar to the Upper Galilee, whereas the Lower Galilee is the most ecologically peripheral area. Despite the harsh ecological conditions in the Lower Galilee, the populations there have much higher genetic diversity than those on Mount Carmel (Table 2, Fig. 9). Thus, Mount Carmel represents an optimal habitat island for these salamanders, and the depurate genetic diversity found on Mount Carmel cannot be explained by harsh ecological conditions. Overall, the Mount Carmel populations indicate the importance of historical factors in geographically disjunct peripheral populations living in an optimal habitat island.

Ecologically marginal conditions tend to enhance population subdivision

The Maxent analysis indicates that the lower Galilee is the most ecologically peripheral area in our study and has the least suitable environment for these salamanders in Northern Israel (Fig. 7). The F_{st} index was higher in the Lower Galilee compared to the other areas (Table 2), thereby indicating greater population subdivision in the Lower Galilee compared to the Upper Galilee and Mount Carmel. This inference is also supported by the NetStruct which indicate much more population subdivision in the Lower Galilee compared to the Upper Galilee and Mount Carmel regions.

Ecologically marginal conditions could result in more population subdivision by creating local barriers to gene flow. The Lower Galilee has less dense maquis and woodland (Table 4) and higher temperatures and less precipitation (Fig. 7) than the other regions. Shaded, vegetated areas that maintain moisture in the soil and air seem to have great importance for dispersal in terrestrial amphibians like salamanders (Hartel et al. 2008; Hocking et al. 2013; Manenti et al. 2009; O'Donnell et al. 2014). Thus, we expect that the fire salamanders can disperse more readily in the higher elevation areas that have lower temperatures, greater precipitation and more vegetative coverage. This interpretation is consistent with the isolation by distance results that indicate no significant isolation in the Upper Galilee even though it is the largest geographical area, whereas there is significant isolation by distance in the smaller Lower Galilee and

Carmel areas (Fig. 6), both of which have less favorable ecological conditions compared to the Upper Galilee (Fig. 8). By all of these environmental criteria, the Lower Galilee (Figs. 1, 7; Table 4) would represent the environment least favorable for dispersal by a terrestrial amphibian.

Additionally, there is a significant linear increase of allelic richness with increasing Maxent scores in the Upper Galilee (Fig. 8), indicating that decreasing temperature and increasing precipitation in a shaded environment may promote increased local dispersal and/or greater population densities even in the region closest to the core. The Lower Galilee has the lowest Maxent scores overall, indicating that the Lower Galilee is approaching an ecological edge for this species (Figs. 7, 8). In the Lower Galilee, there is no relationship between allelic richness and Maxent score (Fig. 9) that may be explained by dispersal in this ecologically marginal environment being so low that extensive population fragmentation has occurred. Such fragmentation can induce extreme local genetic drift that obscures any geographical or ecological signal, as has occurred in peripheral populations of the collared lizard (*Crotaphytus collaris*) in a fragmented peripheral environment (Hutchison and Templeton 1999). The lack of a relationship between allelic richness and Maxent score on Mount Carmel (Fig. 9) is not surprising due to the extreme genetic homogeneity these populations display (Figs. 3, 4 5) and their overall low levels of allelic richness (Fig. 9), which makes it virtually impossible to have any significant correlation using allelic richness as the response variable. The genetic homogeneity among the Mount Carmel populations could arise from increased dispersal due to an overall more favorable environment (Figs. 7, 8) in an area much smaller than the Upper Galilee (Fig. 1b). Support for this explanation stems from mark/recapture studies that document long-distance dispersal on Mount Carmel that indicate potential connectivity between breeding sites (Bar-David et al. 2007). However, the Carmel populations do display significant isolation by distance (Fig. 6) that indicates that dispersal may not be increased in this area that is intermediate environmentally and ecologically between the Upper and Lower Galilees (Figs. 7, 8). An alternative explanation for the genetic homogeneity of the Carmel populations stems from the genetic evidence discussed above that indicates a recent founder event on Mount Carmel. A recent founder event into a new geographical area followed by range expansion promotes genetic uniformity in that new area, as has occurred in other salamanders (Larson 1984; Larson et al. 1984).

The patterns discussed above lead to a general conclusion: *Genetic diversity is influenced by a combination of geographical, historical, and ecological factors.* The genetic and ecological data suggest that our study included different types of peripheral populations: a geographically disjunct peripheral isolate in an ecologically optimal habitat island

(Mount Carmel) that has a strong genetic signature of an historical founder event and extensive genetic homogeneity, an ecologically peripheral population on the edge of the species continuous range in the Lower Galilee displaying much local population subdivision, and a population continuous with the core in the Upper Galilee in an ecologically optimal habitat with no significant subdivision. All of these diverse types of peripheral populations are found close together in a limited area in northern Israel, yet they display different patterns of genetic diversity and subdivision.

The Lower Galilee populations of *S. infraimmaculata* are the ones most likely to be severely affected by the predicted changes in precipitation and temperature (Givati and Rosenfeld 2013; Hartel et al. 2008). The Lower Galilee currently represents an ecologically marginal environment that is also less optimal for dispersal. This combination increases local genetic drift and decrease gene flow, resulting in the observed pattern of increased population subdivision. Lower elevations in the Lower Galilee are the least optimal environments at present, and these lower elevations will likely become even worse for salamanders under climate change. Hence, under climate change, there would be even less dispersal and the inability to reach more optimal environments. However, species can adapt to changing conditions, and the reservoir of high genetic diversity preserved by population subdivision and allele sharing with the core may allow the Lower Galilee populations to successfully adapt to these changing conditions. Indeed, population subdivision increases the variance effective size of the total population and thereby promotes increased genetic diversity in the total population (Chesser et al. 1980, 1993; Wright 1943). Hence, the evolutionary potential of this contiguous peripheral population is high, and this might ameliorate through local adaptation the chances of extinction due to climate change.

In contrast, the Mount Carmel populations may be less affected by climate change, but would probably experience fewer suitable areas and more subdivision as precipitation declines. Given that the Mount Carmel populations seem to be isolated from the core and have a depurate genetic reservoir, they may also be at great risk for extinction under climate change due to a lack of evolutionary flexibility and restricted habitable area.

These diverse genetic, ecological, and historical factors not only highlight the diversity of types of peripheral populations, but they also indicate the complexity of conservation efforts directed at peripheral populations. Such conservation efforts are particularly important for amphibian species in danger of local extinction at their lower-latitude boundaries because of climate change (Givati and Rosenfeld 2013; Griffiths et al. 2010; Mac Nally et al. 2017). Populations on the lower latitude periphery of a species' range often provide an important genetic reservoir for the species as whole, display unique adaptations, and have historically played a

disproportionate role in the species' survival and evolution (Hampe and Petit 2005)—a combination that makes such peripheral populations important in conservation planning. Adding to their importance in conservation is that these low-latitude peripheral populations are the ones most at risk for local extinction under climate change and yet remain understudied (Cahill et al. 2013; Chen et al. 2011). Which ecological/evolutionary/historical forces will be more influential in the future in this complex metapopulation are difficult to predict (Duncan et al. 2015). A more thorough investigations of the genetics, ecology, and history of these peripheral salamander populations in this interesting region is needed in order to make a better assessment of their conservation needs.

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Data Accessibility The full microsatellite genotyping data can be found at: <http://spatialepidemiologylab.weebly.com/data.html>.

References

- Bar-David S, Segev O, Peleg N et al (2007) Long-distance movements by Fire Salamanders (*Salamandra atra*) and implications for habitat fragmentation. *Israel Journal of Ecology and Evolution* 53:143–159
- Blank L, Blaustein L (2012) Using ecological niche modeling to predict the distributions of two endangered amphibian species in aquatic breeding sites. *Hydrobiologia* 693:157–167
- Blank L, Blaustein L (2014) A multi-scale analysis of breeding site characteristics of the endangered fire salamander (*Salamandra atra*) at its extreme southern range limit. *Hydrobiologia* 726:229–244
- Blank L, Sinai I, Bar-David S et al (2013) Genetic population structure of the endangered fire salamander (*Salamandra atra*) at the southernmost extreme of its distribution. *Anim Conserv* 16:412–421
- Blaustein L, Segev O, Rovelli V et al (2017) Compassionate approaches for the conservation and protection of fire salamanders. *Israel Journal of Ecology and Evolution* 63:43–51
- Bogaerts S, Sparreboom M, Pasmans F et al (2013) Distribution, ecology and conservation of *Ommatotriton vittatus* and *Salamandra atra* in Syria. *Salamandra* 49:87–96
- Brussard PF (1984) Geographic patterns and environmental gradients: the central-marginal model in *Drosophila* revisited. *Ann Rev Ecol Syst* 15:25–64
- Cahill AE, Aiello-Lammens ME, Fisher-Reid MC et al (2013) How does climate change cause extinction? *Proc R Soc B-Biol Sci* 280(1750):20121890
- Carson HL (1955) The genetic characteristics of marginal populations of *Drosophila*. *Cold Spring Harb Symp Quant Biol* 20:276–287
- Chen I-C, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. *Science* 333:1024–1026
- Chesser RK, Smith MH, Brisbin IL Jr. (1980) Management and maintenance of genetic variability in endangered species. *Int Zoo Yearb* 20:146–154
- Chesser RK, Rhodes OE, Sugg DW, Schnabel A (1993) Effective sizes for subdivided populations. *Genetics* 135:1221–1232
- Dorchin A, Shanas U (2010) Assessment of pollution in road runoff using a *Bufo viridis* biological assay. *Environ Pollut* 158:3626–3633
- Dubey S, Pike DA, Shine R (2013) Predicting the impacts of climate change on genetic diversity in an endangered lizard species. *Clim Change* 117:319–327
- Duncan SI, Crespi EJ, Mattheus NM, Rissler LJ (2015) History matters more when explaining genetic diversity within the context of the core-periphery hypothesis. *Mol Ecol* 24:4323–4336
- Eckert CG, Samis KE, Loughheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol Ecol* 17:1170–1188
- Elith J, Phillips SJ, Hastie T et al (2011) A statistical explanation of MaxEnt for ecologists. *Divers Distrib* 17:43–57
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* 14:2611–2620
- Fahrig L, Rytwinski T (2009) Effects of roads on animal abundance: an empirical review and synthesis. *Ecol Soc* 14(1):21
- Garriga N, Santos X, Montori A et al (2012) Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodivers Conserv* 21:2761–2774
- Gaston KJ (2003) The structure and dynamics of geographic ranges. Oxford University Press, Oxford
- Givati A, Rosenfeld D (2013) The Arctic Oscillation, climate change and the effects on precipitation in Israel. *Atmos Res* 132:114–124
- Goldberg T, Nevo E, Degani G (2011) Genetic diverseness and differential ecological conditions in *Salamandra atra* larvae from various breeding sites. *Anim Biol* 2:37–49
- Gorodkov KB (1986) Three-dimensional climatic model of potential range and some of its characteristics. II. *Entomol Rev* 65:1–18
- Greenbaum G, Templeton AR, Zarmi Y, Bar-David S (2014) Allelic richness following population founding events—a stochastic modeling framework incorporating gene flow and genetic drift. *PLoS ONE* 9:e115203
- Greenbaum G, Templeton AR, Bar-David S (2016) Inference and analysis of population structure using genetic data and network theory. *Genetics* 202:1299–1312
- Griffiths RA, Sewell D, McCrea RS (2010) Dynamics of a declining amphibian metapopulation: survival, dispersal and the impact of climate. *Biol Conserv* 143:485–491
- Haan SS, Desmond MJ, Gould WR, Ward JP (2007) Influence of habitat characteristics on detected site occupancy of the New Mexico endemic Sacramento Mountains Salamander, *Aneides hardii*. *J Herpetol* 41:1–8
- Hall JK, Schwartz E, Cleave RL (2013) The Israeli DTM (digital terrain map) project. *Microcomput Appl Geol* 2:111–118
- Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecol Lett* 8:461–467
- Hardy R (1945) The influence of types of soil upon the local distribution of some mammals in southwestern Utah. *Ecol Monogr* 15:71–108
- Harless ML, Huckins CJ, Grant JB, Pypker TG (2011) Effects of six chemical deicers on larval wood frogs (*Rana sylvatica*). *Environ Toxicol Chem* 30:1637–1641

- Hartel T, Nemes S, Demeter L, Ollerer K (2008) Pond and landscape characteristics—which is more important for common toads (*Bufo bufo*)? A case study from central Romania. *Appl Herpetol* 5:1–12
- Hendrix R, Hauswaldt JS, Veith M, Steinfartz S (2010) Strong correlation between cross-amplification success and genetic distance across all members of ‘True Salamanders’ (Amphibia: Salamandridae) revealed by *Salamandra salamandra*-specific microsatellite loci. *Mol Ecol Resour* 10:1038–1047
- Hernandez PA, Franke I, Herzog SK et al (2008) Predicting species distributions in poorly-studied landscapes. *Biodivers Conserv* 17:1353–1366
- Hocking DJ, Connette GM, Conner CA et al (2013) Effects of experimental forest management on a terrestrial, woodland salamander in Missouri. *For Ecol Manag* 287:32–39
- Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives. *Trends In Evol Ecol* 9:223–237
- Holt RD, Keitt TH (2005) Species’ borders: a unifying theme in ecology. *Oikos* 108:3–6
- Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* 53:1898–1914
- Jombart T (2008) Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24:1403–1405
- Kalinowski ST (2011) The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. *Heredity* 106:625–632
- Kawecki TJ (2008) Adaptation to marginal habitats. *Annu Rev Ecol Evol Syst* 39:321–342
- Kershenbaum A, Blank L, Sinai I et al (2014) Landscape influences on dispersal behaviour: a theoretical model and empirical test using the fire salamander, *Salamandra atra*. *Oecologia* 175:509–520
- Larson A (1984) Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. *Evol Biol* 17:119–217
- Larson A, Wake DB, Yanev KP (1984) Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics* 106:293–308
- Leshner A, Ramon O (2013) The Israeli mapping guide for Mediterranean vegetation. Ministry of the Environment, Kharkov
- Levin N, Harari-Kremer R, Carmel Y (2011) Remote sensing of Israel’s Natural Habitats. Unpublished Report for the Israeli Nature and Parks Authority, Jerusalem
- Mac Nally R, Horrocks GFB, Lada H (2017) Anuran responses to pressures from high-amplitude drought-flood-drought sequences under climate change. *Clim Change* 141:243–257
- Manenti R, Ficetola GF, De Bernardi F (2009) Water, stream morphology and landscape: complex habitat determinants for the fire salamander *Salamandra salamandra*. *Amphibia-Reptilia* 30:7–15
- Navarro-Cerrillo RM, Hernandez-Bermejo JE, Hernandez-Clemente R (2011) Evaluating models to assess the distribution of *Buxus balearica* in southern Spain. *Appl Veg Sci* 14:256–267
- Nevo E (1998) Genetic diversity in wild cereals—regional and local studies and their bearing on conservation ex situ and in situ. *Genet Resour Crop Evol* 45:355–370
- O’Donnell KM, Thompson FR, Semlitsch RD (2014) Predicting variation in microhabitat utilization of terrestrial salamanders. *Herpetologica* 70:259–265
- Peleg N (2009) Studies on the conservation of the fire salamander (*Salamandra atra*) in Israel. University of Haifa, Haifa
- Perez MF, Franco FF, Bombonato JR et al (2018) Assessing population structure in the face of isolation by distance: are we neglecting the problem? *Divers Distrib* 24:1883–1889
- Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecol Model* 190:231–259
- Pironon S, Papuga G, Vilellas J et al (2017) Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. *Biol Rev* 92:1877–1909
- Pisa G, Orioli V, Spilotros G et al (2015) Detecting a hierarchical genetic population structure: the case study of the Fire Salamander (*Salamandra salamandra*) in Northern Italy. *Ecol Evol* 5:743–758
- Ray N, Lehmann A, Joly P (2002) Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. *Biodivers Conserv* 11:2143–2165
- Rosenberg MS, Anderson CD (2011) PASSAGE: pattern analysis, spatial statistics and geographic exegesis. Version 2. *Met Ecol Evol* 2:229–232
- Rousset F (2008) genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Res* 8:103–106
- Roy DP, Wulder MA, Loveland TR et al (2014) Landsat-8: science and product vision for terrestrial global change research. *Remote Sens Environ* 145:154–172
- Schneider S, Roessli D, Excoffier L (2000) Arlequin ver. 2.000. A software for population genetics data analysis Genetics and Biometry Laboratory, University of Geneva, Switzerland
- Segev O, Polevikova A, Blank L et al (2015) Effects of tail clipping on larval performance and tail regeneration rates in the Near Eastern Fire Salamander, *Salamandra atra*. *PloS one* 10:e0128077
- Segev O, Hill N, Templeton AR, Blaustein L (2010) Population size, structure and phenology of an endangered salamander at temporary and permanent breeding sites. *J Nat Conserv* 18:189–195
- Semlitsch RD, Anderson TL (2016) Structure and dynamics of Spotted Salamander (*Ambystoma maculatum*) populations in Missouri. *Herpetologica* 72:81–89
- Sepulveda AJ, Lowe WH (2009) Local and landscape-scale influences on the occurrence and density of *Dicamptodon aterrimus*, the Idaho Giant Salamander. *J Herpetol* 43:469–484
- Steinfartz S, Veith M, Tautz D (2000) Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of *Salamandra salamandra*. *Mol Ecol* 9:397–410
- Steinfartz S, Kusters D, Tautz D (2004) Isolation and characterization of polymorphic tetranucleotide microsatellite loci in the fire salamander *Salamandra salamandra* (Amphibia: Caudata). *Mol Ecol Notes* 4:626–628
- Swets JA (1988) Measuring the accuracy of diagnostic systems. *Science* 240:1285–1293
- Templeton AR, Routman E, Phillips C (1995) Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* 140:767–782
- Tucker CJ (1979) Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sens Environ* 8:127–150
- Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across the species’ range: null hypotheses for landscape conservation genetics. *Conserv Genet* 4:639–645
- Warburg MR (2011) Changes in recapture rate of a rare salamander in an isolated metapopulation studied for 25 years. *Rus J Herpetol* 15:11–18
- Wright S (1943) Isolation by distance. *Genetics* 28:114–138