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

Running Title: Core-Peripheral Populations of Fire Salamanders

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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

47 INTRODUCTION

48 The contrast between core (central) populations of a species versus peripheral
49 (marginal) populations has attracted the attention of evolutionary biologists ever since Darwin,
50 but particularly since the 1950's (Pironon *et al.* 2017). The most straightforward manner of
51 classifying core and peripheral populations is geographically. In the classification given in
52 Gaston (2003), following Gorodkov (1986)(Gorodkov 1986a, b), the geography of permanent
53 populations of a species fall into four categories: 1) a zone of continuous distribution, but with
54 the possibility of lacuna (areas where the species is absent but surrounded by an otherwise
55 continuous distribution), 2) the limit of the zone of continuous distribution (an edge or
56 periphery), 3) a zone of disjunct distribution in which populations can be found that are
57 geographically separated from each other and from the continuous distribution area, and 4) the
58 limit of the zone of disjunct distribution. Not all species display all four types of these
59 geographical range features, but one that does is the fire salamander, *Salamandra*
60 *infraimmaculata* (Figure 1). The zone of continuous distribution is found in the higher
61 elevations along the eastern Mediterranean region (Figure 1a), with the southern part of the
62 continuous distribution extending into the Galilee region of Northern Israel (Figure 1b) (Bogaerts
63 *et al.* 2013; Steinfartz *et al.* 2000). The Galilee is subdivided geologically into the Lower and
64 Upper Galilee. The Upper Galilee is located at a higher elevation than the Lower Galilee and has
65 a more mesic and cooler climate – and thereby also denser vegetation cover - than the Lower
66 Galilee. The limit of continuous distribution is the edge of the lower Galilee (Figure 1b). There
67 is then a zone of disjunct distribution, with many populations found on Mount Carmel that is
68 geographically separated from the Galilee by a low-elevation valley (Figure 1b). The Mount

69 Carmel populations represent the southernmost limit for this species, and indeed the entire genus
70 *Salamandra*, so Mount Carmel also represents the limit of disjunct distribution (Blank et al.
71 2013).
72 A geographical classification of a species' range is of heuristic value, but it is more
73 useful, particularly for conservation planning of endangered species such as *S. infraimmaculata*,
74 to determine what limits the geographic range and positions of the borders (Gaston 2003). First,
75 there could be abiotic and/or biotic factors that prevent further spread, such as physical barriers
76 (e.g., seas, rivers, mountains, and valleys), climatic factors, absence of essential resources, and
77 the impact of other species. Another complication that has become increasingly important in this
78 era of climate change is the low-latitude edges of a species range that may becoming less
79 optimal. Hampe and Petit (2005) reviewed studies from the fossil record, phylogeography and
80 ecology, and concluded that these low-latitude peripheral populations are disproportionately
81 important for the survival and evolution of biota, yet these are the very populations that remain
82 understudied despite having the highest chances for local extinction under climate change (Cahill
83 *et al.* 2013; Chen *et al.* 2011). Second, there can be historical factors (Duncan *et al.* 2015). For
84 example, suppose past climatic conditions changed, resulting in a contraction of the species
85 range but leaving isolated populations in favorable habitat islands in the previous range to create
86 a zone of disjunct distribution. On the other hand, suppose a zone of disjunct distribution is
87 created by past colonization events of habitat islands through founders derived from the zone of
88 continuous distribution. Many of these historical events leave genetic signatures such that
89 inferences about the past can be made from current genetic surveys, as has been shown in other
90 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 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: Brussard 1984; Eckert *et al.* 2008; Hoffmann & Blows 1994; Kawecki 2008; Nevo 1998; Pironon *et al.* 2017; Vucetich & 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 & 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 enable 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 692 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 8 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

156 (Qiagen). The annealing temperatures for each primer pair were optimized using gradient PCR.
157 PCR products were visualized with a MegaBACE 1000 automated sequencer (Amersham
158 Biosciences) and the microsatellite allele sizes were determined with the ET-ROX 400 size
159 standard (Amersham Biosciences). Alleles were scored using visual inspection and manual
160 corrections of alleles with MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004).
161 Microsatellite genotypes were checked for the presence of null alleles, stutter products, or allelic
162 dropout using MICRO-CHECKER. Linkage disequilibrium and deviations from Hardy-
163 Weinberg equilibrium were investigated using GENEPOP on the web (Rousset 2008).
164
165 Data analyses
166 Quantifying genetic diversity
167 To interpret patterns in genetic diversity between regions, we calculated the average
168 values of allelic richness, number of unique alleles, and observed and expected heterozygosity
169 for each of the regions. We used a randomization test to evaluate the differences in observed and
170 expected heterozygosity, inbreeding index within local populations (F_{is}), and a measure of
171 between population differentiation (F_{st}) between each pair of regions (999 permutations,
172 implemented in FSTAT).
173
174 Analysis of population structure
175 We used the program STRUCTURE to cluster the individuals into a finite number of
176 populations based solely on genetic data. STRUCTURE requires the number of populations to
177 be specified *a priori*, and we used the delta *K* method of Evanno *et al.* (2005), a widely used

178 method for determining *K*, the number of populations.

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

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

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

200 can be tested for significance using permutation tests.

201 The significant clusters found by NetStruct reflect only genetic similarity among
202 individuals and are not necessarily geographic regions, particularly when gene flow and
203 admixture occur. Accordingly, more than one genetic cluster may be found at a single
204 geographic site, and a single genetic cluster may be found at multiple geographic sites. When
205 this occurs, we test the null hypothesis that the NetStruct clusters are homogeneously distributed
206 geographically by constructing a G by C table, where G is the number of geographic sites, C is
207 the number of genetic clusters, and the elements are the number of individuals at geographic site
208 *g* that are also members of genetic cluster. We then test the null hypothesis of geographic
209 homogeneity in this G by C table by an exact permutation test with 10,000 random permutations
210 to determine the p-value under the null hypothesis as well as a 99% confidence interval for the p-
211 value with the program StatExact (Cytel Studio, Cambridge, MA, v 9.0). A rejection of the null
212 hypothesis indicates that assignment of individuals to clusters in the region is biased, and gene
213 flow within the region is not panmictic.

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

219

220 <u>Characterization of geographic and environmental variation</u>

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 (Figure 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 & 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 & Blaustein 2012; Blank & Blaustein 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

243 data instead of presence-absence data (Elith *et al.* 2011; Hernandez *et al.* 2008; Navarro-Cerrillo
244 *et al.* 2011). Maxent predicts the probability distribution across all the cells in the study area. We
245 implemented Maxent using version 3.3.3e of the software developed by Phillips *et al.* (2006).
246 Recommended default values were used for the convergence threshold (10^5) and maximum
247 number of iterations (500). Model performance was evaluated using "Area under the curve"
248 (AUC with a range from 0.0 to 1.0; Swets 1988).
249 We considered 10 environmental variables as potential predictor variables of *S.*
250 *infraimmaculata* distribution in the Maxent analysis: Elevation (meters asl), Northness (degrees),
251 Eastness (degrees), Slope (degrees), Soil type (categorical), Land cover including vegetation type
252 (categorical), Precipitation (mm), Distance to nearest road (meters), Distance to nearest built area
253 (meters), and mean daily temperature in January (°C) (the mid-point of the active breeding
254 season). Previous studies on salamander distributions have indicated the importance of elevation
255 and slope (Blank & Blaustein 2012; Blank & Blaustein 2014; Blank *et al.* 2013; Bogaerts *et al.*
256 2013; Kershenbaum *et al.* 2014), precipitation (Haan *et al.* 2007; Semlitsch & Anderson 2016),
257 temperature (Goldberg *et al.* 2011; Peleg 2009), and land-cover (Hocking *et al.* 2013; Manenti *et*
258 *al.* 2009; O'Donnell *et al.* 2014; Pisa *et al.* 2015; Sepulveda & Lowe 2009). Aspect (Northness
259 and Eastness) is expected to affect the overall radiation reaching the ground. Solar radiation is a
260 direct ecological factor affecting habitat conditions, such as water temperature and soil and
261 hydroperiod of the ponds. Soil was previously found to be an important environmental variable
262 explaining the distribution of *S. infraimmaculata* (Blank & Blaustein 2012). Quickly drained
263 soils limit the time length that water is available for breeding (Hardy 1945). Roads could affect
264 amphibians for three main reasons. First, roads pose mortality risk for individuals crossing the

roads (Fahrig & 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 & 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 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 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 1,000 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

309 allele sharing similarity measures within each cluster. No additional significant clusters were
310 found within Mount Carmel for any edge-pruning threshold (Fig. 5b and c), indicating a high
311 degree of genetic homogeneity among individuals within this geographic region. However, in
312 the Galilee, at an edge-pruning threshold of 0.12, three significant genetic clusters emerged, as
313 indicated by the three colors in Fig. 5b. All three genetic clusters were found both in the Upper
314 and Lower Galilee, and Table 3 presents the results of testing the null hypothesis of geographic
315 homogeneity in the distribution of these clusters. The null hypothesis of geographic homogeneity
316 was strongly rejected for the Galilee as a whole, and equally strongly for just the Lower Galilee
317 sites (Table 3). However, note that in the Upper Galilee, the null hypothesis of geographic
318 homogeneity is not rejected (Table 3). Many individuals from the Lower Galilee site of Zalmon,
319 clustered with individuals from the Upper Galilee sites near tributaries of an Upper Galilee
320 stream that descends to the valley between the Upper and Lower Galilee close to Zalmon. Thus,
321 we also tested the null hypothesis that Zalmon plus the Upper Galilee sites are homogeneous and
322 found that the hypothesis of geographic homogeneity among these sites was not rejected (Table
323 3).

324 The next significant change in NetStruct clustering occurs at edge-pruning threshold of
325 0.22, with the Galilee populations now consisting of five significant clusters (Fig. 5c). Table 3
326 shows that the null hypothesis of geographic homogeneity is still strongly rejected both for the
327 Galilee as a whole, as well as for the Lower Galilee. However, the null hypothesis of geographic
328 homogeneity is now strongly rejected for the Upper Galilee sites as well (Table 3). As can be
329 seen from Table 3, the null hypothesis of geographic homogeneity is accepted for Zalmon and
330 these four Upper Galilee sites. This pattern of geographic homogeneity indicates that this stream

331 from the Upper Galilee is likely a dispersal corridor that genetically connects the Lower Galilee
332 to the Upper Galilee.

333 Because the results given above indicate restricted gene flow among the three geographic
334 regions in our study, we tested for isolation by distance separately using Mantel test within each
335 of these three regions. The pairwise standardized F_{st} among subpopulations correlated positively
336 with Euclidian distance within the Lower Galilee ($r = 0.42$, $p < 0.05$) and Mt. Carmel regions (r
337 $= 0.43$, $p < 0.05$), but there was no significant correlation in the Upper Galilee ($r = 0.16$, $p =$
338 0.29), as shown in Figure 6 (see Appendix for full pairwise tables). The Mantel test for all the
339 populations together resulted with significant correlation ($r = 0.72$, $p < 0.05$).

340

341 Environmental variation

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

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

351

352 Habitat suitability

353 The results of the Maxent modelling are shown in Figure 8. The AUC for the replicate
354 runs was 0.857, indicating a high level of accuracy for the Maxent predictions. Generally, most
355 of the Lower Galilee is represented with low suitability values (<0.4), while the Upper Galilee
356 and Mount Carmel regions were more suitable (Fig. 8). Four variables collectively contributed
357 86% to this optimal Maxent model: soil (36.1%), precipitation (24.1%), temperature (14.3%)
358 and altitude (11.7%). As can be seen from Figure 7, the last three of these variables differ
359 considerably in the three geographic areas that are in our survey.
360 There was a significant linear increase of allelic richness with increasing Maxent
361 suitability scores in the Upper Galilee, but not in Lower Galilee or Mount Carmel (Fig. 9). On
362 Mount Carmel, the Maxent scores were generally higher than those in the Lower Galilee, but the
363 allelic richness was consistently lower in Mount Carmel as compared to the Lower Galilee (Fig.
364 9).
365
366 DISCUSSION
367 We set out to test three hypotheses: 1) that genetic diversity will decrease towards the
368 geographical periphery of a species' range; 2) that genetic diversity in geographically disjunct
369 peripheral areas (Mount Carmel) will be low compared to the core (Upper Galilee) or peripheral
370 populations that are contiguous to the core (Lower Galilee); and 3) that ecologically marginal
371 conditions tend to enhance population subdivision. The results gave support for all these
372 hypotheses.
373
374 **Genetic diversity will decrease towards the geographical periphery of a species' range**

375 Going from the Upper Galilee to the Lower Galilee defines an increasingly peripheral
376 geographical gradient and a decreasing latitude gradient. Our results clearly show that this
377 gradient is associated with declining genetic diversity as measured by allelic richness, observed
378 and expected heterozygosity, and number of unique alleles (Table 2, Fig. 2). Allelic richness and
379 the number of unique alleles are particularly sensitive indicators of how well the balance of gene
380 flow versus local genetic drift can maintain genetic diversity in a species' gene pool (Greenbaum
381 *et al.* 2014). Allelic richness showed a significant decline across this entire gradient and also
382 across the latitudinal gradient confined just to the contiguous core-periphery in the Galilee (Fig.
383 2). The number of unique alleles shows an even more dramatic pattern, with 18 alleles unique to
384 the Upper Galilee, and only one in the Lower Galilee. The low frequency of unique alleles in the
385 Lower Galilee population indicates a significant decrease in gene flow, an increase in local
386 genetic drift in traversing this core-peripheral gradient, both the entire gradient and just the
387 contiguous portion in the Galilee (Fig. 2), and/or historical founder or bottleneck effects during
388 colonizations of peripheral areas. Overall, this pattern supports the hypothesis of decreased
389 genetic diversity at the periphery.
390
391 **Genetic diversity in geographically disjunct peripheral areas will be low compared to the**
392 **core or peripheral populations that are contiguous to the core**
393 Figure 2 suggest that the low measures of genetic diversity found in Mount Carmel are
394 not simply an extrapolation of the trends seen in the contiguous Galilee regions, but rather
395 represent a more extreme drop in genetic diversity. The STRUCTURE, PCA and NetStruct
396 analyses also indicated that the Mount Carmel populations are genetically homogeneous and

397 highly differentiated from the Galilean populations. Allelic diversity was consistently lower in
398 Mount Carmel than in the Galilee, and no unique alleles were found in Mount Carmel. All these
399 patterns are consistent with a recent colonization event associated with a strong founder effect
400 (Blank *et al.* 2013). Another possibility is that the continuous range of the species has been
401 regressing towards the north, stranding the Mt. Carmel populations on a habitat island.
402 Stranding alone would not explain the extreme drop in genetic diversity observed in the Mt.
403 Carmel populations unless coupled with extremely small population size that persisted for many
404 generations on Mt. Carmel. We do not have estimates for the total population size on Mt.
405 Carmel, but it is possible to collect several hundreds of individuals in just a small portion of Mt.
406 Carmel (Bar-David *et al.* 2007). Moreover, our MaxEnt analysis indicates that Mt. Carmel
407 represents an optimal habitat. These observations suggest that small population size for many
408 generations on Mt. Carmel is unlikely. The MaxEnt analysis also demonstrates that this species
409 only inhabits the higher elevation areas, which makes it unlikely that a continuous population
410 ever existed between the Lower Galilee and Mt. Carmel that are separated by a low and wide
411 valley. An isolation by resistance analysis also indicated that low elevations represent a
412 significant and strong dispersal barrier (Kershenbaum *et al.* 2014). These results and
413 observations favor a colonization event of Mt. Carmel with few founders rather than Mt. Carmel
414 being a stranded habitat island after regression of a continuously distributed population.
415 This genetic pattern of low diversity and great homogeneity over all of Mount Carmel
416 cannot be explained by this disjunct population living in an ecologically marginal environment
417 for the species. The environmental conditions on Mount Carmel are more similar to those in the
418 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.*

441 2009; O'Donnell *et al.* 2014). Thus, we expect that the fire salamanders can disperse more
442 readily in the higher elevation areas that have lower temperatures, greater precipitation and more
443 vegetative coverage. This interpretation is consistent with the isolation by distance results that
444 indicate no significant isolation in the Upper Galilee even though it is the largest geographical
445 area, whereas there is significant isolation by distance in the smaller Lower Galilee and Carmel
446 areas (Fig. 6), both of which have less favorable ecological conditions compared to the Upper
447 Galilee (Fig. 8). By all of these environmental criteria, the Lower Galilee (Figs 1 and 7; Table 4)
448 would represent the environment least favorable for dispersal by a terrestrial amphibian.
449 Additionally, there is a significant linear increase of allelic richness with increasing
450 Maxent scores in the Upper Galilee (Fig. 8), indicating that decreasing temperature and
451 increasing precipitation in a shaded environment may promote increased local dispersal and/or
452 greater population densities even in the region closest to the core. The Lower Galilee has the
453 lowest Maxent scores overall, indicating that the Lower Galilee is approaching an ecological
454 edge for this species (Figs 7 and 8). In the Lower Galilee, there is no relationship between allelic
455 richness and Maxent score (Fig. 9) that may be explained by dispersal in this ecologically
456 marginal environment being so low that extensive population fragmentation has occurred. Such
457 fragmentation can induce extreme local genetic drift that obscures any geographical or ecological
458 signal, as has occurred in peripheral populations of the collared lizard (*Crotophytus collaris*) in a
459 fragemented peripheral environment (Hutchison & Templeton 1999). The lack of a relationship
460 between allelic richness and Maxent score on Mount Carmel (Fig. 9) is not surprising due to the
461 extreme genetic homogeneity these populations display (Figs 3-5) and their overall low levels of
462 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 and 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 and 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

484 limited area in northern Israel, yet they display different patterns of genetic diversity and
485 subdivision.

486 The Lower Galilee populations of *S. infraimmaculata* are the ones most likely to be
487 severely affected by the predicted changes in precipitation and temperature (Givati & Rosenfeld
488 2013; Hartel *et al.* 2008). The Lower Galilee currently represents an ecologically marginal
489 environment that is also less optimal for dispersal. This combination increases local genetic drift
490 and decrease gene flow, resulting in the observed pattern of increased population subdivision.
491 Lower elevations in the Lower Galilee are the least optimal environments at present, and these
492 lower elevations will likely become even worse for salamanders under climate change. Hence,
493 under climate change, there would be even less dispersal and the inability to reach more optimal
494 environments. However, species can adapt to changing conditions, and the reservoir of high
495 genetic diversity preserved by population subdivision and allele sharing with the core may allow
496 the Lower Galilee populations to successfully adapt to these changing conditions. Indeed,
497 population subdivision increases the variance effective size of the total population and thereby
498 promotes increased genetic diversity in the total population (Chesser *et al.* 1993; Chesser *et al.*
499 1980; Wright 1943). Hence, the evolutionary potential of this contiguous peripheral population
500 is high, and this might ameliorate through local adaptation the chances of extinction due to
501 climate change.

502 In contrast, the Mount Carmel populations may be less affected by climate change, but
503 would probably experience fewer suitable areas and more subdivision as precipitation declines.
504 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 & 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 & 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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696

697 DATA ACCESSIBILITY

698 - Microsatellite genotypes: Upon acceptance we will archive the microsatellite genotypes in

699 Dryad and accession number will be added to the paper

700

701 TABLES

702 Table 1. The 32 study sites and basic information on the sample sizes (N) and genetic variability

703 in 15 microsatellite loci at each site; A = allelic richness; H_O = observed heterozygosity; H_E =

704 expected heterozygosity. Samples were taken from adults only except for two sites noted below,

705 ● = road kills, •• = Larvae only, ••• = Larvae and adults.

706

Region and Site	Longitude	Latitude	N	A	H_0	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 (10 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 (9 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

707

708

709 Table 2. Summary of genetic diversity from the major sampling regions. Significance is based
710 on permutation tests (999 permutations). Different superscripted letters signify statistically
711 significant differences.
712

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}

713 a and b represent significant differences between regions; p value<0.05. Areas sharing a
714 superscript are not significantly different from each other.
715

716 Table 3. Results of exact permutation tests of the null hypothesis of geographic homogeneity for
717 several regions with respect to the geographic distribution of the genetic clusters found by
718 NetStruct in the Galilee.
719

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

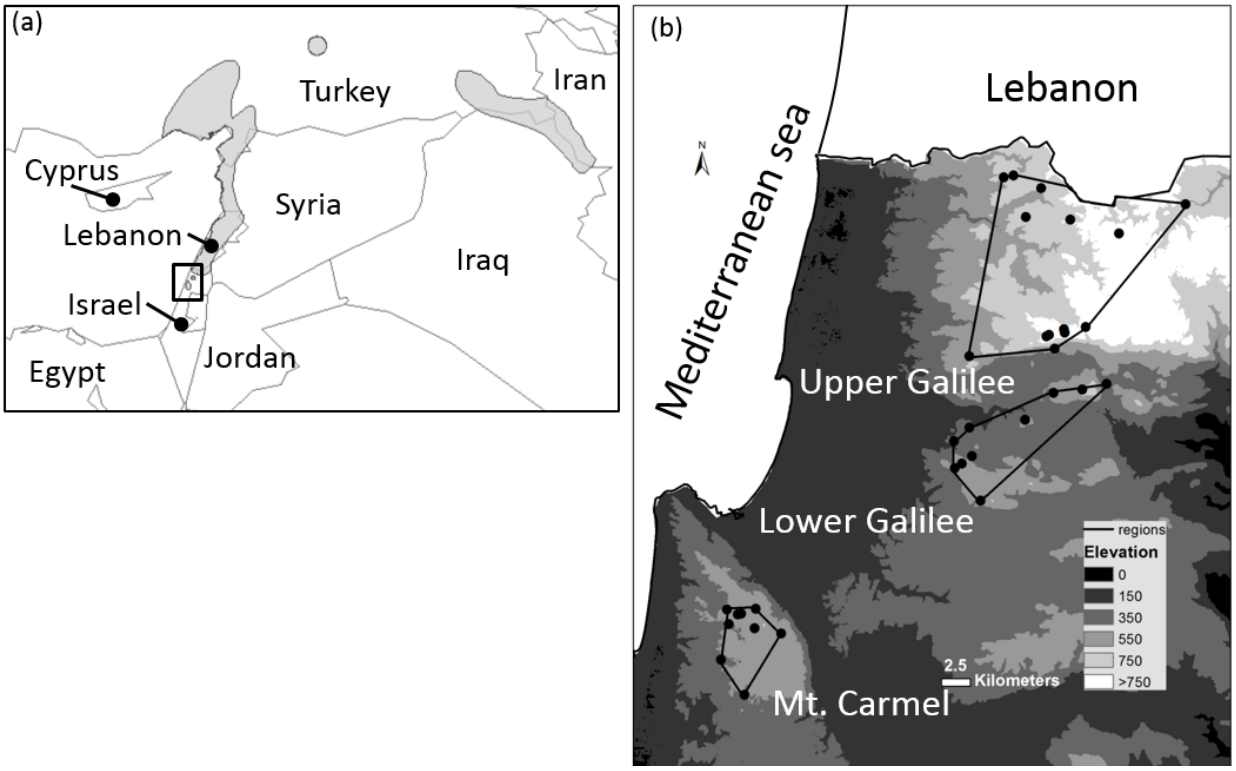
720

721 Table 4. The percentages of the vegetation types found in the Upper Galilee, Mount Carmel,
722 and Lower Galilee. Quantified from the vegetation cover map described in the Methods section.
723

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

724
725

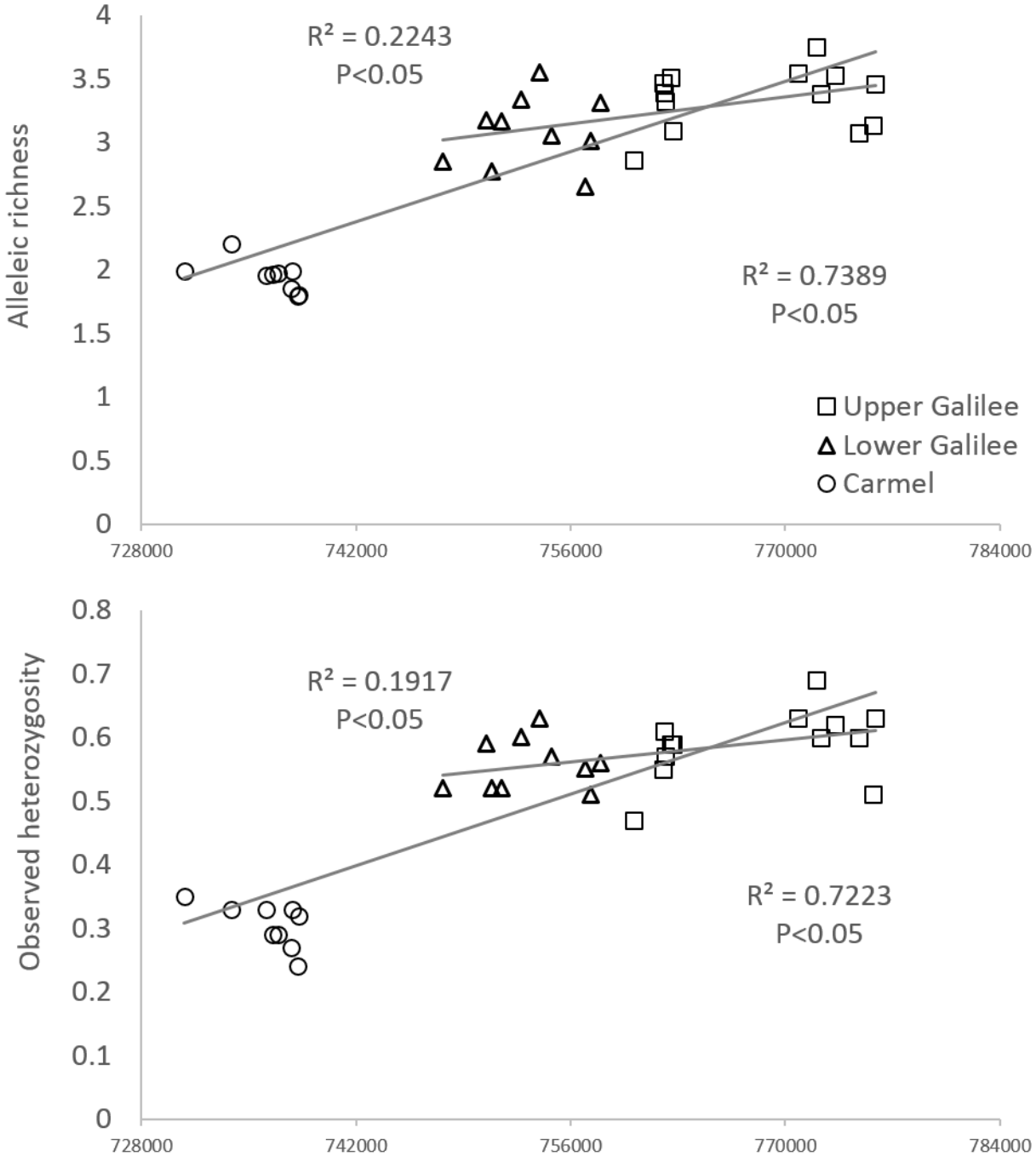
726 FIGURES



727

728 Figure 1. (a) *Salamandra infraimmaculata* distribution range according to the IUCN (IUCN
729 2018). Black frame denotes the study area. (b) The three studied regions: Mount Carmel, the
730 Lower Galilee, and the Upper Galilee Black points represent the 32 breeding sites that were
731 sampled (see Table 1 for their names and coordinates).

732



733

734 Figure 2. A regression analyses of Allelic richness and observed heterozygosity as a function of

735 latitude (°N) in different sampling regions: Mount Carmel sites (circles), Lower Galilee sites

736 (triangles), Upper Galilee sites (squares).

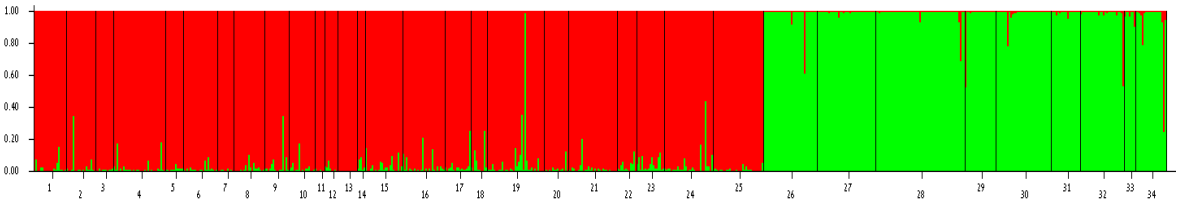
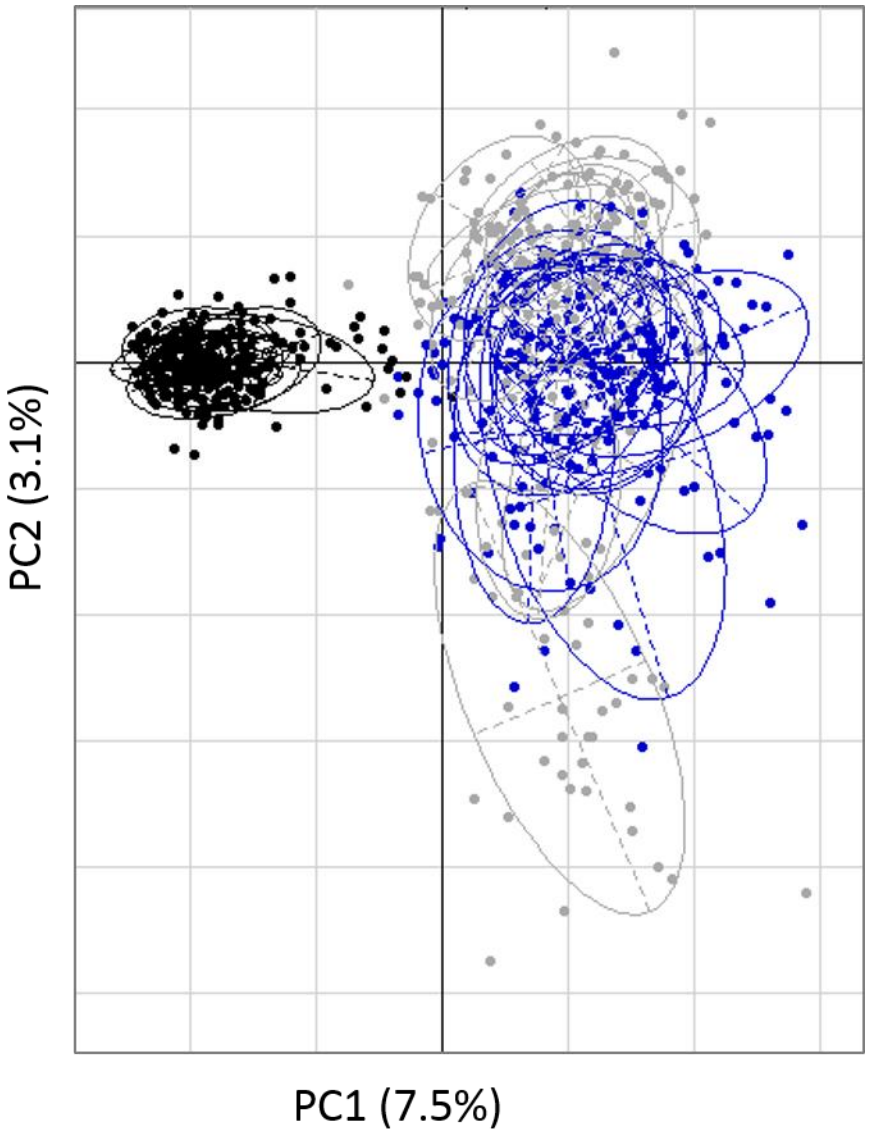


Figure 3. Genetic clustering in the study area obtained with STRUCTURE with *K* =2, the optimal *K* under the delta *K* method. Identical colors identify populations with a homogeneous genetic composition, while different colors represent genetically differentiated populations. The red color is associated with individuals sampled from the Galilee, and green from Mount Carmel.



757

758 Figure 4. Results of the PCA analysis. First and second axes are presented. The dots shows

759 individual salamanders. Ovals represent 95% inertia ellipses. Blue- Upper Galilee; Gray- Lower

760 Galilee; Black- Mount Carmel

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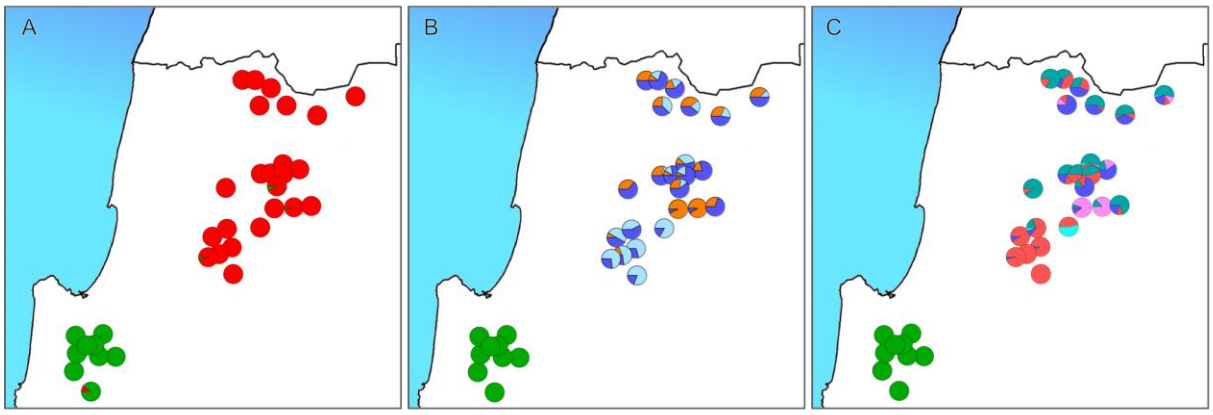
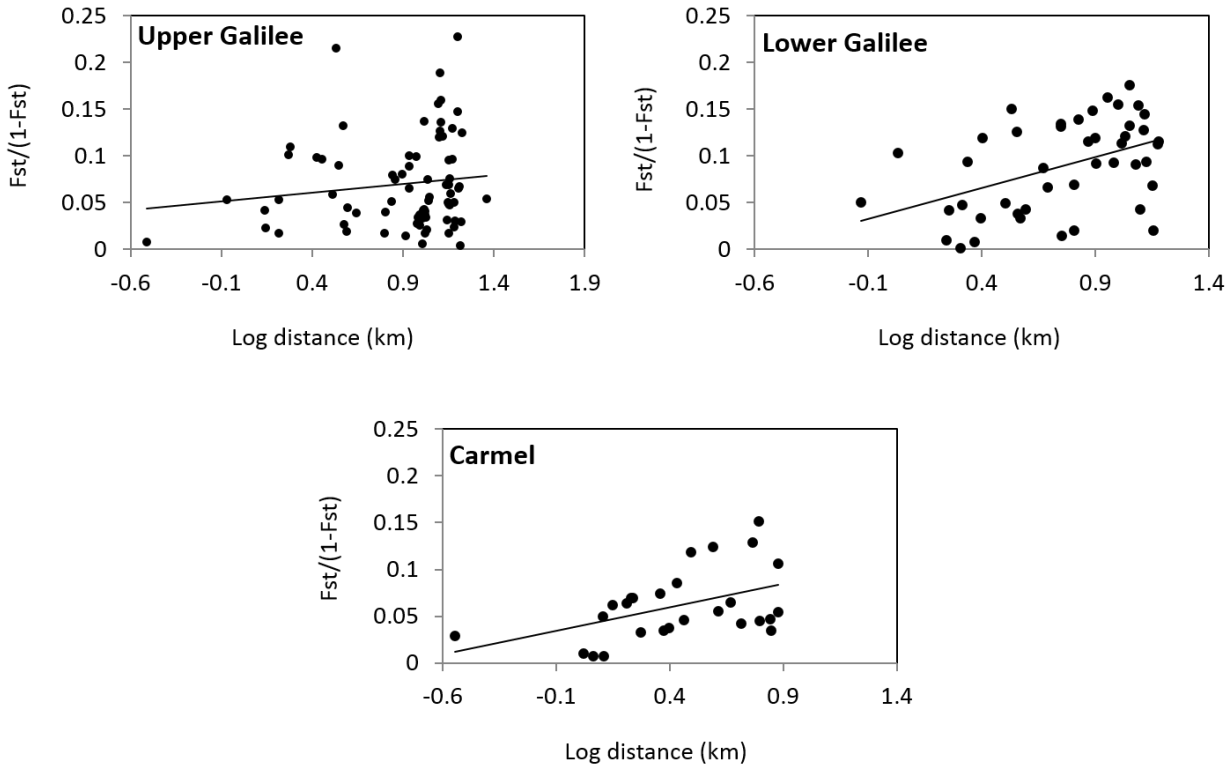


Figure 5. Genetic clustering in the study at three hierarchical levels obtained with NetStruct. Different colors represent different genetic clusters. At each sampling site, the distribution of assignments of individuals to clusters is shown. (A) The highest hierarchical level, obtained by analyzing 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 discernable 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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778

779 Figure 6. Isolation by distance within the three major geographic regions. The Mantel test was

780 not significant for the Upper Galilee (top panel), but was significant for the Lower Galilee

781 (middle panel) and Carmel (lower panel).

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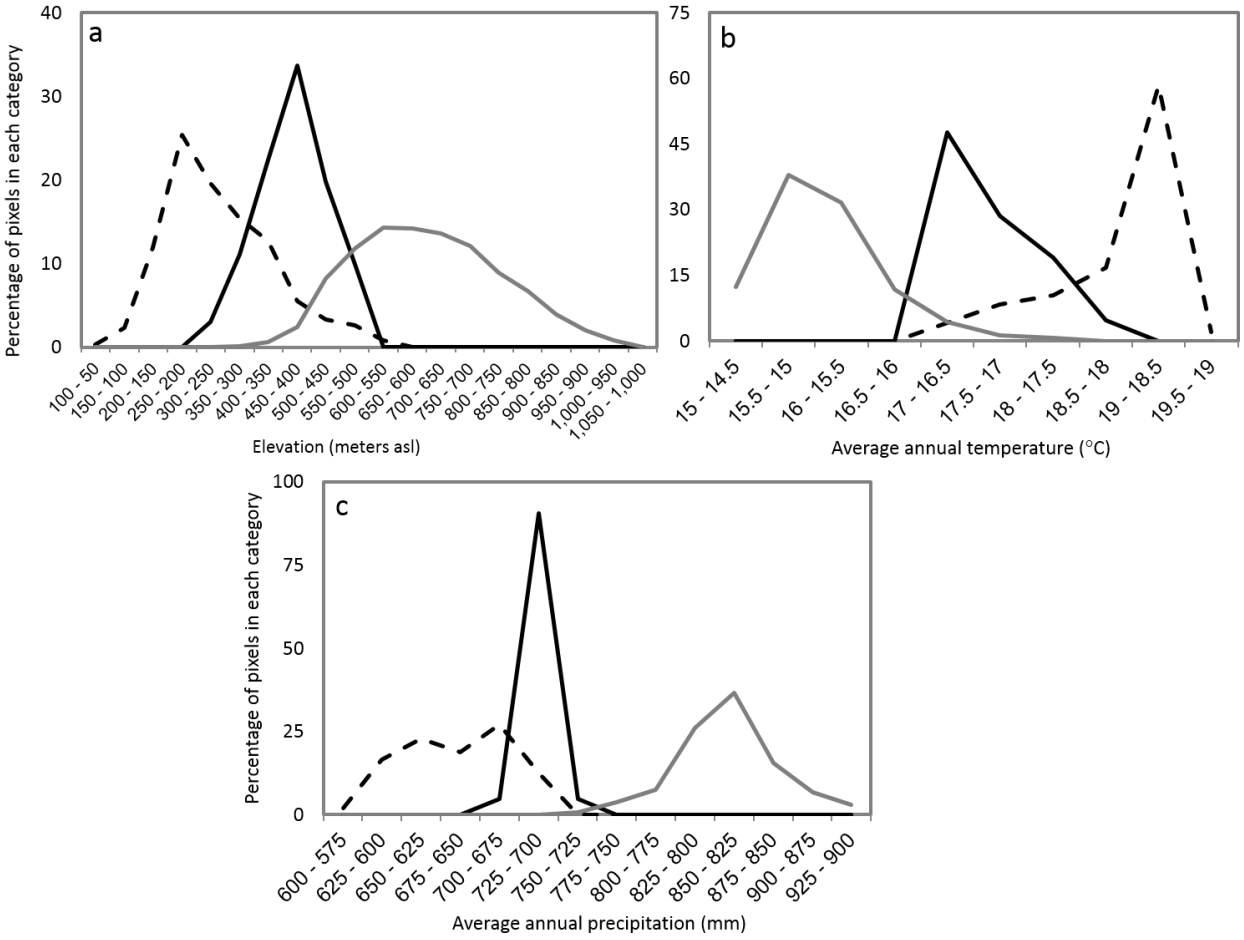

784

785 Figure 7. The distributions of (a) elevation, (b) annual average temperature and average annual
786 precipitation (c) in the three regions. The black lines indicate the distributions on Mount
787 Carmel, the dashed lines the distributions in the Lower Galilee, and the gray lines in the Upper
788 Galilee.

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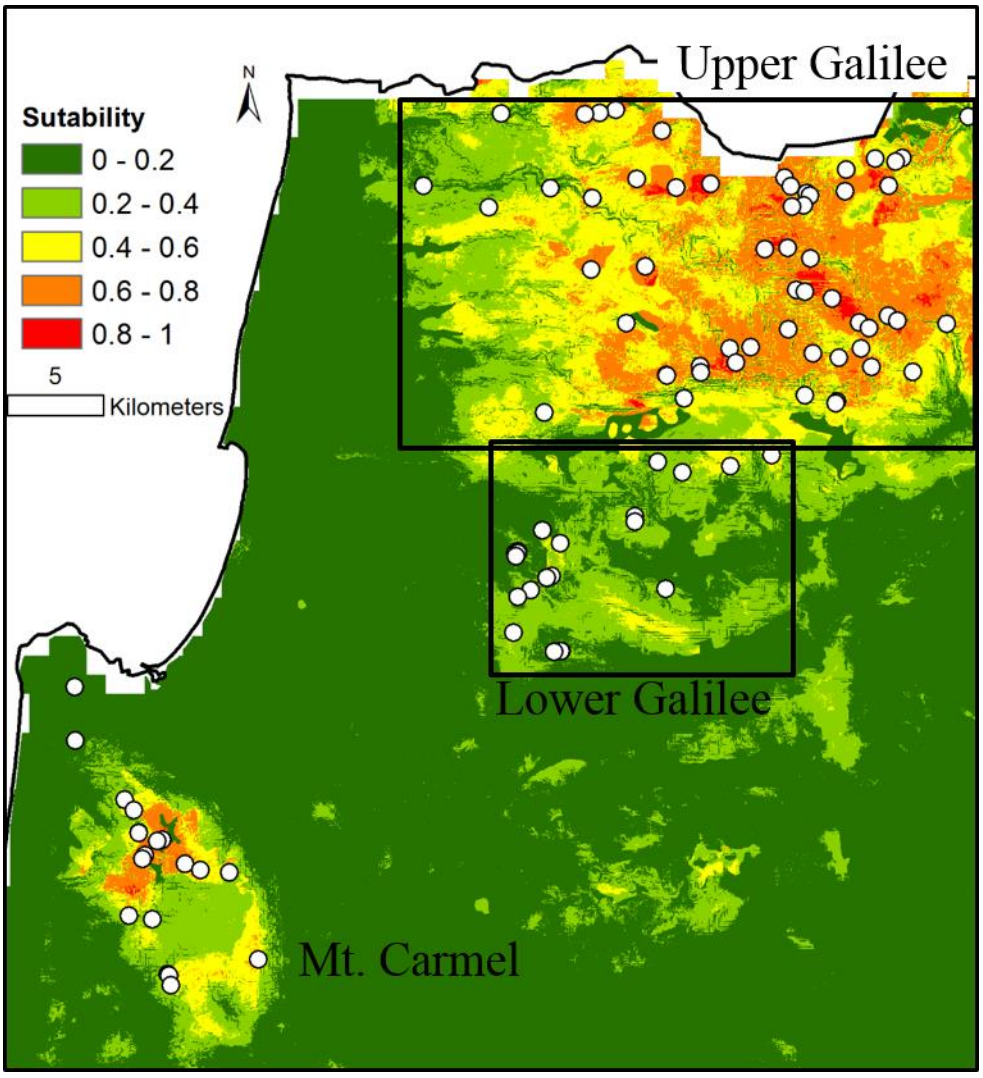

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791 Figure 8. Maxent habitat suitability scores over the three major regions sampled. Mount Carmel
792 is shown in the lower left-hand corner, the Upper Galilee in the upper right-hand corner, and the
793 Lower Galilee just south of the Upper Galilee.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

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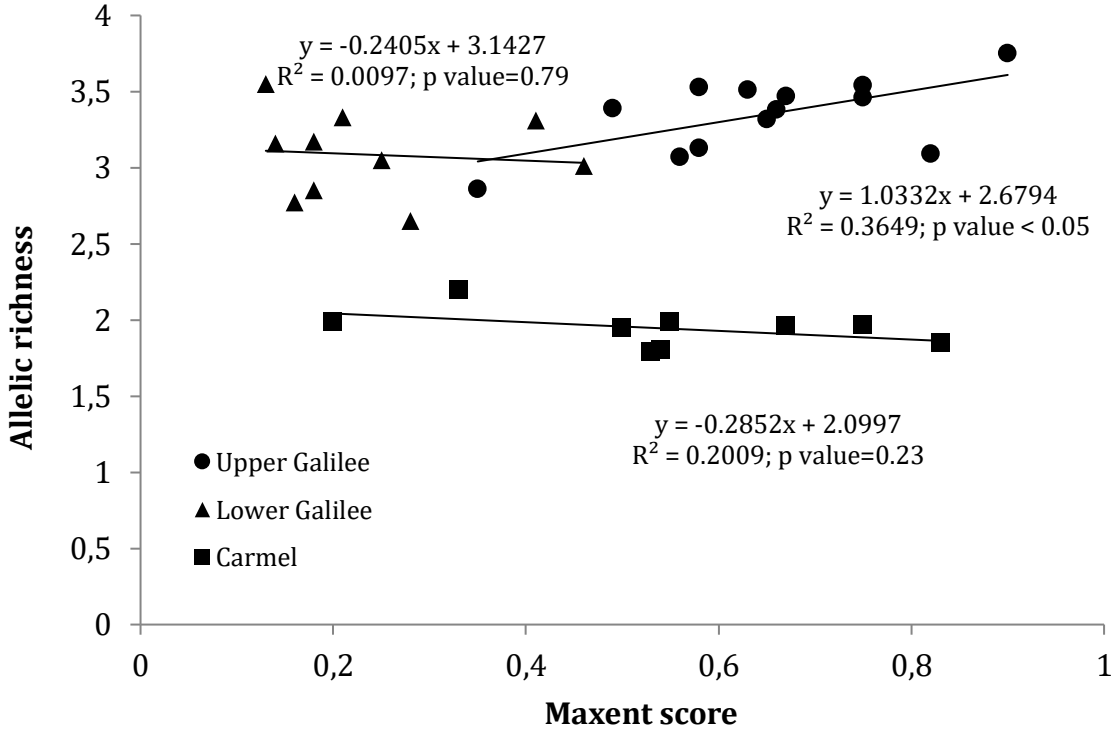

796

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

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