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

Sinai, Iftah

2019-08

unspecified
acceptedVersion

1   The role of landscape and history on the genetic structure of peripheral populations of the Near

2   Eastern fire salamander, *Salamandra infraimmaculata*, in Northern Israel

3

4   Running Title: Core-Peripheral Populations of Fire Salamanders

5

6   Iftah Sinai[1,2*], Ori Segev[1], Gilad Weil[2], Talya Oron[2], Juha Merilä[3], Alan R. Templeton[1,4], Leon

7   Blaustein[1], Gili Greenbaum[5], and Lior Blank[6]

8

9   [1] Institute of Evolution and Department of Evolutionary and Environmental Biology, Faculty of

10   Natural Sciences, University of Haifa, Haifa, 3498838, Israel

11   [2] Israel Nature and Parks Authority, Israel

12   [3] Ecological Genetics Research Unit, Organismal and Evolutionary Biology Research

13   Programme, Faculty Biological & Environmental Sciences, University of Helsinki, Finland

14   [4] Department of Biology, Washington University, St. Louis, MO 63130, USA

15   [5] Department of Biology, Stanford University, Palo Alto, CA 94305-5020 USA

16   [6] Department of Plant Pathology and Weed Research, ARO, Volcani Center, Bet Dagan 50250,

17   Israel

18

19   ORCID:

20   Iftah Sinai: https://orcid.org/0000-0003-1166-610X
21
22   Lior Blank: https://orcid.org/0000-0002-8753-0113
23

24   *Corresponding author:* Phone: 972-0537762082; E-mail: Iftahs@npa.org.il

25 ABSTRACT

26 Genetic studies on core versus peripheral populations have yielded many patterns. This diversity

27 in genetic patterns may reflect diversity in the meaning of "peripheral populations" as defined by

28 geography, gene flow patterns, historical effects, and ecological conditions. Populations at the

29 lower latitude periphery of a species' range are of particular concern because they may be at

30 increased risk for extinction due to global climate change. In this work we aim to understand the

31 impact of landscape and ecological factors on different geographical types of peripheral

32 populations with respect to levels of genetic diversity and patterns of local population

33 differentiation. We examined three geographical types of peripheral populations of the

34 endangered salamander, *Salamandra infraimmaculata,* in Northern Israel, in the southernmost

35 periphery of the genus *Salamandra*, by analyzing the variability in 15 microsatellite loci from 32

36 sites. Our results showed that: 1) genetic diversity decreases towards the geographical periphery

37 of the species' range; 2) genetic diversity in geographically disjunct peripheral areas is low

38 compared to the core or peripheral populations that are contiguous to the core and most likely

39 affected by a founder effect; 3) ecologically marginal conditions enhance population subdivision.

40 The patterns we found lead to the conclusion that genetic diversity is influenced by a

41 combination of geographical, historical, and ecological factors. These complex patterns should

42 be addressed when prioritizing areas for conservation.

43

44 Keywords: endangered salamander, genetic diversity, gene flow, ecology, peripheral populations,

45 conservation

46

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

3

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

91   limit the range. For example, suppose the populations at the border are small in variance

92   effective size and have little to no genetic variation, thereby limiting the ability of these

93   populations to adapt to local conditions (Carson 1955). Alternatively, suppose there is much

94   gene flow from the core to the periphery that can also impede local adaptation (Kawecki 2008).

95   Hence, patterns of genetic variation and gene flow/population subdivision can play important

96   roles in understanding the nature of the periphery of a species' range (for reviews, see: Brussard

97   1984; Eckert *et al.* 2008; Hoffmann & Blows 1994; Kawecki 2008; Nevo 1998; Pironon *et al.*

98   2017; Vucetich & Waite 2003). These considerations indicate the need to take an

99   interdisciplinary approach that integrates genetics, ecology, history, and geography to understand

100 the multifaceted nature of species' borders (Holt & Keitt 2005).

101   The purpose of this paper is to perform such an integrative analysis on the southernmost

102 part of the species' range of the endangered salamander *S. infraimmaculata.* A previous genetic

103 survey revealed significant genetic differentiation between the Mount Carmel and the Lower

104 Galilee populations and lower genetic diversity in Mount Carmel (Blank *et al.* 2013). Blank *et*

105 *al.* (2013) argued that this pattern indicates that the non-contiguous Mount Carmel populations

106 represent an isolated peripheral region that had experienced bottleneck and/or founder effects in

107 its recent demographic history. This earlier survey only included Mount Carmel, the Lower

108 Galilee and the southern edge of the Upper Galilee region. To understand better the potential

109 diversity of peripheral populations with respect to genetic diversity, gene flow patterns, and

110 recent evolutionary history, a more complete genetic sampling across the entire core–periphery

111 gradient would be needed, and this was a major goal of the current study. A better understanding

112 of the edge of the species' range also requires an ecological assessment of the factors that explain

113 the species' distribution in a geographic context, as well as how gene flow patterns relate to

114 landscape and other environmental features. We therefore analyze how the genetic structure of *S.*

115 *infraimmaculata* populations is influenced by geographical, ecological, and landscape factors at

116 the southernmost edge of its global distribution. We then test the impact of landscape and

117 ecological factors on different geographical types of peripheral populations with respect to levels

118 of genetic diversity and patterns of local population differentiation. Specifically, we test three

119 hypotheses commonly made in the core-peripheral population literature by analyzing the

120 variability in 15 microsatellite loci from 32 sites: 1) genetic diversity will decrease towards the

121 geographical periphery of a species' range; 2) genetic diversity in geographically disjunct

122 peripheral areas will be low compared to the core or peripheral populations that are contiguous to

123 the core; and 3) ecologically marginal conditions tend to enhance population subdivision. By

124 addressing these hypotheses, we will be enable to elucidate the relative roles ecological,

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

126 populations.

127

128 MATERIALS AND METHODS

129 Sample collection and DNA extraction

130   We sampled salamanders in three regions: the Upper Galilee, the Lower Galilee, and

131 Mount Carmel (Fig. 1b). We collected genetic samples from 692 fire salamanders (mostly adults

132 with some postmetamorphic juveniles) from 32 breeding sites (Table 1, Fig. 1b). Mount Carmel

133 is a disjunct peripheral region, the Lower Galilee is largely a contiguous peripheral area, and the

134 Upper Galilee is continuous with the core area that extends through Lebanon, Syria and Turkey

135 (Fig. 1a).

136 Tissue samples for molecular analysis were collected by capturing adults (larvae in two

137 cases; see Table 1) during rainy nights and cutting a small tip of the tail (2-3 mm) with a sterile

138 scalpel, placing it in an eppendorf tube with 99% ethanol, and then storing at -20°C until further

139 processing. Tail-tip tissue in salamander larvae was found to have only little effect on fitness

140 (Segev et al. 2015; Blaustein et al. 2017). Other genetic samples were collected early in the

141 morning from fresh road kills of salamander adults in 8 sites in the Upper Galilee. Our goal was

142 to collect samples from at least 20 individuals per site, but lower numbers were obtained for

143 many of the sites due to their small population sizes (Table 1).

144 Each sampled adult individual was photographed in order to identify dorsal spot patterns

145 to ensure that the same individuals were not sampled on different sampling nights (Blank *et al.*

146 2013; Segev *et al.* 2010; Warburg 2011). Genomic DNA was extracted using QIAamp DNA

147 minikit (Qiagen) with the following modifications: protocol-devised RNA free option and

148 incubation with proteinase K.

149

150 Microsatellite genotyping

151 Allelic variation in nuclear markers was assessed using 15 microsatellite loci using primers

152 described earlier (Sal E2, Sal E5, Sal E6, Sal E7, Sal E8, Sal E11, Sal E12, Sal E14, Sal 3, Sal

153 23, SST-A6-I, SST-A6-II, SSTC3, SST-E11 and SST-G6: (Hendrix *et al.* 2010; Steinfartz *et al.*

154 2004). Each forward primer was labeled with a fluorescent dye (HEX ,FAM, or TET) for

155 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>

221   We quantified the altitude (obtained from Hall *et al.* (2013)), average precipitation, and

222   average annual day and night temperatures (data obtained from the Israeli Meteorological

223   Service) at each of the 32 sites. We also quantified the differences in these environmental factors

224   between Mount Carmel, the Lower Galilee, and the Upper Galilee (Figure 1b). We used

225   radiometric and geometric corrected LANDSAT8 satellite imagery data (Roy *et al.* 2014) for

226   producing Normalized Difference Vegetation Index data (NDVI) (Levin *et al.* 2011; Tucker

227   1979). NDVI was computed for two different seasons - winter (February 2014) and summer

228   (July 2014) in order to differentiate between evergreen vegetation and annual vegetation. The

229   continuous NDVI values from both seasons was classify into several discrete categories of

230   Mediterranean flora. The output classes were adjusted to the accepted vegetation cover type

231   names after field validations in four locations along the climatic gradient of the Mediterranean

232   ecosystem. The names of the vegetation cover classes were given according to the Israeli guide

233   for Mediterranean vegetation mapping (Leshner & Ramon 2013).

234

235   Maximum entropy modeling

236   We used data on 97 salamander breeding sites to examine the landscape and

237   environmental characteristics that can explain the distribution of these salamanders in the three

238   regions. We learned of these 97 potential breeding sites based on previous surveys done in the

239   area (Blank & Blaustein 2012; Blank & Blaustein 2014, Sinai and Oron unpublished data) and

240   interviews with Nature and Park Authority rangers. For these 97 sites, we employed maximum

241   entropy distribution (Maxent) modeling to infer the suitable areas for *S. infraimmaculata*.

242   Maxent, unlike other distributional modeling techniques, uses only presence and background

11

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

265   roads (Fahrig & Rytwinski 2009; Garriga *et al.* 2012, T. Oron, personal communication), and

266   indeed many of our samples came from road kills. Second, avoidance of roads restricts dispersal

267   and migration (Ray *et al.* 2002). Third, pollution from road runoff was identified as a threat to

268   aquatic habitats (Dorchin & Shanas 2010; Harless *et al.* 2011). Segev *et al.* (2010) found a

269   positive correlation between built areas and *S. infraimmaculata* population size but suggested

270   that this was because human settlements tended to be established close to springs.

271   Given the Maxent model based on 97 sites that cover more uniformly the distribution of

272   these salamanders within Israel (Fig. 7), we assigned Maxent scores (Dubey *et al.* 2013) to the

273   32 salamander breeding sites surveyed genetically. Such scores are a measure of local habitat

274   suitability for the species.

275

276   RESULTS

277   MICRO-CHECKER analyses revealed no evidence of null alleles or scoring issues across loci.

278   Only three of 105 pairwise loci Fisher exact probability tests of deviation from genotypic

279   equilibrium were significant at $P < 0.05$. Significant linkage disequilibrium was found at only

280   5.86% of loci combinations at the 32 sites.

281

282   <u>Genetic diversity and population structure</u>

283   There were 18 alleles unique to the Upper Galilee, only one to the Lower Galilee, and

284   none unique to Mount Carmel. In the Upper and Lower Galilee, the average allelic richness and

285   the observed and expected heterozygosity were significantly higher than Mount Carmel (Table

286   2). Although the two Galilee regions were not statistically different from one another in genetic

287 diversity measures (Table 2), the F_{st} estimated among the Lower Galilee sites was greater than

288 zero and exceeded that estimated for the Upper Galilee and the Mount Carmel regions, both of

289 which had F_{st} estimates not significantly different from zero (Table 2). We observed moderate

290 decreases in allelic richness and observed heterozygosity when moving from the Upper Galilee

291 to the Lower Galilee, and sharp decreases in these parameters in the Mount Carmel region

292 (Tables 1 & 2). Allelic richness and observed heterozygosity declined significantly with

293 decreasing latitude when the regression included all three multi-site regions, but also when it was

294 restricted just to the sites in the Galilee (Fig. 2).

295   STRUCTURE analyses revealed that the optimal K using the delta K criterion was two.

296 Most individuals fell in one of the two clusters that corresponded geographically to the Mount

297 Carmel region and the Galilee sites, with few admixed individuals between these two geographic

298 clusters (Fig. 3). Like STRUCTURE, the first two PCA axes clearly divided the Galilee region

299 from the Mount Carmel region (Fig. 4).

300   NetStruct provided further insight into population structure. At the lowest edge-pruning

301 threshold (coarse-scale structure), two significant clusters emerged- the Mount Carmel

302 populations and the Galilee populations (Fig. 5a). Hence, this analysis captured the same

303 subdivision as the STRUCTURE analysis, but now with added information that these two

304 clusters are statistically significant. Indeed, not a single random permutation out of 1,000

305 equaled or exceeded the observed modularity for these two clusters, indicating a strong degree of

306 genetic differentiation between these two geographic areas. Because the allele frequencies were

307 so different between these two clusters, we decided to separate them for the subsequent analyses

308 because these large allele frequency differences would dominate the weights assigned to the

14

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

15

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

419   ecological suitability of Mount Carmel is high and similar to the Upper Galilee, whereas the

420   Lower Galilee is the most ecologically peripheral area. Despite the harsh ecological conditions

421   in the Lower Galilee, the populations there have much higher genetic diversity than those on

422   Mount Carmel (Table 2, Fig. 9). Thus, Mount Carmel represents an optimal habitat island for

423   these salamanders, and the depurate genetic diversity found on Mount Carmel cannot be

424   explained by harsh ecological conditions. Overall, the Mount Carmel populations indicate the

425   importance of historical factors in geographically disjunct peripheral populations living in an

426   optimal habitat island.

427

428   **Ecologically marginal conditions tend to enhance population subdivision**

429   The Maxent analysis indicates that the lower Galilee is the most ecologically peripheral

430   area in our study and has the least suitable environment for these salamanders in Northern Israel.

431   (Fig. 7). The F_{st} index was higher in the Lower Galilee compared to the other areas (Table 2),

432   thereby indicating greater population subdivision in the Lower Galilee compared to the Upper

433   Galilee and Mount Carmel. This inference is also supported by the NetStruct which indicate

434   much more population subdivision in the Lower Galilee compared to the Upper Galilee and

435   Mount Carmel regions.

436   Ecologically marginal conditions could result in more population subdivision by creating

437   local barriers to gene flow. The Lower Galilee has less dense maquis and woodland (Table 4)

438   and higher temperatures and less precipitation (Fig. 7) than the other regions. Shaded, vegetated

439   areas that maintain moisture in the soil and air seem to have great importance for dispersal in

440   terrestrial amphibians like salamanders (Hartel *et al.* 2008; Hocking *et al.* 2013; Manenti *et al.*

20

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

463   using allelic richness as the response variable. The genetic homogeneity among the Mount

464   Carmel populations could arise from increased dispersal due to an overall more favorable

465   environment (Figs 7 and 8) in an area much smaller than the Upper Galilee (Fig. 1b). Support

466   for this explanation stems from mark/recapture studies that document long-distance dispersal on

467   Mount Carmel that indicate potential connectivity between breeding sites (Bar-David *et al.*

468   2007). However, the Carmel populations do display significant isolation by distance (Fig. 6) that

469   indicates that dispersal may not be increased in this area that is intermediate environmentally and

470   ecologically between the Upper and Lower Galilees (Figs 7 and 8). An alternative explanation

471   for the genetic homogeneity of the Carmel populations stems from the genetic evidence

472   discussed above that indicates a recent founder event on Mount Carmel. A recent founder event

473   into a new geographical area followed by range expansion promotes genetic uniformity in that

474   new area, as has occurred in other salamanders (Larson 1984; Larson *et al.* 1984).

475   The patterns discussed above lead to a general conclusion: **Genetic diversity is**

476   **influenced by a combination of geographical, historical, and ecological factors.** The genetic

477   and ecological data suggest that our study included different types of peripheral populations: a

478   geographically disjunct peripheral isolate in an ecologically optimal habitat island (Mount

479   Carmel) that has a strong genetic signature of an historical founder event and extensive genetic

480   homogeneity, an ecologically peripheral population on the edge of the species continuous range

481   in the Lower Galilee displaying much local population subdivision, and a population continuous

482   with the core in the Upper Galilee in an ecologically optimal habitat with no significant

483   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

505   genetic reservoir, they may also be at great risk for extinction under climate change due to a lack

506   of evolutionary flexibility and restricted habitable area.

507   These diverse genetic, ecological, and historical factors not only highlight the diversity of

508   types of peripheral populations, but they also indicate the complexity of conservation efforts

509   directed at peripheral populations. Such conservation efforts are particularly important for

510   amphibian species in danger of local extinction at their lower-latitude boundaries because of

511   climate change (Givati & Rosenfeld 2013; Griffiths *et al.* 2010; Mac Nally *et al.* 2017).

512   Populations on the lower latitude periphery of a species' range often provide an important

513   genetic reservoir for the species as whole, display unique adaptations, and have historically

514   played a disproportionate role in the species' survival and evolution (Hampe & Petit 2005) – a

515   combination that makes such peripheral populations important in conservation planning. Adding

516   to their importance in conservation is that these low-latitude peripheral populations are the ones

517   most at risk for local extinction under climate change and yet remain understudied (Cahill *et al.*

518   2013; Chen *et al.* 2011). Which ecological/evolutionary/historical forces will be more influential

519   in the future in this complex metapopulation are difficult to predict (Duncan *et al.* 2015). A more

520   thorough investigations of the genetics, ecology, and history of these peripheral salamander

521   populations in this interesting region is needed in order to make a better assessment of their

522   conservation needs.

523

534

535   REFERENCES

536   Bar-David S, Segev O, Peleg N*, et al.* (2007) Long-distance movements by Fire Salamanders
537   (*Salamandra infraimmaculata*) and implications for habitat fragmentation. *Israel Journal*
538   *of Ecology and Evolution* **53**, 143 - 159.
539   Blank L, Blaustein L (2012) Using ecological niche modeling to predict the distributions of two
540   endangered amphibian species in aquatic breeding sites. *Hydrobiologia* **693**, 157-167.
541   Blank L, Blaustein L (2014) A multi-scale analysis of breeding site characteristics of the
542   endangered fire salamander (*Salamandra infraimmaculata*) at its extreme southern range
543   limit. *Hydrobiologia* **726**, 229-244.
544   Blank L, Sinai I, Bar-David S*, et al.* (2013) Genetic population structure of the endangered fire
545   salamander (*Salamandra infraimmaculata*) at the southernmost extreme of its
546   distribution. *Animal Conservation* **16**, 412-421.
547   Bogaerts S, Sparreboom M, Pasmans F*, et al.* (2013) Distribution, ecology and conservation of
548   *Ommatotriton vittatus* and *Salamandra infraimmaculata* in Syria. *Salamandra* **49**, 87-96.
549   Brussard PF (1984) Geographic patterns and environmental gradients: the central-marginal
550   model in *Drosophila* revisited. *Ann. Rev. Ecol. Syst.* **15**, 25-64.
551   Cahill AE, Aiello-Lammens ME, Fisher-Reid MC*, et al.* (2013) How does climate change cause
552   extinction? *Proceedings of the Royal Society B-Biological Sciences* **280**.
553   Carson HL (1955) The genetic characteristics of marginal populations of *Drosophila*. *Cold*
554   *Spring Harb Symp Quant Biol* **20**, 276-287.
555   Chen I-C, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species
556   associated with high levels of climate warming. *Science* **333**, 1024-1026.
557   Chesser RK, Rhodes OE, Sugg DW, Schnabel A (1993) Effective sizes for subdivided
558   populations. *Genetics* **135**, 1221-1232.
559   Chesser RK, Smith MH, I.L. Brisbin J (1980) Management and maintenance of genetic
560   variability in endangered species. *Internatl. Zoo Yearbook* **20**, 146-154.

561   Dorchin A, Shanas U (2010) Assessment of pollution in road runoff using a *Bufo viridis*
562   biological assay. *Environmental Pollution* **158**, 3626-3633.
563   Dubey S, Pike DA, Shine R (2013) Predicting the impacts of climate change on genetic diversity
564   in an endangered lizard species. *Climatic Change* **117**, 319-327.
565   Duncan SI, Crespi EJ, Mattheus NM, Rissler LJ (2015) History matters more when explaining
566   genetic diversity within the context of the core-periphery hypothesis. *Molecular Ecology*
567   **24**, 4323-4336.
568   Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical
569   ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**, 1170-1188.
570   Elith J, Phillips SJ, Hastie T*, et al.* (2011) A statistical explanation of MaxEnt for ecologists.
571   *Diversity and Distributions* **17**, 43-57.
572   Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the
573   software structure: a simulation study. *Molecular Ecology* **14**, 2611-2620.
574   Fahrig L, Rytwinski T (2009) Effects of Roads on Animal Abundance: an Empirical Review and
575   Synthesis. *Ecology and Society* **14**.
576   Garriga N, Santos X, Montori A*, et al.* (2012) Are protected areas truly protected? The impact of
577   road traffic on vertebrate fauna. *Biodiversity and Conservation* **21**, 2761-2774.
578   Gaston KJ (2003) *The Structure and Dynamics of Geographic Ranges* Oxford University Press,
579   Oxford.
580   Givati A, Rosenfeld D (2013) The Arctic Oscillation, climate change and the effects on
581   precipitation in Israel. *Atmospheric Research* **132**, 114-124.
582   Goldberg T, Nevo E, Degani G (2011) Genetic diverseness and different ecological conditions in
583   *Salamandra infraimmaculata* larvae from various breeding sites. *Animal Biology Journal*
584   **2**, 37-49.
585   Gorodkov KB (1986) Three-dimensional climatic model of potential range and some of its
586   characteristics. II. *Entomological Review* **65**, 1–18.
587   Greenbaum G, Templeton AR, Bar-David S (2016) Inference and analysis of population
588   structure using genetic data and network theory. *Genetics* **202**, 1299-1312.
589   Greenbaum G, Templeton AR, Zarmi Y, Bar-David S (2014) Allelic richness following
590   population founding events – A stochastic modeling framework incorporating gene flow
591   and genetic drift. *Plos One* **9**, e115203.
592   Griffiths RA, Sewell D, McCrea RS (2010) Dynamics of a declining amphibian metapopulation:
593   Survival, dispersal and the impact of climate. *Biological Conservation* **143**, 485-491.
594   Haan SS, Desmond MJ, Gould WR, Ward JP (2007) Influence of habitat characteristics on
595   detected site occupancy of the New Mexico endemic Sacramento Mountains Salamander,
596   *Aneides hardii*. *Journal of Herpetology* **41**, 1-8.
597   Hall JK, Schwartz E, Cleave RL (2013) The Israeli DTM (digital terrain map) project.
598   *Microcomputer Applications in Geology* **2**, 111-118.
599   Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters.
600   *Ecology Letters* **8**, 461-467.
601   Hardy R (1945) The influence of types of soil upon the local distribution of some mammals in
602   southwestern Utah. *Ecological Monographs* **15**, 71-108.
603   Harless ML, Huckins CJ, Grant JB, Pypker TG (2011) Effects of six chemical deicers on larval
604   wood frogs (*Rana sylvatica*). *Environmental Toxicology and Chemistry* **30**, 1637-1641.

605 Hartel T, Nemes S, Demeter L, Ollerer K (2008) Pond and landscape characteristics - which is
606   more important for common toads (*Bufo bufo*)? A case study from central Romania.
607   *Applied Herpetology* **5**, 1-12.
608 Hendrix R, Hauswaldt JS, Veith M, Steinfartz S (2010) Strong correlation between cross-
609   amplification success and genetic distance across all members of 'True Salamanders'
610   (Amphibia: Salamandridae) revealed by *Salamandra salamandra*-specific microsatellite
611   loci. *Molecular Ecology Resources* **10**, 1038-1047.
612 Hernandez PA, Franke I, Herzog SK, *et al.* (2008) Predicting species distributions in poorly-
613   studied landscapes. *Biodiversity and Conservation* **17**, 1353-1366.
614 Hocking DJ, Connette GM, Conner CA, *et al.* (2013) Effects of experimental forest management
615   on a terrestrial, woodland salamander in Missouri. *Forest Ecology and Management* **287**,
616   32-39.
617 Hoffmann AA, Blows MW (1994) Species borders: ecological and evolutionary perspectives.
618   *Trends In Evolution and Ecology* **9**, 223-237.
619 Holt RD, Keitt TH (2005) Species' borders: a unifying theme in ecology. *Oikos* **108**, 3-6.
620 Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance
621   measures: Inferring the relative influences of gene flow and drift on the distribution of
622   genetic variability. *Evolution* **53**, 1898-1914.
623 Kalinowski ST (2011) The computer program STRUCTURE does not reliably identify the main
624   genetic clusters within species: simulations and implications for human population
625   structure. *Heredity* **106**, 625-632.
626 Kawecki TJ (2008) Adaptation to Marginal Habitats. *Annual Review of Ecology Evolution and*
627   *Systematics* **39**, 321-342.
628 Kershenbaum A, Blank L, Sinai I, *et al.* (2014) Landscape influences on dispersal behaviour: a
629   theoretical model and empirical test using the fire salamander, *Salamandra*
630   *infraimmaculata*. *Oecologia* **175**, 509-520.
631 Larson A (1984) Neontological inferences of evolutionary pattern and process in the salamander
632   family Plethodontidae. *Evolutionary Biology* **17**, 119-217.
633 Larson A, Wake DB, Yanev KP (1984) Measuring gene flow among populations having high
634   levels of genetic fragmentation. *Genetics* **106**, 293-308.
635 Leshner A, Ramon O (2013) The Israeli mapping guide for Mediterranean vegetation, Ministry
636   of the Environment.
637 Levin N, Harari-Kremer R, Carmel Y (2011) Remote Sensing of Israel's Natural Habitats.
638   Unpublished Report for the Israeli Nature and Parks Authority, Jerusalem.
639 Mac Nally R, Horrocks GFB, Lada H (2017) Anuran responses to pressures from high-amplitude
640   drought-flood-drought sequences under climate change. *Climatic Change* **141**, 243-257.
641 Manenti R, Ficetola GF, De Bernardi F (2009) Water, stream morphology and landscape:
642   complex habitat determinants for the fire salamander *Salamandra salamandra*.
643   *Amphibia-Reptilia* **30**, 7-15.
644 Navarro-Cerrillo RM, Hernandez-Bermejo JE, Hernandez-Clemente R (2011) Evaluating models
645   to assess the distribution of *Buxus balearica* in southern Spain. *Applied Vegetation*
646   *Science* **14**, 256-267.
647 Nevo E (1998) Genetic diversity in wild cereals - regional and local studies and their bearing on
648   conservation *ex situ* and *in situ Genetic Resources & Crop Evolution* **45**, 355-370.

27

649 O'Donnell KM, Thompson FR, Semlitsch RD (2014) Predicting variation in microhabitat
650   utilization of terrestrial salamanders. *Herpetologica* **70**, 259-265.
651 Peleg N (2009) *Studies on the conservation of the fire salamander (Salamander*
652   *Infraimmaculata) in Israel*, University of Haifa.
653 Perez MF, Franco FF, Bombonato JR, *et al.* (2018) Assessing population structure in the face of
654   isolation by distance: Are we neglecting the problem? *Diversity and Distributions* **24**,
655   1883-1889.
656 Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species
657   geographic distributions. *Ecological Modelling* **190**, 231-259.
658 Pironon S, Papuga G, Villellas J, *et al.* (2017) Geographic variation in genetic and demographic
659   performance: new insights from an old biogeographical paradigm. *Biological Reviews* **92**,
660   1877–1909.
661 Pisa G, Orioli V, Spilotros G, *et al.* (2015) Detecting a hierarchical genetic population structure:
662   the case study of the Fire Salamander (*Salamandra salamandra*) in Northern Italy.
663   *Ecology and Evolution* **5**, 743-758.
664 Ray N, Lehmann A, Joly P (2002) Modeling spatial distribution of amphibian populations: a GIS
665   approach based on habitat matrix permeability. *Biodiversity and Conservation* **11**, 2143-
666   2165.
667 Roy DP, Wulder MA, Loveland TR, *et al.* (2014) Landsat-8: Science and product vision for
668   terrestrial global change research. *Remote Sensing of Environment* **145**, 154-172.
669 Segev O, Hill N, Templeton AR, Blaustein L (2010) Population size, structure and phenology of
670   an endangered salamander at temporary and permanent breeding sites. *Journal for Nature*
671   *Conservation* **18**, 189-195.
672 Semlitsch RD, Anderson TL (2016) Structure and dynamics of Spotted Salamander (*Ambystoma*
673   *maculatum*) populations in Missouri. *Herpetologica* **72**, 81-89.
674 Sepulveda AJ, Lowe WH (2009) Local and landscape-scale influences on the occurrence and
675   density of *Dicamptodon aterrimus*, the Idaho Giant Salamander. *Journal of Herpetology*
676   **43**, 469-484.
677 Steinfartz S, Kusters D, Tautz D (2004) Isolation and characterization of polymorphic
678   tetranucleotide microsatellite loci in the fire salamander *Salamandra salamandra*
679   (Amphibia : Caudata). *Molecular Ecology Notes* **4**, 626-628.
680 Steinfartz S, Veith M, Tautz D (2000) Mitochondrial sequence analysis of Salamandra taxa
681   suggests old splits of major lineages and postglacial recolonizations of Central Europe
682   from distinct source populations of *Salamandra salamandra*. *Molecular Ecology* **9**, 397-
683   410.
684 Swets JA (1988) Measuring the accuracy of diagnostic systems. *Science* **240**, 1285-1293.
685 Templeton AR, Routman E, Phillips C (1995) Separating population structure from population
686   history: a cladistic analysis of the geographical distribution of mitochondrial DNA
687   haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* **140**, 767-782.
688 Tucker CJ (1979) Red and photographic infrared linear combinations for monitoring vegetation.
689   *Remote Sensing of Environment* **8**, 127-150.
690 Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across the
691   species' range: Null hypotheses for landscape conservation genetics. *Conservation*
692   *Genetics* **4**, 639-645.

693 Warburg MR (2011) Changes in recapture rate of a rare salamander in an isolated
694   metapopulation studied for 25 years. *Russian Journal of Herpetology* **15**, 11-18.
695 Wright S (1943) Isolation by distance. *Genetics* **28**, 114-138.
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
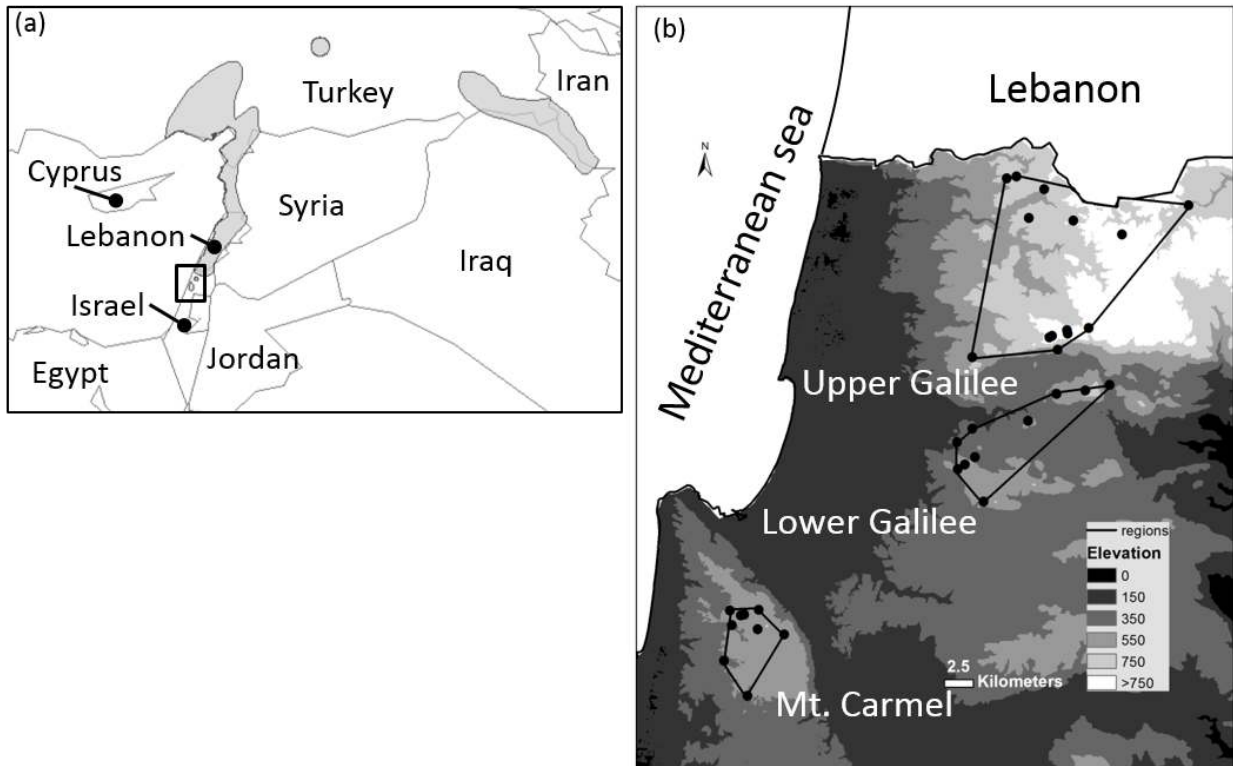
727

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
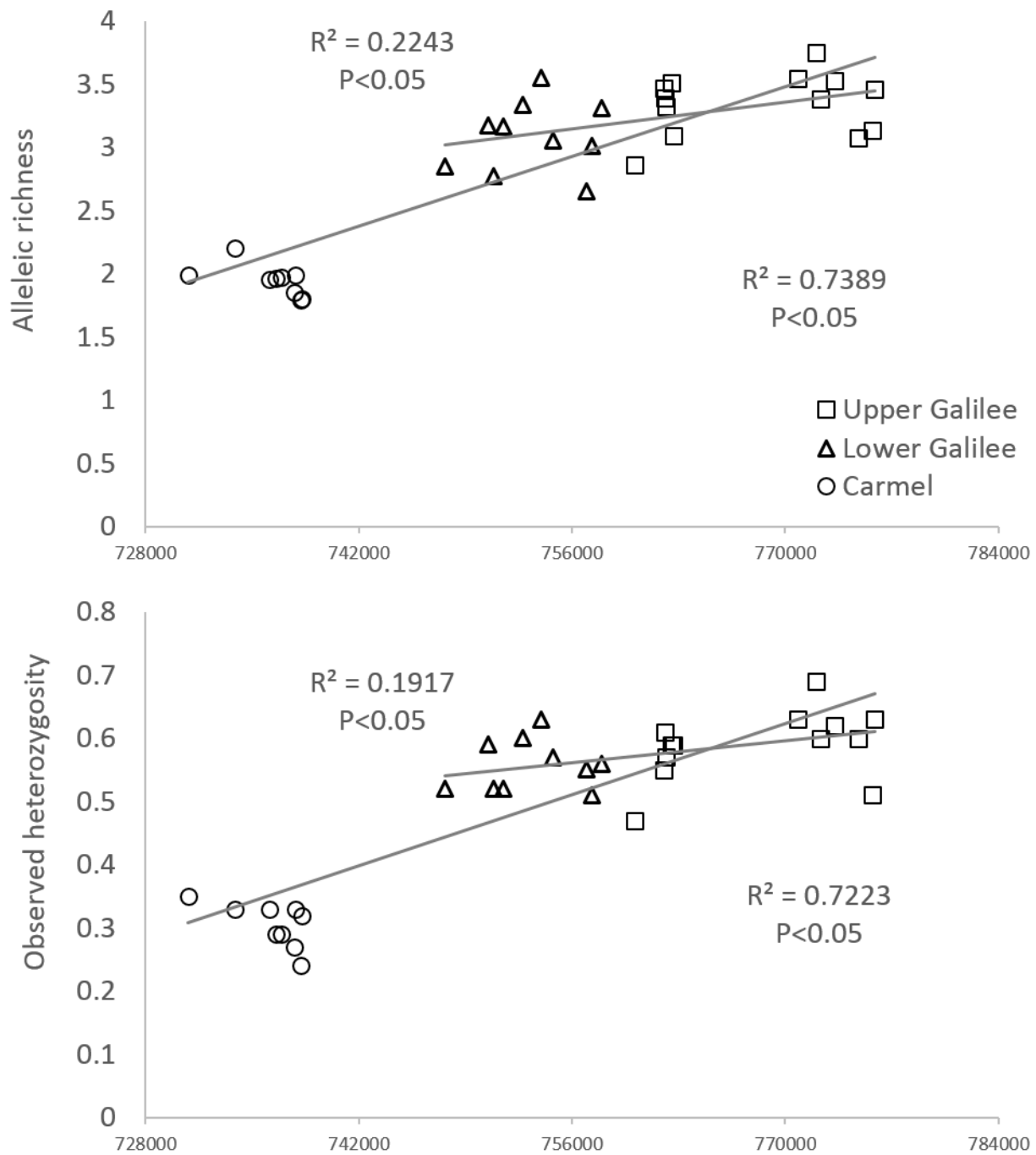
733

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).
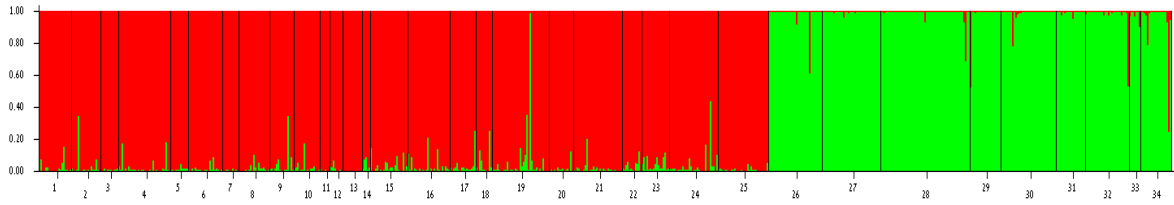
737

738   Figure 3. Genetic clustering in the study area obtained with STRUCTURE with K =2, the

739   optimal K under the delta K method. Identical colors identify populations with a homogeneous

740   genetic composition, while different colors represent genetically differentiated populations. The

741   red color is associated with individuals sampled from the Galilee, and green from Mount Carmel.

742

743

744

745

746

747

748

749

750

751

752

753

754

755

756
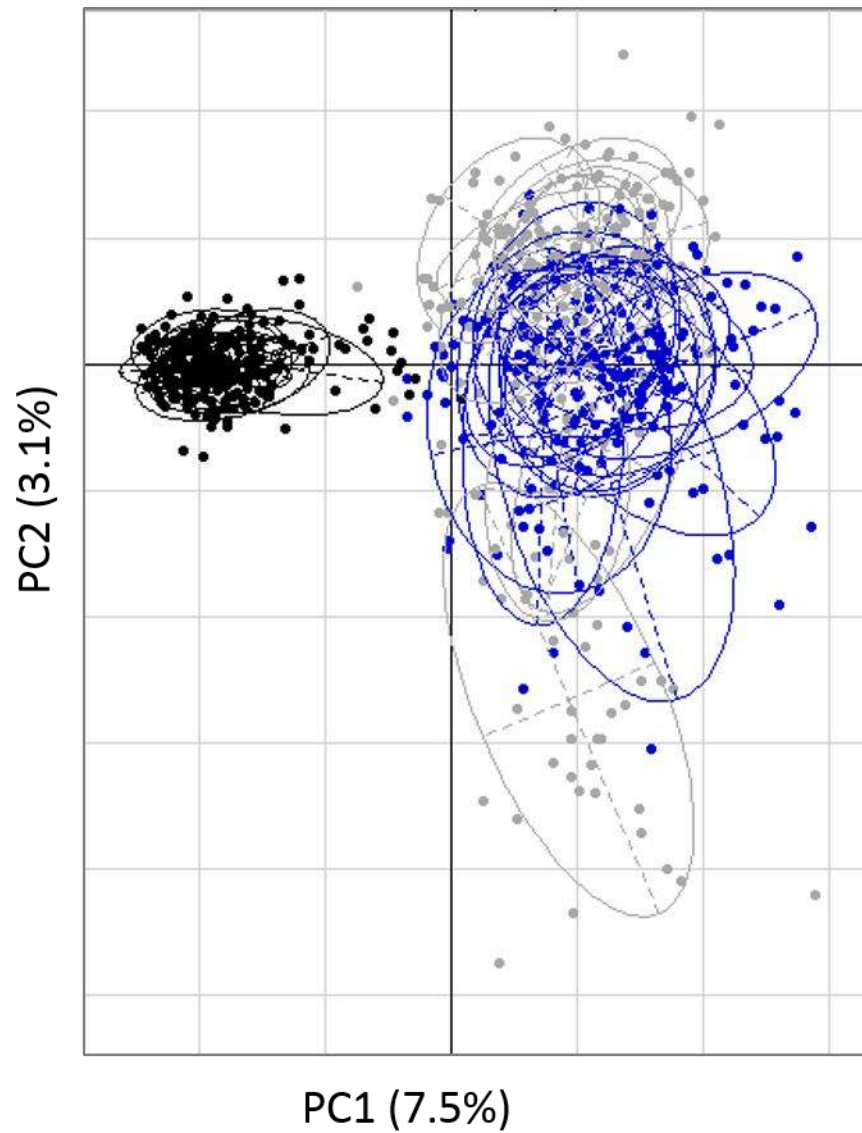
Figure 4. Results of the PCA analysis. First and second axes are presented. The dots shows individual salamanders. Ovals represent 95% inertia ellipses. Blue- Upper Galilee; Gray- Lower Galilee; Black- Mount Carmel
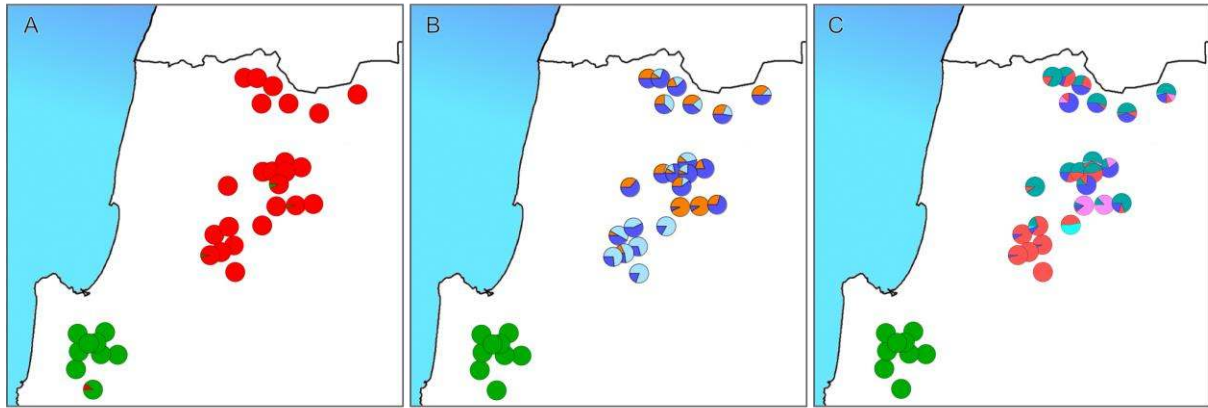
Figure 5. Genetic clustering in the study at three hierarchical levels obtained with NetStruct. Different colors represent different genetic clusters. At each sampling site, the distribution of assignments of individuals to clusters is shown. (A) The highest hierarchical level, obtained by analyzing 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.
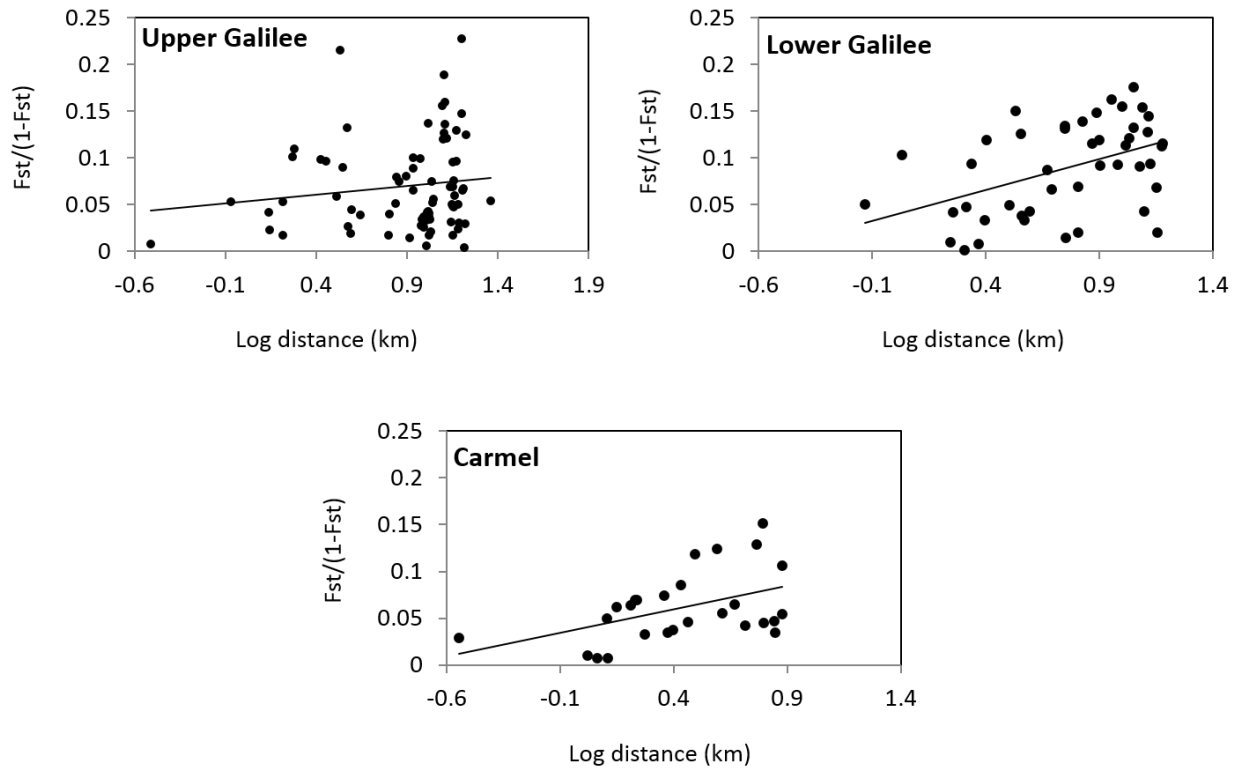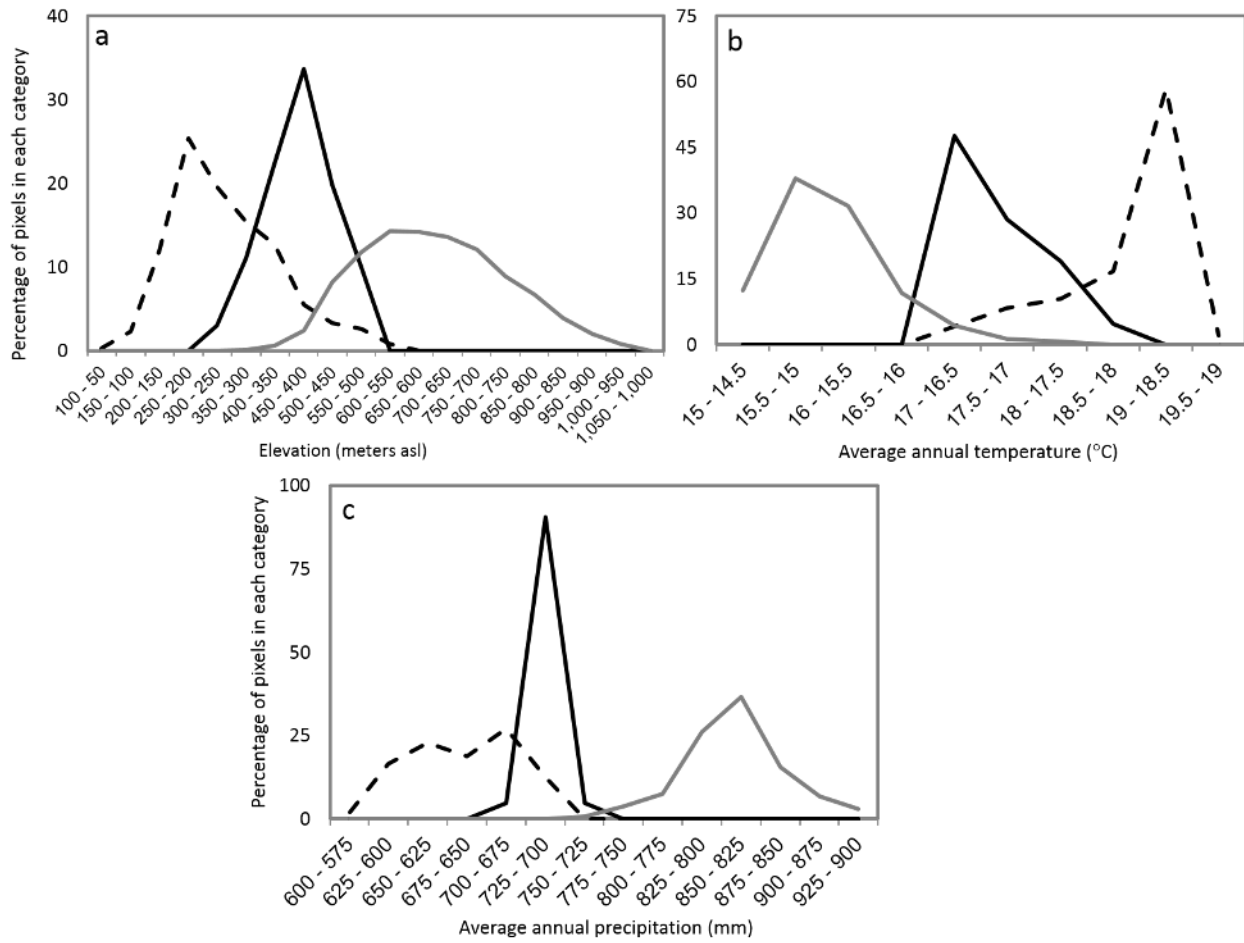
777

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

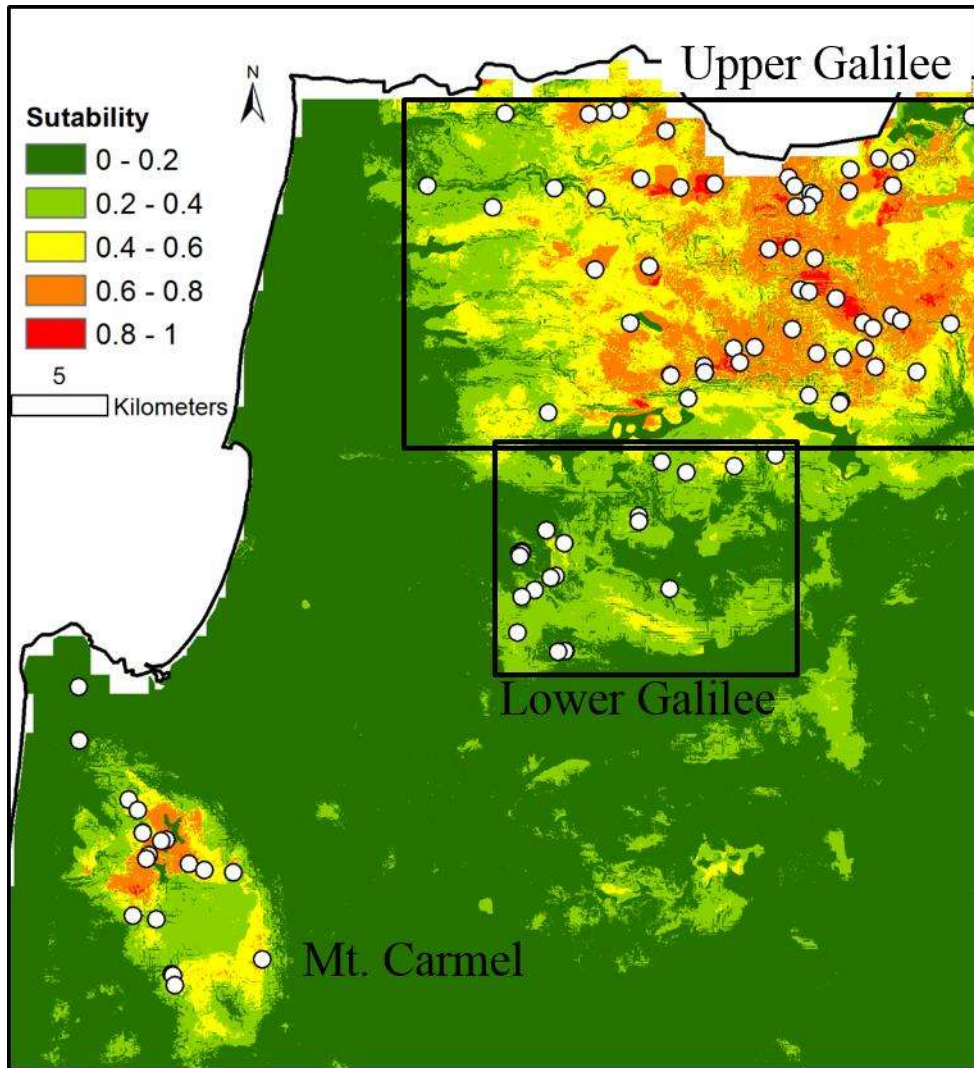781   (middle panel) and Carmel (lower panel).

782

783

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
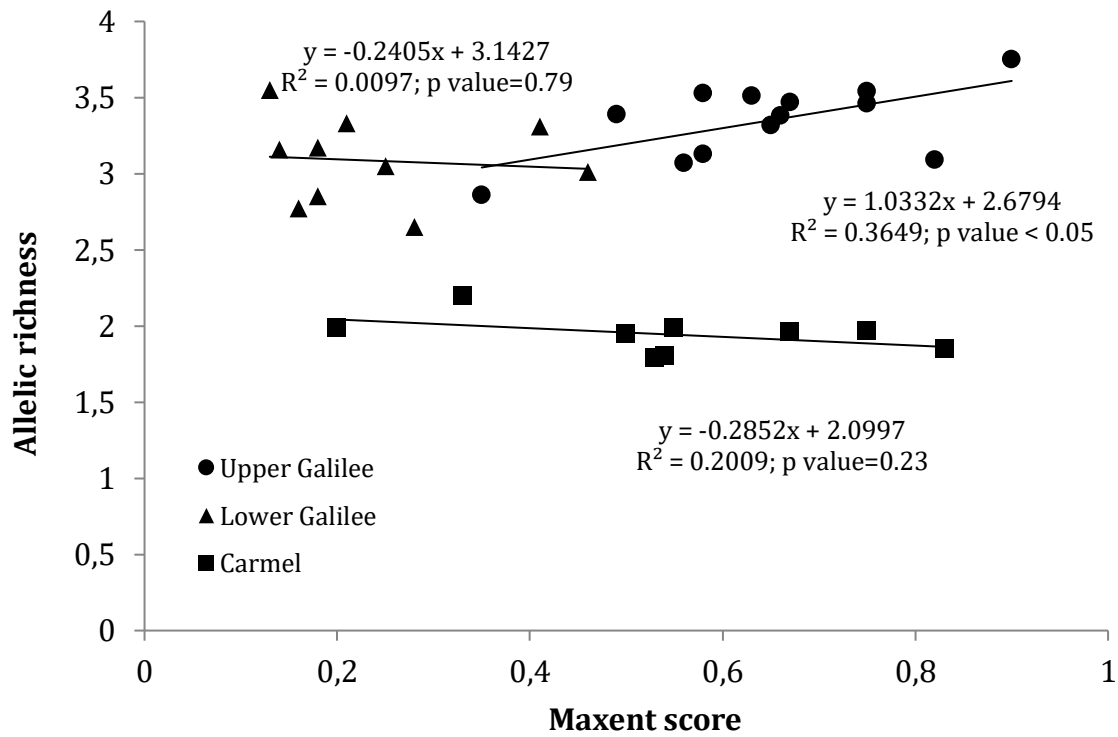
788   Galilee.

789

Figure 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.

$y = -0.2405x + 3.1427$
$R^2 = 0.0097$; p value=0.79

$y = 1.0332x + 2.6794$
$R^2 = 0.3649$; p value < 0.05

$y = -0.2852x + 2.0997$
$R^2 = 0.2009$; p value=0.23

● Upper Galilee
▲ Lower Galilee
■ Carmel

Maxent score

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.

800

801

802

803

804

805

806

807