Animal Conservation

Animal Conservation. Print ISSN 1367-9430

Genetic population structure of the endangered fire salamander (*Salamandra infraimmaculata*) at the southernmost extreme of its distribution

L. Blank[1], I. Sinai[1,2], S. Bar-David[1,3], N. Peleg[1], O. Segev[1], A. Sadeh[1], N. M. Kopelman[4], A. R. Templeton[1,5], J. Merilä[6] & L. Blaustein[1]

1 Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, Institute of Evolution, University of Haifa, Haifa, Israel
2 Israel Nature and Parks Authority, Jerusalem, Israel
3 Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben Gurion University of the Negev, Sede Boqer Campus, Israel
4 Porter School of Environmental Studies, Department of Zoology, Tel Aviv University, Tel Aviv, Israel
5 Department of Biology, Washington University, St. Louis, MO, USA
6 Ecological Genetics Research Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland

Keywords
amphibian; genetic diversity; isolation; microsatellites; peripheral population.

Correspondence
Lior Blank, Department of Evolutionary and Environmental Biology, Faculty of Natural Sciences, Institute of Evolution, University of Haifa, Haifa 31905, Israel. Tel: +972 4 8288328; Fax: +972 4 8246554
Email: liorblank@gmail.com

Editor: Trent Garner
Associate Editor: Robert Jehle

Received 11 March 2012; accepted 8 November 2012

doi:10.1111/acv.12009

Abstract

The negative effects of habitat fragmentation and population isolation on population viability, genetic variability and structuring are well documented, and conservation plans failing to take into account spatial population structure and connectivity can be ineffectual. Of special concern are populations at the periphery of the species range that might show reduced genetic diversity, thus affecting their adaptive potential at environmental margins. We investigated genetic variability and differentiation of the globally near threatened and locally endangered fire salamander *Salamandra infraimmaculata* in northern Israel, an area that represents the periphery of this species' distribution range. Analyses of variability in 15 microsatellite loci from 20 sites revealed substantial population structuring, most of which was due to a strong subdivision between two regions separated by a heavily urbanized valley. In addition, levels of genetic variability within populations were lowest in the peripheral, southernmost populations. These results suggest that the conservation plans for this species should recognize the lower diversity and increased divergence in the peripheral regions, and take into account the observed spatial population structure when devising strategies and measures to ensure the species persistence.

Introduction

Deciphering population genetic structure of endangered species can help to shed light on critical demographic processes, population connectivity and dispersal behavior, and aid conservation efforts (Frankham, Briscoe & Ballou, 2002; Allendorf & Luikart, 2007). Of particular interest are peripheral populations which are inherently more sensitive to human influences than core populations because of their isolation and sensitivity to demographic and environmental stochasticity (Chang *et al*., 2005). Compared with core populations, peripheral populations are typically smaller and reside in fragmented and isolated habitats (Vucetich & Waite, 2003). As a consequence, both the rate of gene flow and effective size of peripheral populations are expected to be lower than those of the populations at the core of the species distribution range (Lesica & Allendorf, 1995). Peripheral populations that are isolated can have a higher extinction risk than populations at the core of the species distribution range (Angelone, 2010, Luquet *et al*., 2011). Peripheral populations may also exhibit local adaptations that are found nowhere else in their species' range making them unique reservoirs of biological diversity (García-Ramos & Kirkpatrick, 1997).

In order to devise sensible conservation plans to alleviate possible threats to species conservation and to prioritize different populations with respect to measures to be taken, a clear understanding of genetic structuring of population can be helpful (Frankham *et al*., 2002). Thus, the basic step in postulating a conservation plan for a species is identification of population boundaries (Rowe & Beebee, 2007) and conservation/management units (Fraser & Bernatchez, 2001). Peripheral populations often constitute distinct conservation units (*sensu* Moritz, 1994), and they are often the focus of conservation programs (Lesica & Allendorf, 1992; Hunter Jr & Hutchinson, 1994; Hamilton & Eckert, 2007).

The fire salamander *Salamandra infraimmaculata* is distributed in northern and eastern parts of the Mediterranean

region. It is classified as endangered in Israel (Dolev & Perevolotsky, 2004) and near threatened worldwide (Papenfuss, 2008). The northern Israeli fire salamander populations are at the edge of the species' distribution range and also occupy the southernmost and most xeric habitats of this genus worldwide (Degani, 1996). In fishless water bodies, *Salamandra* larvae function as top predators and can be considered a keystone species (Blaustein, Friedman & Fahima, 1996), thus increasing the species' conservation concern (Petchey *et al*., 2008). Currently, the major threats to *S. infraimmaculata* populations in Israel are of anthropogenic origin such as habitat transformation, road traffic (T. Oron, unpubl. data) and introduced species (Segev, Mangel & Blaustein, 2009).

The aim of this study was to investigate spatial genetic structuring of *S. infraimmaculata* populations in northern Israel and to shed light on the degree of genetic connectivity among these extant populations at the periphery of the species' range. We did this by analyzing variability in 15 nuclear microsatellite loci at 20 breeding sites (i.e. sites in which salamanders deposit larvae) distributed in two geographic regions in northern Israel: Mt. Carmel and the Galilee mountains.

We hypothesized that the two regions are genetically isolated because they are separated by a low elevation and wide valley either because the sharp elevation differences or because land-use transformation that has taken place in the past 100 years in this valley from wetlands to urban and agricultural areas (Bar-Gal & Shamai, 1983). In addition, we hypothesized that *S. infraimmaculata* in the southern, isolated region of Mt. Carmel should exhibit lower genetic variability than the more northern region, the Galilee. Mt. Carmel represents the southernmost limit of *S. infraimmaculata* distribution worldwide while Galilee is on the contiguous periphery of the species' range and thus is comparatively more central in the species' distribution. If these hypotheses were to be supported by our data, then it would suggest that the Mt. Carmel population would deserve a special conservation status instead of being treated similarly to the Galilee population in the contiguous periphery of species range.

Methods

Study species

In Israel, female *S. infraimmaculata* emerge from their summer estivation site beginning with the fall or winter rains, gravid with developed larvae. They use diverse aquatic habitats to deposit their larvae, including rock pools, springs and wells (Degani, 1996). Larvae remain in pools for at least 2–3 months in temporary habitats and a fraction of individuals remain for as much as a full year in permanent breeding sites (Degani, 1996). In permanent water bodies, fish, including the invasive species *Gambusia affinis* can have strong negative effects (Segev *et al*., 2009). Upon metamorphosing, they leave the water. Mature individuals return to pools to breed after reaching reproductive maturity (age 3–5 years; Warburg, 1994). Little is known about the prereproductive, terrestrial stage of *S. infraimmaculata*. Although adults show considerable fidelity to specific breeding sites (Warburg, 2007, Segev *et al*., 2010), it was found that females spread their progeny among different pools (Segev *et al*., 2011) and there is some movement among breeding sites (Bar-David *et al*., 2007). Given the plan for more and larger urban environments, roads and industry in the region containing *S. infraimmaculata*, habitat destruction, degradation and fragmentation increases the concern over the species' status.

Samples and study sites

We sampled a total of 475 adults from 20 breeding sites during the breeding seasons of 2004–2010. Eleven sampled breeding sites were located in Galilee and nine were located in Mt. Carmel region (Fig. 1 and Table 1). The Galilee mountain regions are at the southern border of the apparently contiguous area of *S. infraimmaculata* distribution extending into Lebanon, Syria and Turkey, while the Mt. Carmel region is not contiguous with the rest of the distribution and represents the southernmost populations of the genus. Galilee consists of low mountain ranges that extend from east to west with several ridges and valleys while Mt. Carmel contains just one long ridge system.

A tissue sample (tail tip) was clipped from adults and preserved in 95% ethanol in the field, and subsequently kept at −20°C in the laboratory until further processing. Individuals were promptly released after taking the tissue sample.

DNA extraction and microsatellite analysis

Total DNA from tissue samples was extracted following the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) protocol with the following modifications: Protocol devised RNA free option, over night incubation with proteinase K and resuspension in 150 μL of double-distilled water. Primers for the genetic analysis of 10 microsatellite loci (Sal E2, Sal E5, Sal E6, Sal E7, Sal E8, Sal E11, Sal E12, Sal E14, Sal 3 and Sal 23) were synthesized following Steinfartz, Kusters & Tautz (2004), and the remaining five (SST-A6-I, SST-A6-II, SST-C3, SST-E11 and SST-G6) following Hendrix *et al*. (2010).

Polymerase chain reactions (PCRs) were carried out using the Qiagen Multiplex PCR Kit (Qiagen) in a total volume of 10 μL containing: 1 × Qiagen Multiplex PCR Master mix, 0.5 × Q-Solution, 0.2–0.3 μM of each primer, dH_2O and 10–20 ng of template DNA. One of each primer was end-labeled with a fluorescent dye for visualization of PCR products. PCR products were diluted 1:100 and electrophoresed on MegaBACE 1000 capillary sequencer (Amersham Biosciences, Sunnyvale, CA, USA) and their sizes were determined using Et-ROX400 size standard (Amersham Biosciences). Genotypes were scored using Fragment Profiler ver. 1.2 (Amersham Biosciences) program.

In order to estimate the error rate in the genotyping data set, an independent random sample of about 13% of the

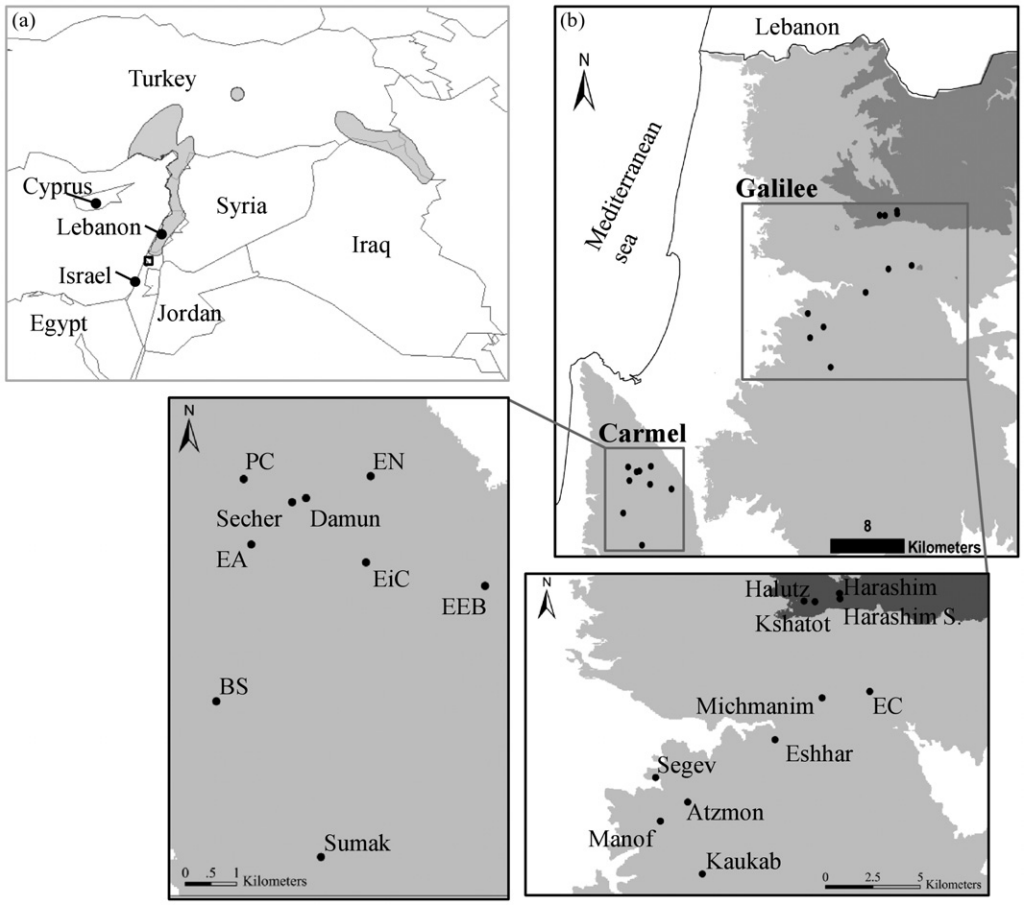

Figure 1 *Salamandra infraimmaculata* distribution range according to International Union for Conservation of Nature. Black frame denotes the study area (a). Location of the study area in northern srael (b). Light gray color represents elevation above 150 m asl and dark gray represent elevation above 700 m asl. Black points represent sampling sites. Abbreviations: Harashim S, Harashim South; EC, Ein Camon; PC, Pine Club; EN, Ein Nesher; EA, Ein Alon; EiC, Ein Chik; EEB, Ein El Balad; BS, Bustan Stream.

individuals ($n = 62$) was rerun and regenotyped. We estimated error rate for each locus by comparing the replicate data sets for differing scores (Pompanon *et al.*, 2005). Estimated error rates (< 6.5%) were deemed acceptable.

Data analyses

We tested for statistically significant linkage disequilibrium among all loci pairs in all subpopulations (i.e. individuals sampled in one breeding site) using FSTAT 2.9.3. Deviations from Hardy–Weinberg equilibrium (HWE) at each locus in each subpopulation were assessed using Genepop (Rousset, 2008) and tested for a heterozygote deficiency within subpopulations for all loci. We adjusted significance levels for multiple tests by using the sequential Bonferroni procedure (Rice, 1989). Possible genotyping errors (i.e. null alleles, large allele dropout and stuttering) were checked using MICRO-CHECKER 2.2.3 software (Van Oosterhout *et al.*, 2004). Frequencies of null alleles were estimated following Brookfield (1996).

To interpret trends in genetic diversity between regions, we calculated the average values of allelic richness, unique alleles and observed (H_O) and expected (H_E) heterozygosities for each of the regions. These statistics were all weighted by sample size. We used a randomization test (1000 permutations, implemented in FSTAT).

To explore the genetic relationships among subpopulations, chord distance (Cavalli-Sforza & Edwards, 1967) was used to construct a neighbor-joining (NJ) tree based on the distance matrix. Bootstrap values were calculated from 1000 matrices of chord distances between the subpopulations. Analyses were done using the Phylip 3.69 package (Felsenstein, 2005). The tree was drawn using TreeView software version 1.6.6 (Page, 1996). STRUCTURE 2.2 (Pritchard, Stephens & Donnelly, 2000) was used to assess population structure without using *a priori* information about an individuals' sampling location. The program uses a model-based Markov chain Monte Carlo algorithm to cluster individuals into metapopulations (clusters) based on multilocus genotype data (Manel, Gaggiotti & Waples, 2005).

Table 1 Study site locations, sample sizes and associated genetic diversity measures

Region	Subpopulation	Longitude	Latitude	*n*	A	Ho	H_E
Galilee	Halutz	32.953°N	35.312°E	23	4.93	0.611	0.624
	Harashim	32.956°N	35.332°E	26	5.4	0.595	0.637
	Harashim South	32.954°N	35.333°E	7	4.2	0.583	0.605
	Kshatot	32.952°N	35.318°E	10	4.4	0.548	0.657
	Ein Camon	32.91°N	35.349°E	35	4.27	0.506	0.599
	Michmanim	32.907°N	35.322°E	6	3.27	0.513	0.535
	Eshhar	32.887°N	35.296°E	30	4.47	0.573	0.592
	Segev	32.869°N	35.229°E	12	4.47	0.604	0.619
	Atzmon	32.857°N	35.247°E	17	4.47	0.519	0.580
	Manof	32.848°N	35.231°E	30	4.93	0.588	0.593
	Kaukab	32.823°N	35.255°E	31	4.2	0.525	0.532
Mt. Carmel	Ein El Balad	32.719°N	35.07°E	33	2.6	0.328	0.301
	Ein Nesher	32.738°N	35.047°E	36	2.13	0.323	0.298
	Ein Chik	32.723°N	35.046°E	55	2.53	0.291	0.314
	Damun	32.734°N	35.033°E	19	2.53	0.326	0.337
	Secher	32.734°N	35.03°E	34	2.53	0.274	0.280
	Pine Club	32.738°N	35.02°E	18	2.07	0.237	0.284
	Ein Alon	32.726°N	35.022°E	27	2.87	0.285	0.317
	Bustan stream	32.698°N	35.014°E	7	2.4	0.330	0.362
	Sumak	32.671°N	35.036°E	19	2.4	0.353	0.341

A, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; *n*, sample size.

For each K (ranging from 1 to 20), we used 10 independent runs to infer the number of genetic populations. Analyses were run using a 50 000 burn-in period, 50 000 iterations and an admixture model assuming correlated allele frequencies. The best fitting model was selected following Evanno, Regnaut & Goudet (2005).

To determine the proportions of the genetic variance due to differences within and among subpopulations, genetic variance was hierarchically assigned according to region using the analysis of molecular variance (AMOVA) using the Arlequin 3.5.1.2 software (Excoffier & Lischer, 2010). This method calculates a standard analysis of variance, in which the total variance is partitioned into covariance components and is used to calculate fixation indices: among clusters relative to the total population (indicated by subscript CT), among subpopulations within clusters (SC), or among subpopulations relative to the total population (ST). AMOVA takes into account the number of mutations between haplotypes, thus we indicated the fixation indices as ϕ_{CT}, ϕ_{SC} and ϕ_{ST}.

Pairwise standard F_{ST} values were used to test for isolation-by-distance among subpopulations within regions. Specifically, we tested the prediction that genetic differentiation would be greater among subpopulations in the isolated Mt. Carmel than among subpopulations in Galilee using conventional F_{ST} (Weir & Cockerham, 1984) and standardized F_{ST} (G'_{ST}). The standardized F_{ST} (G'_{ST}) accounts for heterogeneity in levels of genetic variability and mutation rates among loci (Hedrick, 2005). We determined whether pairwise F_{ST} (and G'_{ST}) between subpopulations correlated with the Euclidian distance using Mantel's test (1000 permutations) implemented in GenAlEx 6 (Peakall & Smouse, 2006). Next, we looked at the genetic differentiation in the Mt. Carmel subpopulations and in the Galilee subpopulations. For this purpose, we evaluated the difference between the regressions of pairwise genetic distances on pairwise Euclidian distances of Mt. Carmel's and Galilee's subpopulations (comparing slope, intercept and mean) using a randomization test (1000 permutations). Because sampling in Galilee covered a broader geographical area than the sampling area in Mt. Carmel, only pairs of subpopulations within the range of Euclidian distances found in Mt. Carmel were used for this test.

Results

No linkage disequilibrium was detected for any pair of loci in the data. Locus L6 deviated significantly from HWE in the Harashim subpopulation and locus L23 in Halutz and Ein Camon subpopulations even after correction for multiple testing. The MICRO-CHECKER analyses suggested that the following loci might be affected by null alleles (null allele frequencies following Brookfield, 1996): locus L14 (0.14) and E11 in Pine Club (0.15), A6II in Chik (0.09), L6 in Harashim (0.11), L8 in Eshhar (0.13) and Harashim (0.1), LG6 in Michmanim (0.23) and Ein Camon (0.24) and L23 in Ein Camon (0.21) and Halutz (0.2). However, this does not suggest a locus-specific problem with null alleles, as for all of these cases, a maximum of two subpopulations of the total 20 were responsible for the significant deviations. Therefore, all the 15 loci were included in the analyses presented below.

Genetic diversity

There were 40 unique alleles (42% of all alleles in this study) in Galilee that were absent in Mt. Carmel, while no unique

alleles were found in Mt. Carmel (Table 2). The average allelic richness and observed and expected heterozygosities were significantly greater in Galilee than in Mt. Carmel (Table 2).

Population structuring

The NJ tree summarizing the overall patterns of genetic distances among subpopulations is shown in Fig. 2. The topology of the NJ tree revealed a distinct clustering of two major groups: one comprising of the subpopulations in the Galilee region and the other consisting of the subpopulations from the Mt. Carmel region (Fig. 2).

The probabilistic clustering of multilocus genotypes to a predefined number of clusters (K) with the program STRUCTURE was congruent with the NJ results; plotting of the values of ln P(X|K), and ΔK, following Evanno *et al.*'s (2005) method indicated that the best fitting model identifies two distinct populations ($K = 2$), which again corresponded to the Galilee and Mt. Carmel subpopulations (Fig. 3).

Table 2 Summary of genetic diversity in fire salamanders from the Galilee and Mt. Carmel

	Galilee	Mt. Carmel	*P* value
Number of sites	11	9	
Number of individuals	227	248	
Unique alleles	40	0	
Allelic richness	3.208	1.944	0.001
Observed heterozygosity	0.563	0.302	0.001
Expected heterozygosity	0.598	0.309	0.001
F_{ST}	0.09	0.064	NS
G'_{ST}	0.231	0.204	NS

NS, not significant.

The AMOVA results provided further evidence for highly significant genetic divergence between Mt. Carmel and Galilee (Table 3). The majority of variation was explained by differences within subpopulations (77.46%, $\phi_{ST} = 0.222$, $P < 0.0001$; Table 3). However, a significant amount of variation was also explained by differences among regions (17.18%, $\phi_{CT} = 0.172$, $P < 0.0001$), with a small, but significant percentage of variation accounted for among subpopulations within regions (5.05%, $\phi_{SC} = 0.061$, $P < 0.0001$; Table 2). This suggests large genetic differences between Mt. Carmel and Galilee with some differences occurring between breeding sites within the two regions.

F_{ST} among subpopulations correlated positively with Euclidian distance across the total study area ($r = 0.80$, $P < 0.001$) and within Galilee ($r = 0.25$, $P = 0.028$) and Mt. Carmel regions ($r = 0.40$, $P < 0.001$) separately (Fig. 4). G'_{ST} estimates gave similar results (results not shown). Thus, when using only the Galilee pairs of subpopulations within the range of Euclidian distances between the Mt. Carmel pairs of subpopulations, both the *y*-intercept and slope did not differ significantly between Galilee and Mt. Carmel ($P = 0.17$ and $P = 0.23$, respectively).

Discussion

Our study demonstrates that there is considerable genetic heterogeneity among the *S. infraimmaculata* populations in

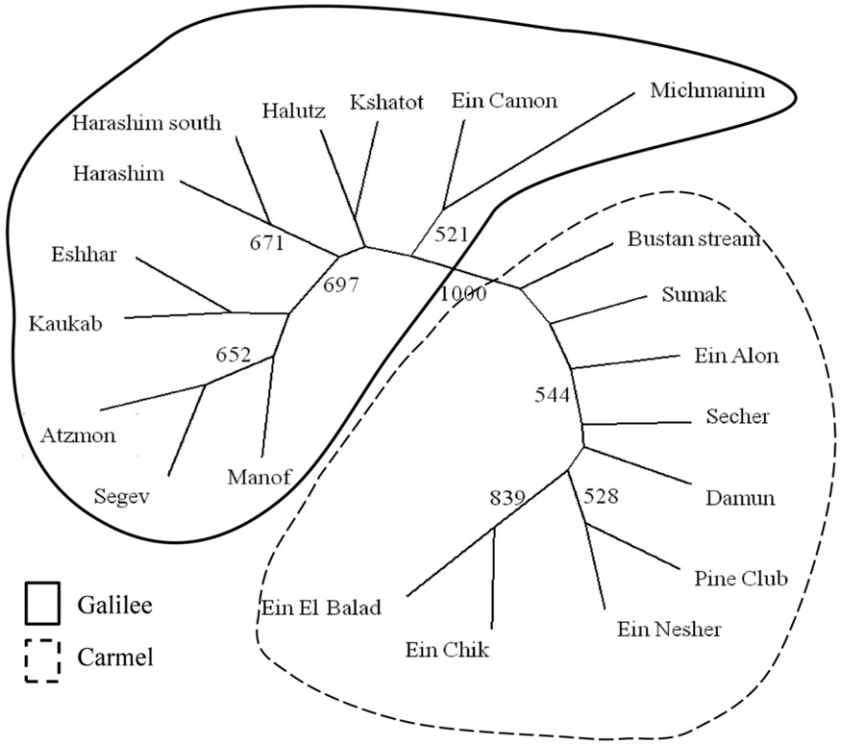

Figure 2 Unrooted neighbor-joining tree comparing all subpopulations based on Cavalli-Sforza and Edwards' chord distances. Numbers correspond to the consensus value of the adjacent node, based on 1000 bootstrap matrices. Only bootstrap values greater than 500 are reported.

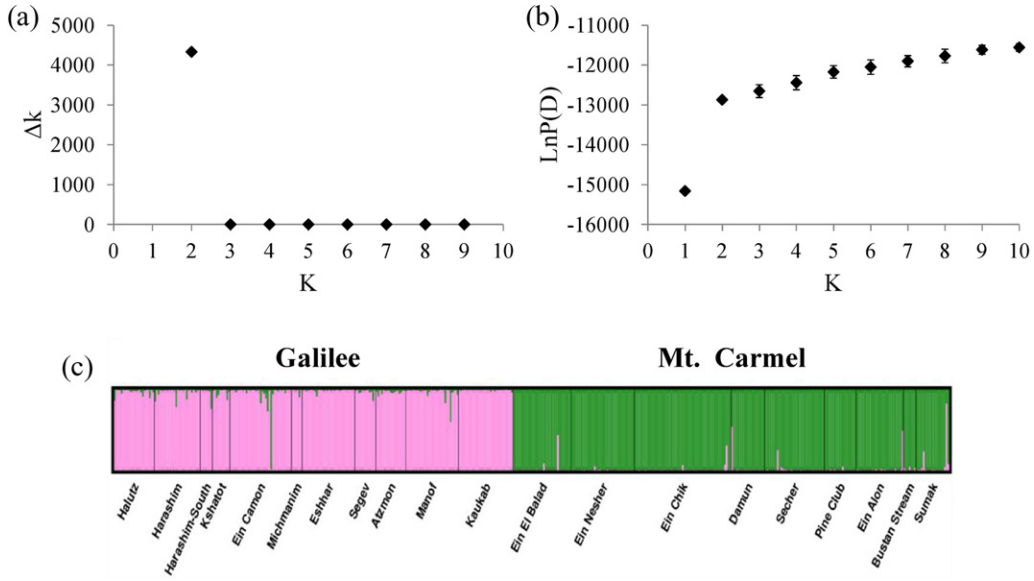

Figure 3 Results of the STRUCTURE analyses showing values of ΔK (a) and ln P(X|K) (±SD), second order rate of change (Evanno *et al.*, 2005), as a function of the number of clusters (K) (b) and population clustering (c). Because a steady asymptote was found, only 1–10 clusters were presented in graphs a and b.

Table 3 Results of the analysis of molecular variance analysis of genetic variability in fire salamanders

	Percentage of variation	Fixation index	*P* value
Among regions (clusters) relative to the total population (ϕ_{CT})	17.18	0.172	< 0.0001
Among subpopulations within regions (clusters) (ϕ_{SC})	5.05	0.061	< 0.0001
Among subpopulations relative to the total population (ϕ_{ST})	77.46	0.222	< 0.0001

northern Israel. The different analyses that we used all lead to the same robust conclusion – the existence of a strong barrier to gene flow between Galilee and Mt. Carmel regions. Additional tests not shown here for brevity [principal component analysis (Goudet, 1999) and barrier analysis (Manni, Guerard & Heyer, 2004)] also yield the same consistent results. Below, we elaborate on these issues as well as discuss some of the assumptions underlying the results.

Population structure

Bayesian clustering, NJ tree analysis and AMOVA, coupled with estimates of allelic richness, expected heterozygosity and the number of unique alleles, all revealed the existence of two fire salamander populations within the area covered in our study. Mt. Carmel represents the southernmost limit of *S. infraimmaculata*'s distribution worldwide and is a geographically isolated region. Our results demonstrate that the Mt. Carmel's population is a genetically isolated peripheral population as compared with the population of the Galilee region. Only 3 out of 475 individuals were assigned to the region for which they were not found by STRUCTURE analysis. This suggests that gene flow between Galilee and Mt. Carmel in both directions is very limited. It is possible that the little gene flow that does occur is due to translocations conducted by humans.

Changes in land use can explain the divergence between the Mt. Carmel and Galilee populations. In the past 150 years, extensive urbanization has taken place in the low area between Galilee and Mt. Carmel regions. Heavy road traffic in this area would present high mortality risk to fire salamanders as has been demonstrated for *S. infraimmaculata* in the upper Galilee (T. Oron, unpubl. data) and for the congeneric *S. salamandra* in the Iberian Peninsula (Garriga *et al.*, 2012). Scattered swamps once located in the valley, which could serve as breeding sites, were drained about 100 years ago when this plain was transformed into agricultural land (Bar-Gal & Shamai, 1983). Thus, a possible explanation for the reduced genetic variation in the Mt. Carmel may be that Mt. Carmel's population became progressively more isolated from the core populations, leading to increased genetic drift and concomitant loss of alleles. This seemingly short time frame can lead to population differentiation, because the degree of genetic differentiation is not just a function of the time since isolation, but also a function of the variance effective sizes. Population subdivision can greatly increase the variance effective size of the total population while simultaneously decrease the variance effective sizes of subpopulations (Templeton, 2006). Research on the collared lizard provides a clear example of rapid genetic differentiation among populations over relatively short spaces and time periods when dispersal is highly restricted. Collared lizards

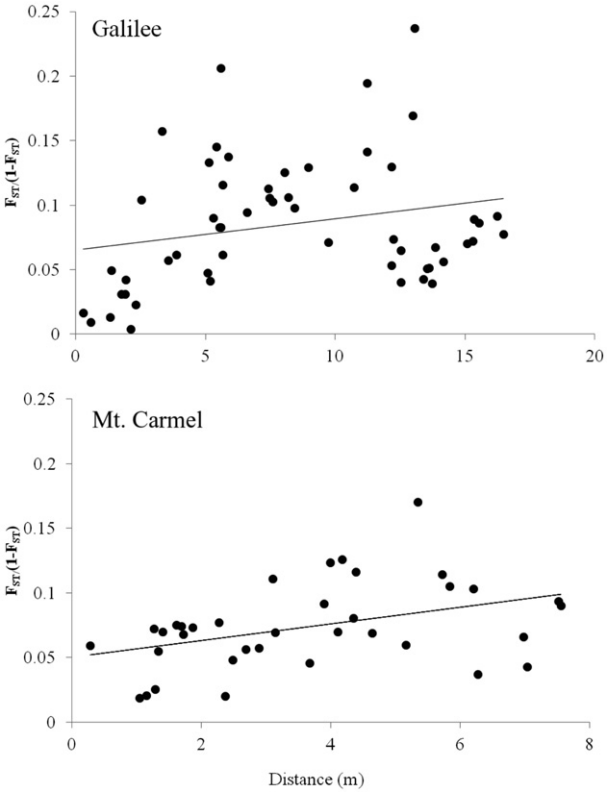

Figure 4 Relationship between pairwise linearized F_{ST} and geographic (Euclidean) distance within the Galilee and the Mt. Carmel regions. Note differences in the scales of the *x*-axes.

use glades (open rocky habitats) that are patchily embedded within a matrix of forest. Interglade dispersal can only occur after normally impenetrable forests are burned. Suppression of forest fires starting in the 1940s lead to extreme interpopulation differentiation within 40–50 years (Hutchison & Templeton, 1999, Templeton *et al*., 2007).

It can be anticipated that the multiridged Galilee would result in more population subdivision than the more homogeneous Mt. Carmel that consists of just one long ridge system. Our results give some indications to support this: in the NJ tree analysis, most sites in Mt. Carmel split from the main branch while all sites in Galilee split from sub-branches. Additionally, the F_{ST} estimate among Galilee subpopulations exceeded that of Mt. Carmel's subpopulations, albeit not significantly so (Table 3). Hence, even if there were no reduction in total population size, we would predict that the total Mt. Carmel population would have a lower variance effective size than the total Galilee population. Mt. Carmel is a smaller geographical area than Galilee, so it is likely to imply a smaller census size, further accentuating a lower total variance effective size in Mt. Carmel relative to Galilee. In addition, there may have been a possible founder event for the Mt. Carmel population, resulting in a lower genetic variation for the Mt. Carmel subpopulations whose effects could persist into the present. All of these factors may have contributed to the comparatively lower diversity on Mt. Carmel and the genetic differentiation between the two regions.

It is also possible that the isolation of Mt. Carmel's population is not the result of a current process and land use developments in the area, but rather, arises from the existence of long-term geographic barriers. A possible geographic barrier could be the low elevation valley that separates Galilee and Mt. Carmel regions. Although there were some observations more than 40 years ago of isolated pockets of Salamandra larvae along the low elevation edge of Mt Carmel, but never in other parts of the extensive lowland (R. Ortal, personal observations), salamanders are currently found only at higher elevations (Goldberg *et al*., 2007; Blank & Blaustein, 2012). Thus, elevation differences among *S. infraimmaculata* populations may have limited gene flow and dispersal. Movement between low to high elevation might be limited because of the energetic costs that accompany crossing ridges, or by increased predation risk associated with it. An additional natural barrier could be the bedrock type. While the dominant bedrocks in Mt. Carmel and Galilee are limestone and dolomite, the area between these regions is mostly chalk. The differences in bedrock affect, for example, the plant community (Kruckeberg, 2004) and water-holding capacity of the soil (Schiller *et al*., 2010) which might explain the *S. infraimmaculata* distribution. Further studies focused on actual dispersal behavior and habitat choice during dispersal could shed light on this possibility.

In contrast to the substantial differentiation between Galilee and Mt. Carmel regions, there was little differentiation between subpopulations within Mt. Carmel and within Galilee regions, which provides evidence for connectivity among subpopulations within each region. Several recent studies have demonstrated that amphibians may move considerably further than thought previously (Trenham, Koenig & Shaffer, 2001; Smith & Green, 2005). Schmidt, Schaub & Steinfartz (2007) found that average distance between successive recaptures of *S. salamandra*, a closely related species to *S. infraimmaculata* (Steinfartz *et al*. 2000), ranged from 4 to 319 m. Bar-David *et al*. (2007) found that the Euclidean distances between capture and recapture sites of *S. infraimmaculata* individuals was as long as 1280 m. Long-distance movements are important components of an organism's life history, and they influence gene flow and population dynamics and persistence. Increased migration rates between populations may lower the probability of local extinction (Brown & Kodric-Brown, 1977) and increase long-term persistence of the total population (Hanski & Gilpin, 1997). As a result, viability of amphibian populations often depends on connectivity between subpopulations (Marsh & Trenham, 2001). In light of the low within-region genetic differentiation in this study, it appears that there is significant gene flow among local fire salamander subpopulations within each region.

Overall, our results indicate low genetic diversity of the Mt. Carmel population that can be explained by strong genetic drift, possibly stemming from a bottleneck and/or a founder effect. The lack of new mutations in the Mt. Carmel population – together with the low degree of differentiation between subpopulations with the region – suggests a relatively recent founding or isolation event for the Mt. Carmel

population. The low degree of differentiation between the Mt. Carmel subpopulations parallels the low differentiation among populations of the salamander *Plethodon cinereus* that formerly colonized glaciated areas in North America (Larson, 1984). Hence, our results can be the results of a twin effect of isolation and bottleneck: extreme genetic differentiation between source (Galilee) and founder (Mt. Carmel) populations, and low degree of genetic differentiation among subpopulations established by an expanding founder population.

Implication for conservation

Currently, the largest threats to populations of *S. infraimmaculata* in Israel are likely posed by anthropogenic activities such as habitat destruction and loss, road traffic (T. Oron, unpubl. data) and introduced species (Segev *et al*., 2009). In order to devise sensible conservation plans to alleviate these threats and prioritize different populations with respect to measures to be taken, a clear understanding of genetic structuring of population can be helpful (Frankham *et al*., 2002). To this end, the observed high degree of genetic differentiation among *S. infraimmaculata* populations in northern Israel should provide relevant information for conservation planning. The isolated Mt. Carmel region is also situated further away from the core population of this species, and retains only a small proportion of the genetic variability present within the Galilee population. When there is no movement between regions – as appears to be the case here in light of our results – the demographic attributes are determined by the local subpopulation sizes, which are substantially smaller than the overall population size. Very limited (or no) movement of the locally endangered *S. infraimmaculata* between Mt. Carmel and Galilee regions, as revealed in our study, might increase the probability of local extinction (Brown & Kodric-Brown, 1977) and decrease long-term persistence of Mt. Carmel population (Hanski & Gilpin, 1997). Considering the low genetic diversity and limited gene flow to the Mt. Carmel region, and because isolated peripheral populations are vulnerable to fitness loss (Angelone, 2010; Luquet *et al*., 2011), conservation measures such as translocations or supplementation of Mt. Carmel population from Galilee sources might be taken into consideration in the future. However, before any translocation are done, it will be important to evaluate whether the Mt. Carmel and Galilee populations have evolved any local adaptations and/or differentiation other than that seen in the neutral markers genes analyzed here. In other words, although our analyses suggests that Mt. Carmel and Galilee populations of fire salamanders constitute two distinct conservation units on the basis of neutral genetic markers (cf. Fraser & Bernatchez, 2001; Frankham *et al*., 2002, Boessenkool *et al*., 2009), it remains unclear to what degree they show ecological exchangeability (Crandall *et al*., 2000). Thus, we suggest that the Galilee and Mt. Carmel fire salamanders should be considered as separate management units for conservation.

Acknowledgments

This study was funded by ISF grant 961–2008 awarded to L. Blaustein and S. Bar-David and grants 129662 and 134728 from the Academy of Finland awarded to J. Merilä. We also acknowledge the Vatat scholarship provided by the Israel Council for Higher Education and the European Erasmus Mundus program provided by the European commission awarded to L. Blank. This research was partly funded by the Israel Nature and Park Authority. The samples were collected under license from the Israel Nature and Parks Authority (permit 2009/36565). We thank Scott McCairns, Arne Nolte, Sebastian Steinfartz for fruitful discussions and Marika Karjalainen, Kirsi Kähkönen, Amelid Fahum and Tamar Krugman for their help with laboratory work.

References

Allendorf, F.W. & Luikart, G. (2007). *Conservation and the genetics of populations*. Malden: Blackwell Publishing.

Angelone, S. (2010). Are differences in fitness traits related to genetic clusters? An empirical test on the European tree frog. *Biol. Conserv.* **143**, 471–478.

Bar-David, S., Segev, O., Peleg, N., Hill, N., Templeton, A.R., Schultz, C.B. & Blaustein, L. (2007). Long-distance movements by fire salamanders (*Salamandra infraimmaculata*) and implications for habitat fragmentation. *Isr. J. Ecol. Evol.* **53**, 143–159.

Bar-Gal, Y. & Shamai, S. (1983). The swamps of Emek Yizre'el – myth and reality. *Cathedra* **27**, 163–174.

Blank, L. & Blaustein, L. (2012). Using ecological niche modeling to predict the distributions of two endangered amphibian species in aquatic breeding site. *Hydrobiologia* **693**, 157–167.

Blaustein, L., Friedman, J. & Fahima, T. (1996). Larval Salamandra drive temporary pool community dynamics: evidence from an artificial pool experiment. *Oikos* **76**, 392–402.

Boessenkool, S., Star, B., Waters, J.M. & Seddon, P.J. (2009). Multilocus assignment analyses reveal multiple units and rare migration events in the recently expanded yellow-eyed penguin (*Megadyptes antipodes*). *Mol. Ecol.* **18**, 2390–2400.

Brookfield, J.F.Y. (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol. Ecol.* **5**, 453–455.

Brown, J.H. & Kodric-Brown, A. (1977). Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology* **58**, 445–449.

Cavalli-Sforza, L.L. & Edwards, A.W.F. (1967). Phylogenetic analysis. Models and estimation procedures. *Am. J. Hum. Genet.* **19**, 233–257.

Chang, C.S., Choi, D.Y., Kim, H., Park, T.Y. & Kim, Y.S. (2005). Patterns of allozyme variation in relation to population size of the threatened plant *Megaleranthis saniculifolia* (Ranunculaceae) in Korea. *J. Plant Biol.* **48**, 339–350.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M. & Wayne, R.K. (2000). Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.* **15**, 290–295.

Degani, G. (1996). *Salamandra salamandra at the southern limit of its distribution*. Kazrin: Laser Pages Publication.

Dolev, A. & Perevolotsky, A. (2004). *The red book – vertebrates in Israel*. Jerusalem: Israel Nature and Parks Authority and the Society for the Protection of Nature in Israel.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620.

Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour.* **10**, 564–567.

Felsenstein, J. (2005). *PHYLIP (phylogeny inference package) version 3.6*. Seattle: Department of Genome Sciences, University of Washington.

Frankham, R., Briscoe, D.A. & Ballou, J.D. (2002). *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Fraser, D.J. & Bernatchez, L. (2001). Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol. Ecol.* **10**, 2741–2752.

García-Ramos, G. & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution* **51**, 21–28.

Garriga, N., Santos, X., Montori, A., Richter-Boix, A., Franch, M. & Llorente, G.A. (2012). Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodivers. Conserv.* **21**, 2761–2774.

Goldberg, T., Pearlson, O., Nevo, E. & Degani, G. (2007). Mitochondrial DNA analysis of *Salamandra infraimmaculata* larvae from habitats in northern Israel. *Progrese şi Perspective in Medicina Veterinară – Lucrări ştiintifice* **50**, 23–31.

Goudet, J. (1999). *PCA-Gen version 1.2*. Lausanne: Institute of Ecology, Biology Building, University of Lausanne (UNIL).

Hamilton, J.A. & Eckert, C.G. (2007). Population genetic consequences of geographic disjunction: a prairie plant isolated on Great Lakes alvars. *Mol. Ecol.* **16**, 1649–1660.

Hanski, I.A. & Gilpin, M.E. (1997). *Metapopulation biology: ecology, genetics and evolution*. San Diego: Academic Press.

Hedrick, P.W. (2005). A standardized genetic differentiation measure. *Evolution* **59**, 1633–1638.

Hendrix, R., Hauswaldt, J.S., Veith, M. & Steinfartz, S. (2010). Strong correlation between cross-amplification success and genetic distance across all members of 'True Salamanders' (Amphibia: Salamandridae) revealed by *Salamandra salamandra* specific microsatellite loci. *Mol. Ecol. Resour.* **10**, 1038–1047.

Hunter, M.L. Jr & Hutchinson, A. (1994). The virtues and shortcomings of parochialism: conserving species that are locally rare, but globally common. *Conserv. Biol.* **8**, 1163–1165.

Hutchison, D.W. & Templeton, A.R. (1999). Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution* **53**, 1898–1914.

Kruckeberg, A.R. (2004). *Geology and plant life: the effects of landforms and rock types on plants*. Seattle, Washington: University of Washington Press.

Larson, A. (1984). Neontological inferences of evolutionary pattern and process in the salamander family Plethodontidae. *Evol. Biol.* **17**, 119–217.

Lesica, P. & Allendorf, F.W. (1992). Are small populations of plants worth preserving? *Conserv. Biol.* **6**, 135–139.

Lesica, P. & Allendorf, F.W. (1995). When are peripheral populations valuable for conservation? *Conserv. Biol.* **9**, 753–760.

Luquet, E., Léna, J.P., David, P., Joly, P., Lengagne, T., Perrin, N. & Plénet, S. (2011). Consequences of genetic erosion on fitness and phenotypic plasticity in European tree frog populations (*Hyla arborea*). *J. Evol. Biol.* **24**, 99–110.

Manel, S., Gaggiotti, O.E. & Waples, R.S. (2005). Assignment methods: matching biological questions with appropriate techniques. *Trends Ecol. Evol.* **20**, 136–142.

Manni, F., Guerard, E. & Heyer, E. (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Hum. Biol.* **76**, 173–190.

Marsh, D.M. & Trenham, P.C. (2001). Metapopulation dynamics and amphibian conservation. *Conserv. Biol.* **15**, 40–49.

Moritz, C. (1994). Defining 'evolutionarily significant units' for conservation. *Trends Ecol. Evol.* **9**, 373–375.

Page, R.D.M. (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **12**, 357–358.

Papenfuss, T. (2008). *Salamandra infraimmaculata*. In: IUCN 2008. Red List of Threatened Species. http://www.iucnredlist.org/details/59466/0.

Peakall, R.O.D. & Smouse, P.E. (2006). GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**, 288–295.

Petchey, O.L., Eklöf, A., Borrvall, C. & Ebenman, B. (2008). Trophically unique species are vulnerable to cascading extinction. *Am. Nat.* **171**, 568–579.

Pompanon, F., Bonin, A., Bellemain, E. & Taberlet, P. (2005). Genotyping errors: causes, consequences and solutions. *Nat. Rev. Genet.* **6**, 847–859.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rousset, F. (2008). genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol. Ecol. Resour.* **8**, 103–106.

Rowe, G. & Beebee, T.J.C. (2007). Defining population boundaries: use of three Bayesian approaches with microsatellite data from British natterjack toads (*Bufo calamita*). *Mol. Ecol.* **16**, 785–796.

Schiller, G., Ungar, E.D., Cohen, S. & Herr, N. (2010). Water use by Tabor and Kermes oaks growing in their respective habitats in the Lower Galilee region of Israel. *For. Ecol. Manage.* **259**, 1018–1024.

Schmidt, B.R., Schaub, M. & Steinfartz, S. (2007). Apparent survival of the salamander *Salamandra salamandra* is low because of high migratory activity. *Front. Zool.* **4**, 19. doi: 10.1186/1742-9994-4-19.

Segev, O., Mangel, M. & Blaustein, L. (2009). Deleterious effects by mosquitofish (*Gambusia affinis*) on the endangered fire salamander (*Salamandra infraimmaculata*). *Anim. Conserv.* **12**, 29–37.

Segev, O., Hill, N., Templeton, A.R. & Blaustein, L. (2010). Population size, structure and phenology of an endangered salamander at temporary and permanent breeding sites. *J. Nat. Conserv.* **18**, 189–195.

Segev, O., Mangel, M., Wolf, N., Sadeh, A., Kershenbaum, A. & Blaustein, L. (2011). Spatiotemporal reproductive strategies in the fire salamander: a model and empirical test. *Behav. Ecol.* **22**, 670–678.

Smith, M.A. & Green, D.M. (2005). Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **28**, 110–128.

Steinfartz, S., Veith, M. & Tautz, D. (2000). Mitochondrial sequence analysis of Salamandra taxa suggests old splits of major lineages and postglacial recolonizations of Central Europe from distinct source populations of *Salamandra salamandra*. *Mol. Ecol.* **9**: 397–410.

Steinfartz, S., Kusters, D. & Tautz, D. (2004). Isolation and characterization of polymorphic tetranucleotide microsatellite loci in the fire salamander *Salamandra salamandra* (Amphibia: Caudata). *Mol. Ecol. Notes* **4**, 626–628.

Templeton, A.R. (2006). *Population genetics and microevolutionary theory*. Hoboken: John Wiley & Sons, Inc.

Templeton, A.R., Neuwald, J.L., Brazeal, H. & Robertson, R.J. (2007). Invited minireview: restoring demographic processes in translocated populations: the case of collared lizards in the Missouri Ozarks using prescribed forest fires. *Isr. J. Ecol. Evol.* **53**, 179–196.

Trenham, P.C., Koenig, W.D. & Shaffer, H.B. (2001). Spatially autocorrelated demography and interpond dispersal in the salamander *Ambystoma californiense*. *Ecology* **82**, 3519–3530.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* **4**, 535–538.

Vucetich, J.A. & Waite, T.A. (2003). Spatial patterns of demography and genetic processes across the species' range: null hypotheses for landscape conservation genetics. *Conserv. Genet.* **4**, 639–645.

Warburg, M.R. (1994). Population ecology, breeding activity, longevity, and reproductive strategies of *Salamandra salamandra* during an 18-year long study of an isolated population on Mt. Carmel, Israel. *Mertensiella* **4**, 399–421.

Warburg, M.R. (2007). The phenology of a rare salamander (*Salamandra infraimmaculata*) in a population breeding under unpredictable ambient conditions: a 25 year study. *Acta Herpetol.* **2**, 147–157.

Weir, B.S. & Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.