Landscape Ecol (2019) 34:2615–2630
https://doi.org/10.1007/s10980-019-00908-6

RESEARCH ARTICLE

Topographical features and forest cover influence landscape connectivity and gene flow of the Caucasian pit viper, *Gloydius caucasicus* (Nikolsky, 1916), in Iran

Roya Adavodi · Rasoul Khosravi · Samuel A. Cushman · Mohammad Kaboli

Received: 21 October 2018 / Accepted: 15 September 2019 / Published online: 27 September 2019

Abstract
Context The populations of the Caucasian pit viper are declining and face genetic threats because of habitat fragmentation. Landscape genetics facilitates effective connectivity conservation actions by providing methods to predict factors affecting gene flow.
Objectives We described the genetic diversity and structure of Caucasian pit viper populations in Iran. We also adopted an individual-based landscape genetics approach to determine the effects of landscape features on gene flow across the species' range.
Methods We evaluated the degree of genetic structuring using spatial and non-spatial clustering methods. Then, we used restricted multivariate optimization and maximum-likelihood population effects modeling to predict landscape resistance to gene flow. Finally, we compared the predictions based on maximum-likelihood population effects modeling and reciprocal causal modelling in the context of multivariate optimization.
Results Strong genetic structure was found between populations. Landscape genetics analysis showed that gene flow was related to forest cover density and topographic roughness. Gene flow was much higher in areas of low topographical roughness and was impeded by closed forests. We found an overall high similarity in multivariate optimization results obtained through reciprocal causal modeling and maximum-likelihood population effects modeling, suggesting that both approaches may produce consistent predictions.
Conclusions Caucasian pit viper has limited dispersal abilities resulting in genetic structuring at short distances and gene flow appears to be influenced by natural features, in particular topographic roughness and forest cover density. Our findings have important implications for the species' conservation and can be used to develop empirically supported prioritization of core habitats and corridors.

Keywords Caucasian pit viper · Gene flow · Landscape genetics · Landscape resistance · Multivariate optimization

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10980-019-00908-6) contains supplementary material, which is available to authorized users.

R. Adavodi · M. Kaboli (✉)
Department of Environmental Sciences, Faculty of Natural Resources, University of Tehran, Karaj, Iran
e-mail: mkaboli@ut.ac.ir

R. Khosravi
Department of Natural Resources and Environmental Engineering, School of Agriculture, Shiraz University, Shiraz, Iran

S. A. Cushman
Rocky Mountain Research Station, USDA Forest Service, Flagstaff, AZ, USA

Introduction

Spatial patterns of genetic variation and gene flow among subpopulations may be affected by geography (Cox et al. 2012), sex-biased dispersal (Fontenot et al. 2011), and natural and anthropogenic landscape factors (Cushman et al. 2006). Identifying factors influencing population connectivity is essential to delineate barriers to gene flow and to mitigate current and future threats to persistence of populations (Cushman et al. 2012). Landscape genetics provides a valuable framework to infer how landscape features and anthropogenic perturbations impede or facilitate gene flow between populations (Balkenhol et al. 2016).

The few studies that have been conducted on the spatial patterns of genetic variation of snake species have shown that genetic structuring in these species may be due to geographic distance (Klug et al. 2011), sex-biased dispersal (Keogh et al. 2007), habitat cover type (Sunny et al. 2015) and urbanization (Gangloff et al. 2017). The cryptic life of most snake species increases difficulty of investigating dispersal and gene flow using mark-recapture methods. Landscape genetics is an alternative method to observational studies of gene flow and population genetic structure that is particularly useful for rare or cryptic species (Schwartz et al. 2009).

Maximum resistance of landscape variables (RMax) and a power function relating the landscape variable to resistance (P) are two main parameters that are often considered in isolation by resistance hypotheses (IBR; McRae 2006). Hence, optimizing resistance models in terms of the correct variables contributing to resistance, their effect size (RMax), and their functional form (P) is important to obtaining reliable and correct inferences in landscape genetics researches (Reddy et al. 2017; Shirk et al. 2018). In recent years, a variety of methods have been developed to identify the correct resistance model among different alternative models of landscape resistances such as regression-based methods (Shirk et al. 2018) and Bayesian statistics (Guillot et al. 2005). The final goal of any statistical method is to select (i) the most important variables in resistance surfaces and (ii) the magnitude of RMax and P of each variable in resistance surfaces (Shirk et al. 2018). Correlation among different hypotheses of landscape resistance, the degree of gene flow and level of genetic structure in the study population, and instability in genetic structure of the populations may lead to difficulty in selecting the best model among different competing resistance models (Cushman et al. 2013; Zeller et al. 2016). Shirk et al. (2018) evaluated seven regression-based selection algorithms based on the accuracy in predicting the correct landscape variables, RMax and P. They suggested that maximum-likelihood population effects model (MLPE) had the best performance in extracting true landscape features. Zeller et al. (2016) and Reddy et al. (2017) suggested that the combination of restricted multivariate optimization (Shirk et al. 2010) with reciprocal causal modelling (RCM; Cushman et al. 2013) in an individual-based landscape genetics framework provides better identification of drivers of genetic differentiation than several other approaches. Also, Shirk et al. (2018) suggested that if landscape genetics analysis done using the indices of model fit instead of significance testing, the evaluation of Mantel-based methods are not sensitive to critiques relating to Mantel-based model selection criteria. Here, we quantitatively compared results of multivariate restricted optimization using MLPE to that of RCM to predict landscape resistance.

The halys pit vipers are a venomous and viviparous group of snakes belonging to the Viperidae family. The genus *Gloydius* is divided into three complex groups including *G. halys/G. intermedius*, *G. blomhoffii* and *G. strauchi* (Wagner et al. 2016). *Gloydius caucasicus* belongs to *G. halys/G. intermedius* complex. These vipers are widely distributed across various biotopes on a broad territory and have a vast distribution in many parts of Asia (Orlov and Barabanov 1999; Wagner et al. 2016). The Caucasian pit viper is the only member of the Crotalinae subfamily present in Iran, and is distributed from northwestern to northeastern Iran in a wide variety of terrestrial and mountainous forest and bush land habitats (Latifi 2000). Two morphs of the Caucasian pit viper are observed in northern Iran including the gray and red morphs (Fig. 1). The populations of the species in Iran are declining and face genetic threats from small population sizes as a result of overhunting, habitat loss and fragmentation. As snake metapopulations often depend on gene flow and dispersal across heterogeneous landscapes, efficient conservation of the remaining populations requires knowledge about landscape effects on genetic variation, population

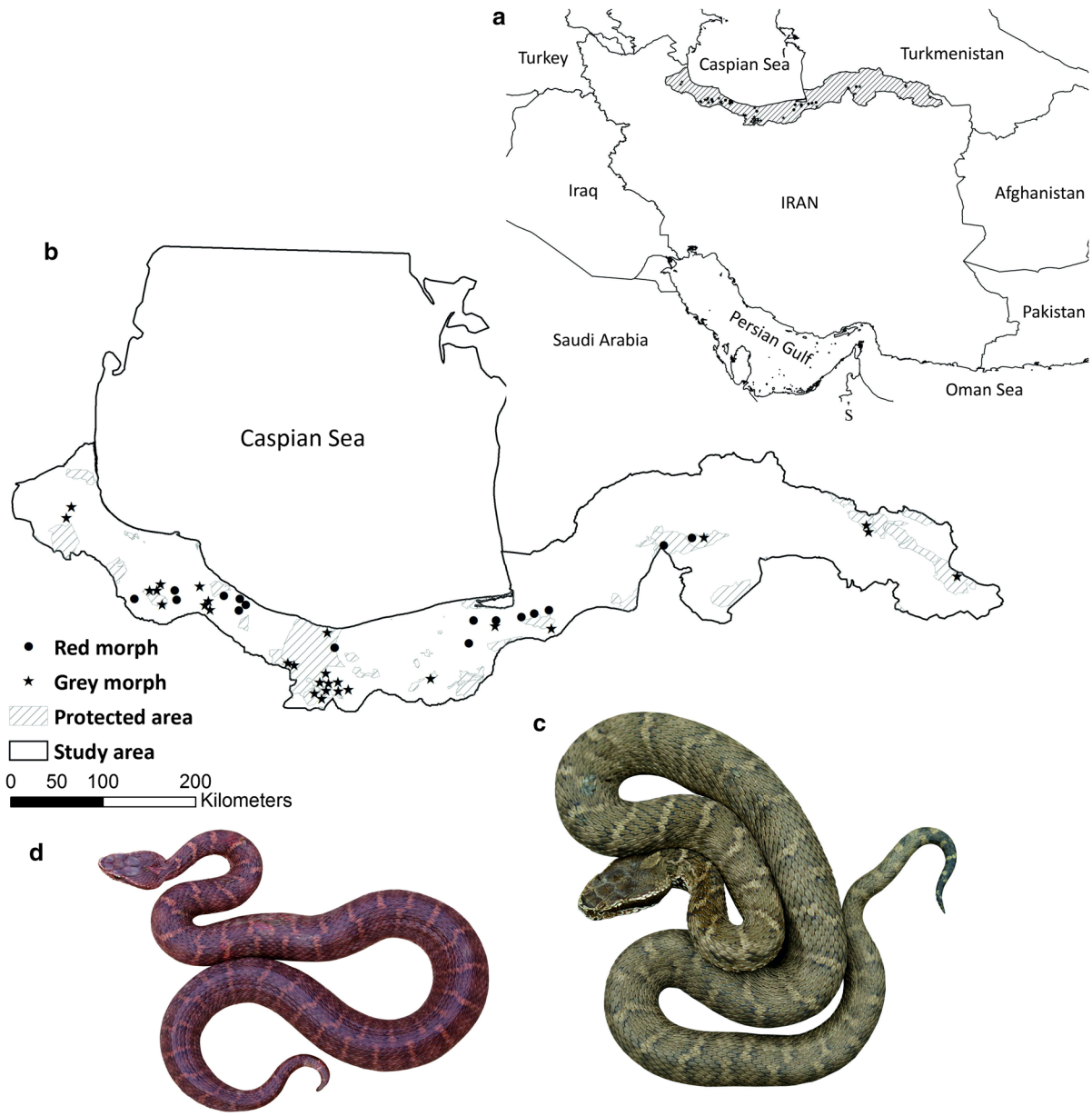

Fig. 1 Hatched area refers to the study landscape in the north of Iran (**a**), location of the 49 genetic samples collected in the study area (**b**), gray (**c**) and red (**d**) morphs of the Caucasian pit viper. (Color figure online)

structure and functional connectivity. As the forest landscape of the species in northern Iran is highly fragmented, we hypothesized that natural and anthropogenic factors would have influenced metapopulation structure.

In this study, we conducted the first fine-scale, individual-based landscape genetics analysis that addresses (1) the level of genetic variation and fine-scale population genetic structure of Caucasian pit viper populations, and (2) the effect of landscape features on gene flow and interaction among subpopulations. We used maximum-likelihood population effects model and reciprocal causal modelling to optimize the landscape parameters (i.e., RMax and P) and to identify the relationship between landscape factors and gene flow. As the Caucasian pit viper

inhabits a wide altitude range (30 to 3000 m), it may act as a surrogate species. Therefore, conservation of viable and ecologically functional populations of the species may provide an umbrella for other co-existing species in northern Iran. Also, the results of this study can provide critical knowledge to fill a gap in scientific knowledge about the genetic structure and gene flow of the Caucasian pit viper and to guide future work to develop effective conservation and management solutions for the species.

Materials and methods

Study area and sampling

The Caucasian pit viper occurs in a variety of habitats in northern Iran. The Alborz Mountain ranges, which extend from the north-west to the north-east, prevent moisture bearing systems to pass through to the south. Hence, the northern slopes of the Alborz Mountains receive high amounts of precipitation and are naturally covered by closed Hyrcanian forests. A detailed description of the studied landscape is given in Asadi et al. (2019).

We collected genetic samples from 49 *G. h. caucasicus* between 2014 and 2017 from all recognized habitats across its distribution range (Fig. 1). Tissue samples were obtained from carcasses of snakes that were victims of poaching, hunting, disease, road accidents and from live specimens collected during foot searches. The samples obtained from live specimens consisted of pieces of ventral scales clips or tail tips. Locations of individual samples were recorded using a global positioning system. All samples were stored in 96% ethanol prior to DNA extraction.

DNA extraction and PCR amplification

Total genomic DNA from ventral scale and tissue samples was extracted using standard proteinase k digestion and phenol–chloroform protocols (Sambrook et al. 1989). The quality and quantity of extracted DNA were evaluated by Nanodrop. We employed seven dinucleotide species-transferred microsatellites developed for Crotalinae species to characterize genetic diversity and structure of the species. The PCR amplification was done using QIAGEN PCR MasterMix in optimized PCR conditions. A detailed description of the PCR conditions is given in Text S1 and Table S1 in Online Resource 1. Fragment analysis was carried out using ABI3730xl (Macrogen Inc., South Korea). Alleles were scored using Geneious R9 (9.0.5; Biomatters Ltd.) under standard run conditions with LIZ500 as the internal size standard.

Genetic structure and dispersal events among populations

The probability of a set of allelic frequencies given K populations was determined using the admixture model with correlated allele frequencies, K-values between 1 and 7, 10 separate iterations for each K with 1,000,000 MCMC iterations and burn-in 100,000 within STRUCRURE as a non-spatially-explicit method (Pritchard et al. 2000) without location priors. In addition, Structure Harvester (Earl and Vonholdt 2012) was used to discern the optimal number of clusters and visualize the results of optimal K. The CLUMPP program (Jakobsson and Rosenberg 2007) was used to summarize individual assignment probabilities and to find a close match among multiple independent runs for the most likely K.

We also used R package GENELAND as a spatially-explicit method to characterize the spatial distribution of genetic diversity of the species across the landscape. We ran GENELAND ten times using Dirichlet allele frequencies, 400,000 MCMC iterations, and by varying the number of putative populations from 1 to 7. The number of Poisson processes was set equal to the number of samples (Guillot 2008). As the assignment tests indicated genetic population structure, we computed pairwise population estimates of F_{ST} (Wright 1969) to describe the genetic structure of Caucasian pit vipers. Pairwise F_{ST} values were tested for significance via 10,000 permutations in FSTAT.

Genetic diversity

We statistically tested genotypic data for null alleles, large allele drop-out, and scoring error using the Brookfield-1 equation within MICRO-CHECKER (Van Oosterhout et al. 2004). All genotypes were screened for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium for all pairs

of loci using Markov chain approximation within GENEPOP (Raymond and Rousset 1995). We controlled for increased Type I error rates due to multiple testing with sequential Bonferonni–adjusted P =0.002. We used GENEPOP to obtain standard indices of genetic diversity for all recognized populations from assignment tests (i.e., mean number of alleles (A), observed heterozygosity (H_O), and expected heterozygosity (H_E)). FSTAT 2.9.3 (Goudet 2001) was used to estimate inbreeding coefficients (F_{IS}) and rarefied allelic richness (A_r).

Landscape genetics analysis

Landscape variables and thematic resolution

We selected elevation, topographic roughness, slope position, density of forest cover, and human footprint as hypothesized landscape drivers of gene flow based on available knowledge of Caucasian pit viper associations and other related species (Behrooz et al. 2015; Asadi et al. 2019). Asadi et al. (2019) showed that proximity to forest cover and topographic roughness are two main variables in predicting habitat suitability of the species. Topographical roughness, elevation and slope position were calculated as continuous landscape variables from a 90 m resolution digital elevation model (DEM; http://srtm.csi.cgiar.org) using the Gradient and Geomorphometric Modelling Toolbox for ArcGIS (Evans et al. 2014) at a focal radius of 1 km. The landscape cover produced by the Iranian Forests, Rangeland and Watershed Management Organization (FRWMO 2009) was used to derive density of forest cover within a 1-km focal radius as another continuous variable. To take into account the anthropogenic effects on gene flow, we used the human footprint layer developed by Sanderson et al. (2002) which integrates data on population density, road density, land transformation, infrastructures and remote sensing analysis of night lights. Roads are often associated with high levels of noise pollution, which can have a negative effect on vibration-sensitive species, in addition to the high percentage of road killings of wildlife. New roads can also facilitate urban expansion, which leads to an increase in human interactions with habitats and consequently habitat destruction and fragmentation. We used 250 m resolution as the smallest resolution of resistance surfaces considering the area of the study landscape (98,000 Km^2), existing landscape data, and computer processing intensity. We were able to add more details to forest landscape by distinguishing between the cover classes of vegetation map, resulting in a local landscape model consisting of 250 × 250 m pixels.

We produced alternative hypothetical resistance surfaces as a function of topographic roughness, elevation and slope position according to the hypotheses that pit vipers' movement may be impeded by rough terrain and resistance may increase with topographic roughness and slope position. We modelled resistance due to density of forest cover according to the hypothesis that the species movements should be impeded by high density of forest cover. Forest cover is associated with large amounts of woody debris and other ground litter that may pose a structural impediment to snake locomotion. Two morphs of the Caucasian pit viper are observed in Northern Iran including gray and red morphs. The gray morph individuals are often found in highlands with rangeland cover; however, the red morph can be found more often in areas with sparse forest cover in lower elevation. Therefore, we hypothesized that the dense forest cover impedes the movements of individuals. We also calculated resistance surfaces for human footprint under the hypothesis that landscape resistance to gene flow would increase with increasing human footprint given increasing physical barriers associated with human development as well as demographic obstacles associated with high mortality and low population density in areas with high human impact.

To address the effects of thematic resolution of resistance surfaces on gene flow, we assessed a range of power functions (P = 0.1, 0.2, 1.0, 2.0, 4.0) and maximum resistance values (RMax = 5, 10, 20, 40, 80) for each landscape variable (e.g., Shirk et al. 2010). The Power function controls the shape of the functional response between the landscape variable and resistance to gene flow (Shirk et al. 2010). Resistance surfaces of all landscape variables were produced across all combinations of the five RMax and P (i.e., 25 resistance surfaces for each variable). Cost distances were calculated among pairs of individuals using R package sGD (Shirk et al. 2012) and a raster of resistance surface as input. We calculated mean inter-individual Rousset's a (Rousset 2000)

genetic distances for each pair of individuals using SPAGeDi 1.5 (Hardy and Vekemans 2002) as the response variable in the landscape genetics analysis.

Univariate and multivariate optimization of variables

We used restricted multivariate optimization, an approached developed by Shirk et al. (2010), implemented in two ways. First, we used the reciprocal causal modeling (Cushman et al. 2013) to rank alternative hypotheses within the optimization loop. Second, we used maximum-likelihood population effects models (MLPE; Clarke et al. 2002) as the objective function to rank hypotheses, which has recently been shown to be the most robust current regression-based method for identifying the landscape factors affecting gene flow (Shirk et al. 2018). For the restricted multivariate optimization using RCM, following the method developed by Shirk et al. (2010) and Reddy et al. (2017), we selected the best univariate thematic resolution parameters for each variable by calculating partial Mantel correlation between genetic distance and each resistance surface partialling out all other candidate resistance surfaces for that variable using R package sGD (Shirk et al. 2012). A fully supported candidate model would have all positive r values in its column and all negative or near zero values in its row (Reddy et al. 2017). In the second step, we re-optimized the thematic resolution parameters for each univariate optimized variable in the context of the effects of all other variables (i.e., multivariate optimization). For multivariate optimization of each variable, we held the parameters of all other landscape variables constant at their univariate optimization and we calculated partial Mantel correlation between genetic distance and each resistance surface partialling out all other candidate resistance surfaces for that variable. The optimization loop continues, re-optimizing all variables, until the parameters stabilize. A fully supported candidate model would have all positive r values in its column and all negative or near zero values in its row.

For optimization of the thematic resolution using MLPE, we selected the best univariate thematic resolution parameters for each variable by calculating Akaike's information criteria (AIC) to rank all candidate resistance surfaces for each variable according to their goodness of fit. For multivariate optimization, we held the parameters of all other landscape variables constant at their univariate optimization and we calculated AIC for all resistance surfaces of each landscape variable. The optimization loop continues, re-optimizing all variables using MLPE in the R package RESISTANCEGA (Peterman 2014).

After optimizing thematic resolution parameters for all landscape variables, we created all combinations of landscape variables at their multivariate optimization that resulted from restricted multivariate optimization with RCM and MLPE (31 resistance models) to determine which subset of optimized variables should be included in the final resistance surface. We used two model selection frameworks to evaluate correlation between landscape resistance matrices and genetic distance. The first was based on the correlation between each optimized landscape resistance and genetic distance using partial Mantel test in a reciprocal causal modelling method (RCM; Cushman et al. 2013). We created an N $\times$ N matrix of correlations where N was the number of alternative optimized resistance surfaces resulted from multivariate optimization (31 $\times$ 31) and each number in the matrix was the correlation coefficient (partial Mantel r). The candidate landscape resistance model with the most positive values in its column (partial Mantel r values) and the most negative or near zero values in its row (partialling out every other candidate resistance surface) was declared the most supported model (Reddy et al. 2017). The R package 'Vegan' (Oksanen et al. 2013) was used to perform all Mantel-based methods. We used a second landscape genetics approach implemented in the R package RESISTANCEGA" (Peterman 2014) to assess which of the 31 resistance surfaces resulting from MLPE optimization best explains genetic structure. We used MLPE to relate genetic distance to resistance distance for all combinations of optimized resistance surfaces. For each candidate resistance surface, we calculated AIC to rank the models according to their goodness of fit and to assess which of the 31 multivariate resistance surfaces best explains genetic structure in the Caucasian pit viper. The best model was selected based on the lowest AIC score fit with maximum likelihood. In addition to landscape resistance models, we also calculated Euclidean geographic distance among pairs of individuals to run an isolation-by-distance (IBD) model.

Results

Population genetic structure in the Caucasian pit viper

The results of STRUCTURE revealed strong evidence for population subdivision in the Caucasian pit viper. We found that likelihood increased with increasing number of clusters (K), but began to plateau at K = 5. According to the maximum value of LnP (D) and delta K, five genetic clusters were inferred from STRUCTURE. The percent membership of each sample in the five clusters correspond to geographic regions of sampled subpopulations suggesting that there is correspondence between geography and genetic variability (Fig. 2). Similar to STRUCTURE, results from GENELAND showed five genetic clusters (Fig. S1, Online Resource 1). Samples from the northwestern part of the study area were grouped into GILAN population (G1), samples from the Behshahr region were grouped into BEHSHAHR (G2), all Lar National Park samples in the central Alborz were grouped into LAR population (G3), samples from Golestan were grouped into GOLESTAN (G4) and samples from Khorasan were grouped into KHORASAN population (G5). Results of pairwise F_{ST} were consistent with the results of assignment tests and showed significant genetic differentiation between population pairs. F_{ST} values were large between pairs of populations, ranging from 0.217 (GILAN and KHORASAN) to 0.387 (LAR and KHORASAN; Table S2, Online Resource 1).

Microsatellite variations in the Caucasian pit viper

The results of Micro-Checker showed no evidence of allele dropout and scoring errors. We also found no significant evidence of null alleles in Micro-Checker. The results of FreeNA showed evidence of null alleles in CwA14, Crti95, Ch7-87 and Crti10 (Table S3, Online Resource 1). As the threshold of null alleles were less than 0.2 (except locus CwA14 in BEHSHAHR population) and considering the low effect of null alleles on assignment tests, we did not remove loci for subsequent analysis (Okello et al. 2005; Carlsson 2008). Although two microsatellite loci (Crti95 and Crti10) revealed deviations from HWE for the whole sample ($P < 0.05$), we did not find evidence of deviation from HWE for the whole sample after Bonferroni's correction ($P > 0.002$). Also, no evidence of deviation from HWE was found at the population level after Bonferroni's correction ($P > 0.002$; Table S4, Online Resource 1). No evidence of linkage disequilibrium was found within each population after correcting for multiple comparisons.

A total of 144 alleles were scored across 7 loci in all samples. Allelic variation per locus ranged from 2 to 12 alleles. Mean allelic richness was similar among populations and the range of allelic richness for all loci was between 2.67 in the BEHSHAHR to 2.16 in the LAR. The observed heterozygosity ranged from 0.73 to 0.95 and expected heterozygosity ranged from 0.55 to 0.75 (Table S5, Online Resource 1). Estimated inbreeding coefficient (F_{IS}) varied from -0.07 in the BEHSHAHR population to − 0.58 in the KHORASAN population.

Landscape genetics analysis

Univariate optimization

Univariate optimization of thematic resolution of landscape variables showed that, after controlling the effect of geographic distance, topographic roughness had the strongest and a significant univariate correlation with genetic differentiation, followed by density of forest cover, elevation, slope position and human footprint (Table 1). Univariate IBR was significant, independent of distance, only for topographic roughness at its optimal univariate thematic resolution. The most optimal univariate thematic resolution for topographic roughness was obtained at RMax 80, indicating high effect of this variable on landscape resistance to gene flow. Also, elevation and slope position had univariate RMax values of 5, indicating substantially weaker effects than topographic roughness. Elevation indicated a highly convex effect on resistance (P = 4), while human footprint and slope position showed a highly concave effect on resistance (P = 0.1). Also, topographic roughness showed a linear effect on resistance (P = 1; Table 1). Univariate optimization of thematic resolution of landscape variables using MLPE also showed that topographic roughness has the strongest univariate correlations with genetic differentiation, after controlling the effect of geographic distance (i.e., univariate IBR), followed

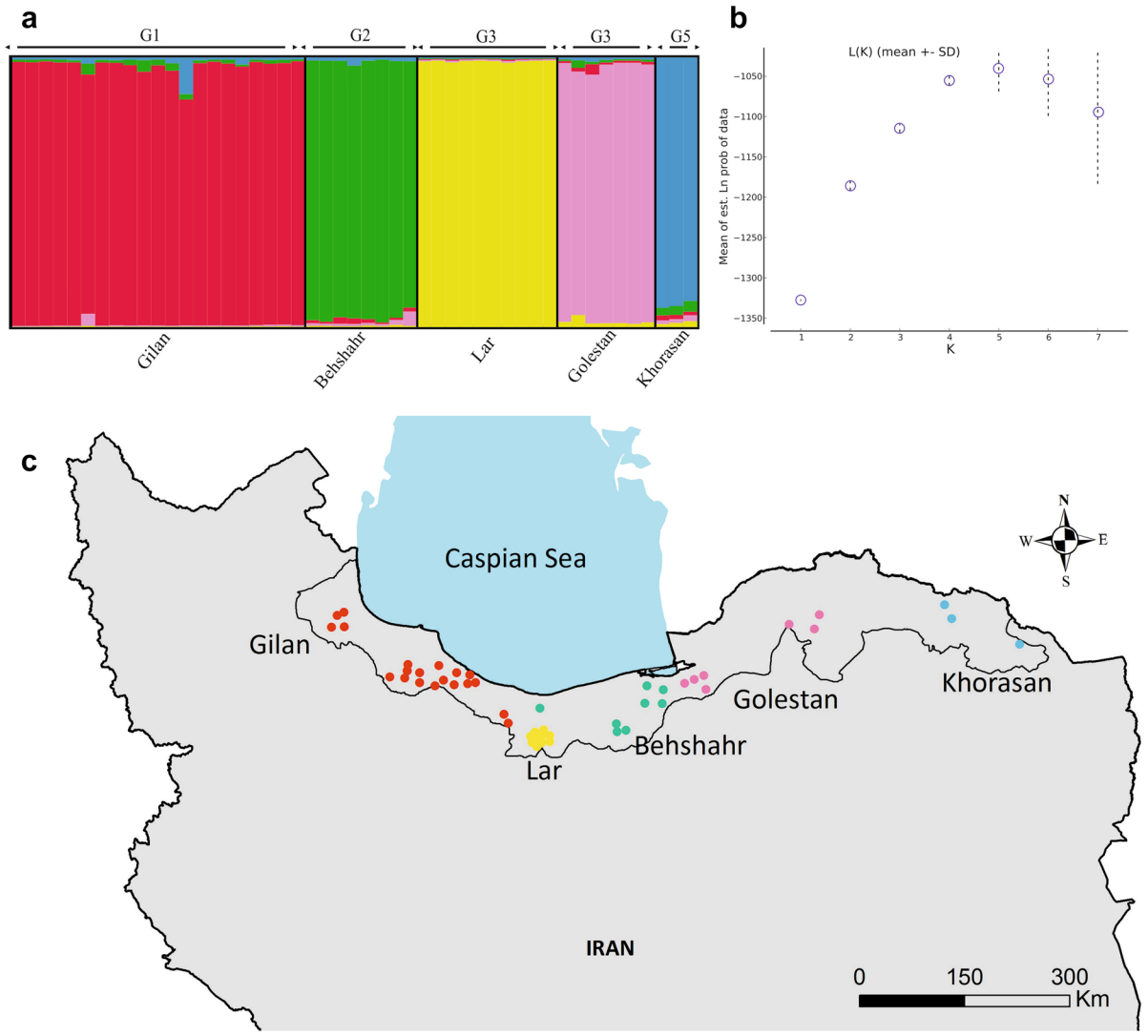

Fig. 2 The most likely number of genetically distinct clusters within the Caucasian pit viper based on **a** Mean L(K) ± SD over 10 runs per K, **b** percentage of individuals assignment to different K and **c** geographical location of the genetically groups identified by STRUCTURE (red = GILAN; yellow = LRA; pink = GOLESTAN, green = BEHSHAHR; and blue = KHORASAN). (Color figure online)

by density of forest cover, elevation, slope position, human footprint and (Table 2).

Multivariate optimization and model selection

The best multivariate thematic resolutions for density of forest cover and topographic roughness using restricted multivariate optimization were the same as the univariate optimization (Table 1). On the other hand, the best multivariate optimal thematic resolutions for human footprint (RMax = 20; P = 4), slope position (RMax = 5; P = 4) and elevation (RMax = 5; P = 0.1) were different than the univariate optimization ($RMax_{human\ footprint} = 20$; $P_{human\ footprint} = 0.1$; $RMax_{slope\ position} = 5$; $P_{slope\ position} = 0.1$; $RMax_{elevation} = 5$; $P_{elevation} = 4$; Table 1). The partial Mantel correlation between the optimized multivariate resistance surface with genetic distance, after controlling the effects of geographic distance (i.e., multivariate IBR) using restricted multivariate optimization, was higher than the combination of resistance surfaces at their univariate optimization (r = 0.363 vs. r = 0.295; Table 1).

Table 1 Univariate and multivariate thematic resolution optimization for each variable using restricted multivariate optimization

Landscape variable	Univariate optimization	IBR model r = partial Mantel P = P-value	Multivariate optimization
Elevation	RMax = 5; power = 4	r = 0.32 P = 0.999	RMax = 5; power = 0.1
Topographic roughness	RMax = 80; power = 1	r = 0.29 P = 0.001	RMax = 80; power = 1
Slope position	RMax = 5; power = 0.1	r = 0.20 P = 0.999	RMax = 5; power = 4
Density of forest cover	RMax = 20; power = 2	r = 0.11 P = 0.078	RMax = 20; power = 2
Human footprint	RMax = 20; power = 0.1	r = − 0.15 P = 0.995	RMax = 20; power = 4
Combined	r = 0.295 P = 0.001		r = 0.363 P = 0.001

r partial Mantel correlation between genetic distance and univariate optimized resistance surface controlling for geographic distance (IBR)

Table 2 Univariate and multivariate thematic resolution optimization for each variable using MLPE method

Landscape variable	Univariate optimization	IBR model r = partial Mantel P = P-value	Multivariate optimization
Elevation	RMax = 5; power = 4	r = 0.32 P = 0.999	RMax = 5; power = 0.1
Topographic roughness	RMax = 80; power = 2	r = 0.27 P = 0.001	RMax = 80; power = 0.2
Slope position	RMax = 5; power = 0.1	r = 0.20 P = 0.999	RMax = 5; power = 4
Density of forest cover	RMax = 20; power = 0.1	r = 0.14 P = 0.092	RMax = 20; power = 4
Human footprint	RMax = 20; power = 0.1	r = − 0.14 P = 0.989	RMax = 20; power = 4
Combined	r = 0.295 P = 0.001		r = 0.344 P = 0.001

r partial Mantel correlation between genetic distance and univariate optimized resistance surface controlling for geographic distance (IBR)

Optimization of thematic resolution of resistance surfaces using MLPE confirmed the results of restricted multivariate optimization with causal modeling, with a few minor differences (Table 2). For example, while topographic roughness indicated a more convex effect on resistance in the MLPE method (P = 2) than restricted multivariate optimization (P = 1) in univariate optimization, this variable showed a more concave effect on resistance in the MLPE (P = 0.2) than restricted multivariate optimization (P = 1) in multivariate optimization. The best multivariate optimal thematic resolutions for all variables was different than univariate optimization in the MLPE method (Table 2). The partial Mantel correlation between the optimized multivariate resistance surfaces produced by MLPE method and genetic distance, after controlling the effects of geographic distance, was higher than the combination of resistance surfaces at their univariate optimization (r = 0.344 vs. r = 0.275; Table 2).

Both methods of model selection (i.e., RCM framework and MLPE) included four landscape variables (topographical roughness, slope position, density of forest cover and human footprint) in the top five multivariate models (Table 3 and 4). The top model selected by RCM included topographic roughness and density of forest cover only and showed a higher correlation with genetic distance than the IBD model ($r_{IBD} = 0.151$ vs. $r_{IBR} = 0.371$; Table 3). Table 4 shows the MLPE model selection framework of the top five models with the lowest AIC. Like the RCM, the selected top model using MLPE included topographic roughness and density of forest cover (Table 4). The combination of optimized topographic roughness (RMax = 80; P = 0.2) and density of forest cover (RMax = 20; P = 4) had the strongest

Table 3 List of five top landscape genetic models ranked by row mean based on RMC framework

Model No.	Variables	Row mean	r	*P*-value
1	Density of forest cover + Topographic roughness	0.239	0.371	0.001
2	Density of forest cover + Topographic roughness + Human footprint	0.228	0.370	0.001
3	Density of forest cover + Elevation + Topographic roughness	0.181	0.371	0.001
4	Density of forest cover + Elevation + Human footprint + Topographic roughness	0.178	0.363	0.001
5	Density of forest cover + Elevation + Human footprint + Topographic roughness + Slope position	0.178	0.362	0.001

The candidate resistance surface with the highest row mean (i.e., the largest average relationship to genetic distance after partialling out the effect of other candidate models) was used as a relative support for each candidate resistance model. r shows the partial Mantel correlation between the cost distance matrix for each resistance model and the genetic distance matrix. P-value shows the significance of the calculated correlation coefficient

Table 4 List of five top landscape genetic models ranked by AIC scores based on MLPE framework

Model no.	Variables	AIC	ΔAIC	r	*P*-value
1	Density of forest cover + Topographic roughness	− 2076.57	0.00	0.294	0.001
2	Density of forest cover + Topographic roughness + Human footprint	− 2073.97	2.60	0.292	0.001
3	Density of forest cover + Topographic roughness + Slope position	− 2071.11	5.46	0.371	0.001
4	Density of forest cover + Human footprint + Topographic roughness + Slope position	− 2068.58	7.99	0.296	0.001
5	Topographic roughness + Slope position	− 2056.10	20.47	0.296	0.001

r shows the partial Mantel correlation between the cost distance matrix for each resistance model and genetic distance matrix. P-value shows the significance of the calculated correlation coefficient

relationship with genetic differentiation based on MLPE framework with an AIC value of − 2076.57 and showed a stronger correlation with genetic distance than the IBD model (r IBD = 0.151 vs. r IBR = 0.294). The top three models selected by MLPE included topographic features (i.e., roughness and slope position), suggesting that topographic features of the studied landscape have a significant effect on gene flow. A pixel–pixel correlation between the top models identified by the two methods showed high correlation (r = 0.75) indicating that the MLPE and RCM models are similar in their predictions (Fig. 3).

Discussion

We adopted existing individual-based landscape genetics frameworks (e.g., Cushman et al. 2013; Reddy et al. 2017; Shirk et al. 2018) to characterize the spatial patterns of genetic diversity and evaluate the effects of habitat heterogeneity on population genetic structure and gene flow for the Caucasian pit viper in Iran. We showed three main results. Firstly, there is significant population genetic structure in the Caucasian pit viper in Iran. Secondly, genetic diversity analyses suggest relatively moderate to high level of genetic diversity in the studied populations. Thirdly, our individual-based landscape genetics analysis showed that gene flow is primarily related to a combination of topographic features and density of forest cover, with resistance lowest in areas of low topographical roughness and low tree cover. We found that the restricted multivariate optimization using reciprocal causal modeling produced very similar results to optimization using maximum-likelihood population effects modeling, confirming the result of Shirk et al. (2018) that, while MLPE is slightly favored

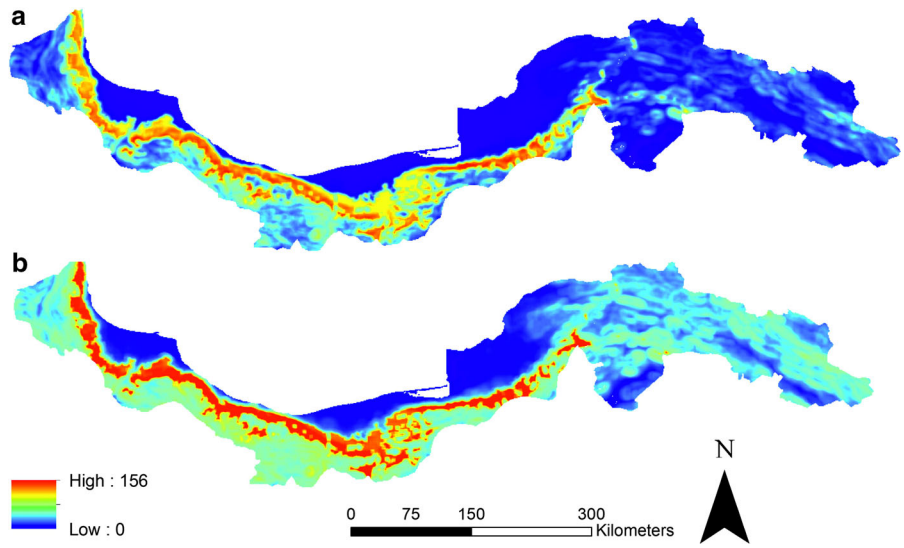

Fig. 3 Map of the best resistance model based on the RCM (**a**) and MLPE (**b**) model selection framework. The resistance map based on both selection frameworks depicts the combined effects of topographical roughness and density of forest cover. Landscape resistance is lowest in parts of the landscape with low topographical roughness and low canopy of forest cover. (Color figure online)

based on model performance, both methods produce similar and robust predictions.

Genetic diversity, population structure and recent gene flow

We found high levels of genetic diversity in this viper despite its limited ability to move and to adapt to environment changes. Although about 38% of the suitable habitats of this species is within the network of protected areas (Asadi et al. 2019), recently, the populations of this species have declined due to a range of anthropogenic factors. Land conversion to agricultural fields, overgrazing, rangeland destruction, as well as large-scale hunting and capturing by vaccine and serum institutes (Rastegar-Pouyani et al. 2018), threaten the viability of the populations. Therefore, re-colonization of new populations in available suitable habitat patches, restoration of degraded habitats, establishment of safe zones in current protected areas, and prevention of hunting and venom collection seems to be vital to maintain the genetic diversity and to assure the long-term persistence of populations.

We also found that gene flow is limited among populations of *G. h. caucasicus* and allele frequency may reflect genetic drift in local and isolated subpopulations. Also, the genetically recognized populations exhibited moderate to high levels of genetic differentiation between the populations based on F_{ST} measure. As large sample sizes are required in order to precisely infer F_{ST}, the small sample size in some populations may lead to overestimates of genetic differentiation among populations. Moreover, microsatellite loci affected by null alleles would probably not alter the overall outcome of assignment testing (Carlsson 2008), therefore we focused only on the results of the assignment tests (i.e., STRUCTURE and GENELAND) to infer genetic differentiation among studied populations.

A previous analysis of genetic structure based on mtDNA + nDNA (Asadi et al. 2019) revealed an ancient split between northeastern, central, and northwestern populations. They showed that there is a significant genetic structure among populations of the Caucasian pit viper in northern Iran including Kopet Dagh-Eastern Alborz, Central Alborz, Lar National Park-Central Alborz, and Western Alborz-Azerbaijan. Comparison of the results of Asadi et al. (2019) with the present study shows that there are differences in the observed genetic structure based on mtDNA (Asadi et al. 2019) and microsatellites (present study). For example, Asadi et al. (2019) found one clade in northeast of Iran (i.e., Kopet Dagh-Eastern Alborz), while we found three genetic structures including KHORASAN, BEHSHAHR, and GOLESTAN in northeast of study area. As mtDNA reaches fixation by genetic drift faster than microsatellites, a possible explanation for higher genetic structuring at microsatellite level compared to mtDNA, could be due to the recent landscape changes in this part of the Alborz Mountains. The second difference is related to

the populations in northwest of Iran. Asadi et al. (2019) found two clades in central (i.e., Central Alborz) and northwest of Iran (i.e., Western Alborz-Azerbaijan), whereas we only found one genetic cluster (GILAN) in this area. It could be concluded that considering the results of all markers, it seems that a combination of historical events and recent landscape changes in northern Iran has influenced the observed genetic structure in populations of the Caucasian pit viper. Although it would be interesting to test the effects of historical events on current genetic structure of the Caucasian pit viper, we did not test this hypothesis in the present study because of the scarcity of data on the historical landscape.

The limited dispersal ability of the Caucasian pit viper may affect gene flow among populations. Also, in species with limited dispersal, genetic structure may emerge even on small geographic scales (Klug et al. 2011). Species habitat selection (Sunny et al. 2015), body size, and biased dispersal (Keogh et al. 2007) are other factors affecting the gene flow and genetic structure among populations of snakes. Also, the probability of genetic structure in specialist species is higher than generalists.

Landscape genetics

This paper compared results of multivariate restricted optimization (Shirk et al. 2010) based on two objective functions: reciprocal causal modeling, and maximum-likelihood population effects modeling. As we used the measures of model fit instead of significance tests for selection of true landscape resistance, our evaluation of Mantel-based correlations (RCM framework) was not sensitive to critiques relating to Mantel-based model selection criteria (Shirk et al. 2018). Shirk et al. (2018) showed that for complex models with multiple variables contributing to resistance, RCM is more reliable than simple Mantel correlations and causal modelling.

However, Castillo et al. (2014) showed that RCM framework is appropriate for determining the correct variables in landscape genetics analysis, but less effective at accurately determining RMax and P. There is an inherent dependency in both genetic and landscape distance matrices that can affect the results of landscape genetic analysis. Assumptions of independence and linearity is one of the sources of model selection error suggested by Zeller et al. (2016). Shirk et al. (2018) confirmed that MLPE is the selection method that had the best performance based on identifying the correct variables, their RMax and functional form (P). They suggested that MLPE provides the greatest probability of selecting the true landscape resistance model from competing alternative models. Little is known about the differences in inferences produced by RCM compared to MLPE modeling in an optimization approach, and this paper provides quantitative comparison of the results produced by both methods on the same data set.

Thematic resolution optimization

The partial Mantel tests were significant for resistance distances and had partial correlation coefficients that were stronger than Euclidean distance. Therefore, it can be inferred that gene flow of the Caucasian pit viper is limited by landscape structure and is not simply a function of IBD. The results of the univariate model optimization suggested that topographic roughness was the landscape factor most strongly correlated with gene flow based on the strength of partial Mantel correlation. This suggests that historical population connectivity of the Caucasian pit viper in the study area was primarily driven by natural landscape factors. However, due to time lag effects (Landguth et al. 2010), recent anthropogenic alterations of the landscape may not be detectable in the genetic data even if they have large effects on population connectivity (e.g., Reddy et al. 2019). In both optimization methods, topographical roughness had optimal multivariate thematic resolutions with RMax values of 80, indicative of the high effect of this variable on resistance to gene flow. Although density of forest cover had RMax value smaller than topographic roughness (RMax = 20), this variable showed higher levels of contrast (P = 2 to 4) than topographic roughness, indicating a highly concave response between the density of forest cover and resistance to gene flow. As multivariate models combine different landscape variables with different Rmax and P values, the effect of each variable on genetic differentiation should be explained through both parameters in multivariate models.

Landscape resistance for Caucasian pit viper

The selected top model using both methods of model selection included topographic roughness and density of forest cover as among the most important drivers of gene flow. These findings reveal that limited gene flow is most strongly related to resistance from high terrain roughness and dense forest cover.

The results suggest that Caucasian pit viper movements are highest in areas with low topographical roughness, reflecting the increased energy efficiency of moving through flat terrain. A careful survey of the study area reveals that as roughness increases, barriers to gene flow increase too. Highlands and low-hill regions offer fewer barriers to dispersal and gene flow of the Caucasian pit viper, and thus genetic differentiation occurs less often. Our observations during field surveys are fully in concordance with the fact that most of the genetic samples were captured in forests with low density cover and rangelands in highlands. High terrain roughness may act as a barrier for the species because of the high energy required to traverse, or because of the challenging ecological and environmental conditions associated with regions of high topographic roughness.

According to our results, limited gene flow is detected among populations of the Caucasian pit viper in areas with highly dense forest cover. The Caucasian pit viper in northern Iran seems to experience increasing difficulty to disperse across the landscape from rangelands and forests with low to moderate canopy cover to forests with dense cover. Proximity to forest cover is one the most important variables in predicting habitat suitability of the species (Asadi et al. 2019). Accordingly, this viper occupies highlands with forest and rangeland cover in northern and southern slopes of the Alborz Mountain. As some studies have shown, habitat suitability may be an important predictor of gene flow, thus high landscape resistance (Fig. 3) in previously predicted suitable habitats (i.e., Asadi et al. 2019) may initially seem surprising. There is limited research comparing connectivity models with habitat suitability of snake species. While forest cover and proximity to this cover influence species habitat suitability, the Caucasian pit viper dispersal may be facilitated through different conditions, such as open canopy and non-forest areas. Ecological factors that drive habitat suitability may be different from those influencing dispersal and gene flow (Mateo-Sánchez et al. 2015; Roffler et al. 2016). Therefore, suitable habitats within a home range may not necessarily equate to areas with low resistance to gene flow. Consequently, dispersing Caucasian pit vipers may select portions of the landscape for dispersal that are relatively low quality for supporting resident populations. Similar results have been found in other taxa as well (e.g., Elliot et al. 2014). Another potential explanations for the observed divergence of habitat suitability and dispersal resistance may be due to the scale of habitat suitability and gene flow modeling. Although proximity to forest cover influences habitat suitability and occurrence of the Caucasian pit viper at a local scale, density of forest cover and topographic roughness are more influential to gene flow and genetic structure at a broader scale. We conclude that in the distribution range of the Caucasian pit viper in Iran, the species selects forests and rangelands with relatively low canopy cover in areas with low topographical roughness for movement and gene flow.

Field observations confirmed that the species inhabit rangelands in high elevation or forest with low canopy cover. Therefore, the species movements may be impeded by high density of forest cover. The Hyrcanian forests on the northern slopes of the Alborz are composed of three distinct altitudinal gradients including lowlands (300–700 m), midlands (700–1800 m), and highlands (1800–2400 m). The highest density of Hyrcanian forests is found in midlands. The Caucasian pit viper is quite difficult to spot as it is well camouflaged and perfectly hides under bushes. Also, these vipers are very alert and constantly stay close to debris, stones, cracked rocks, rodent holes or any space that offers shelter. They quickly escape and take cover before one can observe or capture them. Thus, sampling this species on the northern slopes of the Alborz was extremely difficult. Given our current limited knowledge on the distribution and habitat preferences of the species, we believe inferences about the relationships between pit viper occurrence and density of forest cover cannot be made unless denser sampling is done in midland forests of the northern slope of the Alborz to enrich our understanding of the species' presence/absence and density in this region.

Nevertheless, topographical roughness and density of forest cover are clearly major factors that affect gene flow of the Caucasian pit viper in northern Iran.

Our results show strong support for topographic roughness and elevation (r = − 0.53) as factors driving population connectivity. This finding suggests that dispersal of the Caucasian pit viper is limited primarily by topographically complex areas and secondarily by high forest cover.

Our results suggested weak relationships between human footprint and gene flow (Mantel statistic r = 0.149, *P* value = 0.06). The weak and marginally nonsignificant effect of this landscape factor could be due to several factors. A possible explanation could be related to time-lag effects (Landguth et al. 2010). The landscape in northern Iran has changed rapidly over recent years due to the increase in human footprint resulting in a disequilibrium between the genetic structure and current configuration of the landscape. Landguth et al. (2010) found that a lag-time of 1–15 generations is needed to detect a genetic signal of anthropogenic variables on genetic structure. If we consider a generation time of about 3 years for the species (Fitch 1963), a time frame of 50 years is needed to trace the effects of landscape changes on genetic patterns. Klug et al. (2011) found that dispersal and gene flow of Eastern yellowbelly racer (*Coluber constrictor flaviventris*) was not limited by natural or anthropogenic land cover in northeastern Kansas, even though profound recent landscape change has greatly reduced the extent and connectivity of habitat for that species, suggesting major time lag effects. Therefore, the lack of strong apparent effects of human footprint on gene flow of the Caucasian pit viper based on molecular genetic data is not definitive as to recent or current effects, and other studies using such things as mark-recapture movement monitoring may be needed to look at recent and current patterns of movement (e.g., Elliot et al. 2014).

In contrast to anthropogenic factors, topographical factors have remained stable for thousands of years, and hence there is no time-lag effect for these stable factors (Reddy et al. 2017). Therefore, these factors have stronger apparent relationships with gene flow in the Caucasian pit viper. Likewise, Wasserman et al. (2010), Reddy et al. (2017) and Vergara et al. (2017) found that gene flow of American marten (*Martes americana*), tigers (*Panthera tigris*), stone marten (*Martes fiona*) and pine marten (*Martes martes*), respectively, was driven more by topographic variables than land cover and human footprint. Our finding therefore does not mean that anthropogenic factors will not affect gene flow of the species in the future, or that, given time lag effects, they are not already limiting gene flow.

Conclusions and implications for conservation

The results presented in this study provide the first detailed microsatellite-based analysis of the population genetic structure and population connectivity of the Caucasian pit viper. Topographic roughness and density of forest cover are the two variables that most strongly limit gene flow for the Caucasian pit viper in Iran. Our results also showed that including landscape variables with differential resistance values and functional shapes in a multivariate optimization (Shirk et al. 2010; Reddy et al. 2017) improves the prediction of gene flow in the Caucasian pit viper. This study is therefore an important addition in understanding how landscape variables affect landscape connectivity. Our findings have implications for the long-term persistence of the species in the face of future habitat changes. Given the high genetic structure and limited gene flow observed in our study and the study conducted by Asadi et al. (2019), these populations are likely a set of genetically distinct units suggesting that managers should give high priority to protecting the remaining populations. Further studies based on empirical analyses of genetic patterns and simulation modelling are needed to properly evaluate the potential effect of landscape factors on gene flow.

Acknowledgements This study was licensed by the Iranian Department of Environment under permits Nos. 94/6049 and 96/3631.

References

Asadi A, Montgelard C, Nazarizadeh M, Moghaddasi A, Fatemizadeh F, Simonov E, Kami HG, Kaboli M (2019) Evolutionary history and postglacial colonization of an Asian pit viper (*Gloydius halys caucasicus*) into Transcaucasia revealed by phylogenetic and phylogeographic analyses. Sci Rep 9(1):1224

Balkenhol N, Cushman SA, Storfer A, Waits LP (2016) Introduction to landscape genetics–concepts, methods, applications. Landsc Genet. https://doi.org/10.1002/9781118525258.ch01

Behrooz R, Kaboli M, Nourani E, Ahmadi M, Shabani AA, Yousefi M, Asadi A, Rajabizadeh M (2015) Habitat modeling and conservation of the endemic Latifi's Viper

(*Montivipera latifii*) in Lar National Park, Northern Iran. Herpetol Conserv Biol 10(2):572–582
Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. J Hered 99(6):616–623
Castillo JA, Epps CW, Davis AR, Cushman SA (2014) Landscape effects on gene flow for a climate-sensitive montane species, the American pika. Mol Ecol 23(4):843–856
Clarke RT, Rothery P, Raybould AF (2002) Confidence limits for regression relationships between distance matrices: estimating gene flow with distance. J Agric Biol Environ Stat 7(3):361
Cox CL, Streicher JW, Sheehy CM III, Campbell JA, Chippindale PT (2012) Patterns of genetic differentiation among populations of *Smilisca fodiens*. Herpetologica 68(2):226–235
Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. Am Nat 168(4):486–499
Cushman SA, Shirk A, Landguth EL (2012) Separating the effects of habitat area, fragmentation and matrix resistance on genetic differentiation in complex landscapes. Landscape Ecol 27(3):369–380
Cushman SA, Wasserman TN, Landguth EL, Shirk AJ (2013) Re-evaluating causal modeling with Mantel tests in landscape genetics. Diversity 5(1):51–72
Earl DA, Vonholdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4(2):359–361
Elliot NB, Cushman SA, Macdonald DW, Loveridge AJ (2014) The devil is in the dispersers: predictions of landscape connectivity change with demography. J Appl Ecol 51(5):1169–1178
Evans JS, Oakleaf J, Cushman SA, Theobald D (2014) An ArcGIS toolbox for surface gradient and geomorphometric modeling, version 2.0-0. https://evansmurphy.wixsite.com/evansspatial. Accessed 21 Mar 2007
Fitch HS (1963) Natural history of the racer Coluber constrictor. University of Kansas, Lawrence
Fontenot BE, Makowsky R, Chippindale PT (2011) Nuclear–mitochondrial discordance and gene flow in a recent radiation of toads. Mol Phylogenet Evol 59(1):66–80
Gangloff EJ, Reding DM, Bertolatus D, Reigel CJ, Gagliardi-Seeley JL, Bronikowski AM (2017) Snakes in the city: population structure of sympatric Gartersnakes (*Thamnophis* spp.) in an urban landscape. Herpetol Conserv Biol 12(2):509–521
Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9. 3. http://www2.unil.ch/popgen/softwares/fstat.htm
Guillot G (2008) Inference of structure in subdivided populations at low levels of genetic differentiation—the correlated allele frequencies model revisited. Bioinformatics 24(19):2222–2228
Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. Mol Ecol Notes 5(3):712–715
Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2(4):618–620
Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23(14):1801–1806
Keogh JS, Webb JK, Shine R (2007) Spatial genetic analysis and long-term mark–recapture data demonstrate male-biased dispersal in a snake. Biol Lett 3(1):33–35
Klug PE, Wisely SM, Kimberly WA (2011) Population genetic structure and landscape connectivity of the Eastern Yellowbelly Racer (*Coluber constrictor flaviventris*) in the contiguous tallgrass prairie of northeastern Kansas, USA. Landscape Ecol 26(2):281–294
Landguth E, Cushman S, Schwartz M, McKelvey K, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. Mol Ecol 19(19):4179–4191
Latifi M (2000) Snakes of Iran. Department of the Environment, Tehran
Mateo-Sánchez MC, Balkenhol N, Cushman S, Pérez T, Domínguez A, Saura S (2015) A comparative framework to infer landscape effects on population genetic structure: are habitat suitability models effective in explaining gene flow? Landscape Ecol 30(8):1405–1420
McRae BH (2006) Isolation by resistance. Evolution 60(8):1551–1561
Okello JBA, Wittemyer G, Rasmussen H,Douglas-Hamilton I, Nyakaana S, Arctander P, Siegismund HR (2005) Noninvasive genotyping and Mendelian analysis of microsatellites in African savannah elephants. J Hered 96(6):679–687
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara R, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) Package 'vegan'. Community ecology package, version 2(9)
Orlov NL, Barabanov AV (1999) Analysis of nomenclature, classification, and distribution of the *Agkistrodon halys–Agkistrodon intermedius* complexes: a critical review. Russ J Herpetol 6(3):167–192
Peterman WE (2014) ResistanceGA: an R package for the optimization of resistance surfaces using genetic algorithms. Methods Ecol Evol 9(6):1638–1647
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2):945–959
Rastegar-Pouyani E, Oraie H, Khosravani A, Akbari A (2018) Phylogenetic position of Iranian pitvipers (Viperidae, Crotalinae, *Gloydius*) inferred from mitochondrial cytochrome b sequences. Trop Zool 31(2):55–67
Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49(6):1280–1283
Reddy PA, Cushman SA, Srivastava A, Sarkar MS, Shivaji S (2017) Tiger abundance and gene flow in Central India are driven by disparate combinations of topography and land cover. Divers Distrib 23(8):863–874
Reddy P, Puyravaud JP, Cushman S, Segu H (2019) Spatial variation in the response of tiger gene flow to landscape features and limiting factors. Anim Conserv. https://doi.org/10.1111/acv.12488
Roffler GH, Schwartz MK, Pilgrim KL, Talbot SL, Sage GK, Adams LG, Luikart G (2016) Identification of landscape features influencing gene flow: how useful are habitat selection models? Evol Appl 9(6):805–817

Rousset F (2000) Genetic differentiation between individuals. J Evol Biol 13:58–62

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Sanderson EW, Jaiteh M, Levy MA, Redford KH, Wannebo AV, Woolmer G (2002) The human footprint and the last of the wild: the human footprint is a global map of human influence on the land surface, which suggests that human beings are stewards of nature, whether we like it or not. Bioscience 52(10):891–904

Schwartz MK, Copeland JP, Anderson NJ, Squires JR, Inman RM, McKelvey KS, Pilgrim KL, Waits LP, Cushman SA (2009) Wolverine gene flow across a narrow climatic niche. Ecology 90(11):3222–3232

Shirk A, Cushman S, Landguth E (2012) Simulating pattern-process relationships to validate landscape genetic models. Int J Ecol 2012:8

Shirk AJ, Landguth EL, Cushman SA (2018) A comparison of regression methods for model selection in individual-based landscape genetic analysis. Mol Ecol Resour 18(1):55–67

Shirk A, Wallin D, Cushman S, Rice C, Warheit K (2010) Inferring landscape effects on gene flow: a new model selection framework. Mol Ecol 19(17):3603–3619

Sunny A, Monroy-Vilchis O, Zarco-González MM, Mendoza-Martínez GD, Martínez-Gómez D (2015) Genetic diversity and genetic structure of an endemic Mexican Dusky Rattlesnake (*Crotalus triseriatus*) in a highly modified agricultural landscape: implications for conservation. Genetica 143(6):705–716

Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4(3):535–538

Vergara M, Cushman SA, Ruiz-González A (2017) Ecological differences and limiting factors in different regional contexts: landscape genetics of the stone marten in the Iberian Peninsula. Landscape Ecol 32(6):1269–1283

Wagner P, Tiutenko A, Mazepa G, Borkin LJ, Simonov E (2016) Alai! Alai!–a new species of the Gloydius halys (Pallas, 1776) complex (Viperidae, Crotalinae), including a brief review of the complex. Amphibia-Reptilia 37(1):15–31

Wasserman TN, Cushman SA, Schwartz MK, Wallin DO (2010) Spatial scaling and multi-model inference in landscape genetics: Martes americana in northern Idaho. Landscape Ecol 25(10):1601–1612

Wright S (1969) Evolution and the genetics of populations: Vol. 2. In: Cook LM, Harris H (eds) The theory of gene frequencies. University of Chicago Press, Chicago

Zeller KA, Creech TG, Millette KL, Crowhurst RS, Long RA, Wagner HH, Balkenhol N, Landguth EL (2016) Using simulations to evaluate Mantel-based methods for assessing landscape resistance to gene flow. Ecolo Evol 6(12):4115–4128