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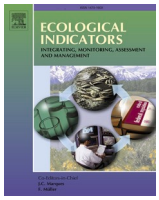

Anthropogenic and natural fragmentations shape the spatial distribution and genetic diversity of roe deer in the marginal area of its geographic range

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ABSTRACT

Habitat destruction and fragmentation are major factors in the destruction of genetic diversity and affect the movement behavior of the Roe deer population in the remaining habitats. Here, we study the population and landscape genetics of *Capreolus capreolus* (roe deer) in northern and northwestern Iran using twelve polymorphism microsatellite markers. From 111 total specimens, 63 had successful extraction (6 feces, 35 tissues, 9 bones, and 13 antlers). We considered 30 microsatellite polymorphic loci, of which only 12 were amplified for our further analysis. For genetic diversity analysis, the Weir-Cockerham method was applied to measure the inbreeding coefficient (FIS) and fixation index (FST) for each locus as well as for each population. For landscape genetics, the susceptibility patterns of genetic variations were assessed using three hypotheses including isolation by distance (IBD), isolation by environment (IBE), isolation by resistance (IBR), and individual landscape genetic analysis. A habitat suitability map as an indicator of landscape resistance was constructed from several species distribution models (SDMs) algorithms including Generalized Boosting Models (GBM), Maximum Entropy (Maxent), Random Forest (RF), Generalized Linear Model (GLM), Multivariate Adaptive Regression Splines (MARS) and artificial neural networks (ANN) and an ensemble model. Our estimated FIs index showed that the Golestan, Arasbaran, and Guilan populations had the highest and lowest genetic diversity among roe deer populations. According to the Fst criterion, our results showed that Golestan and East Azarbaijan (Arasbaran) had the highest and Mazandaran had the lowest genetic distance patterns. Our results do not suggest that there is high genetic differentiation for roe deer in the region, with high levels of gene flow between study areas. We found that geographic distance has no significant relationship with genetic distance and that there is no significant relationship between the ecological niche non-similarity matrix and the genetic distance matrix. The most influential factors affecting gene flow in roe deer were aspect and elevation variables. The analysis suggests that the landscape has no significant influence on the structuring of the studied population and shows little genetic differentiation.

1. Introduction

Roe deer (*Capreolus capreolus*), one of the 44 deer species in the world, is classified as Least Concern by the IUCN Red List due to its widespread distribution and increasing trend in the number of individuals (IUCN, 2019). Still, deer are reported as a protected species, according to the Iranian Ministry of Environment (DoE). The species is sensitive to habitat fragmentation (Chastagner et al., 2017). Because

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deer are considered ecosystem engineers (Côté et al., 2004; Martin, 2018), they can physically remodel ecosystems. A lack of species knowledge directly affects the conservation and management of protected species (Hepenstricka et al., 2012; Long et al., 2010). Based on recent studies, the range of roe deer in Iran consists of the Hyrcanian and Arasbaran forests and a small population in the western part of Iran.

Habitat destruction, degradation, and fragmentation are major threats to biodiversity (Baur and Erhardt, 1995; Sala et al., 2000; Wilson et al., 2016). Fragmentation of natural and anthropogenic habitats can severely affect the genetic structure, reducing genetic diversity and viability of small and isolated animal populations (Wang et al., 2017). Previous studies have highlighted and focused on the identification of mutation rates in animals (Allio et al., 2017), naturally induced fragmentation (Sergio et al., 2018), and adverse consequences of biodiversity loss and species extinction (Heilpern et al., 2018).

Anthropogenic habitat fragmentation affects spatial patterns of species movement and functional connectivity, gene flow, patterns of spatial genetic variation, and population size in threatened and endangered species (Taylor et al., 1993; Lande, 1998; Fahrig, 2003; Cushman, 2006; Fahrig, 2007). Some studies have recognized that forest discontinuity leads to declines in animal populations by reducing the amount of viable core habitat area and increasing edge effects (Reh and Seitz, 1990; Gerlach and Musolf, 2000). This has also been explored in previous studies of how natural and anthropogenic chronic fragmentation can alter the behavior and practice of genome face and population genetics of wild species (Keller and Largiader, 2003; Keller et al., 2004, 2005). Landscape resistance and geographic distance are the two key factors that can negatively affect patterns of genetic diversity (Landguth et al., 2010; Cushman, 2006; Fahrig, 2007). , the low-fragmentation landscape has greater genetic diversity than the high-fragmentation landscape, but larger habitat areas can provide genetic refuge for threatened wild species (McRae et al., 2008).

In short, landscape fragmentation can alter distribution and population size, patterns of genetic diversity, and structure through gene flow mechanisms (Forge et al., 2003). Increased random genetic drift and inbreeding as well as reduced gene flow increase the genetic divergence between populations (Schlaepfer et al., 2018). In the short term, the loss of genetic diversity expands the level of homozygosity and the development of deleterious recessive alleles that can reduce individual fitness through inbreeding depression (Charlesworth and Willis, 2009; Schlaepfer et al., 2018). In the long term, reduced genetic diversity can impair a population's potential to adapt to changing environmental conditions (Manel and Holderegger, 2013).

Landscape genetics provides a research framework to study the influence of landscape and environmental traits on genetic structure, genetic discontinuities, and gene flow (Balkenhol et al., 2016; Storfer, 2007; Manel and Holderegger, 2013; Cushman et al., 2006). Research into DNA-based markers or sequences with known positions on a chromosome for genetic diversity has a long history and is useful for quantifying diversity in nuclear DNA (Zhang and Hewitt, 2003). Historically, microsatellite is an ancient term used to describe cryptically simple repeated short sequence motifs (no longer than six base pairs) and has been distributed in coding and non-coding regions of every mammalian genome studied to date (Metzgar et al., 2000) They can be highly polymorphic, especially when long and uninterrupted, and therefore represent useful genetic markers (Chistiakov et al., 2006; Buschiazzo and Gemmell, 2006; Guichoux et al., 2011; Bhargava and Fuentes, 2010).

There is currently little knowledge about the distribution of roe deer in highly fragmented landscapes (Debeffe et al. 2012; Benoit et al., 2020; Ducros et al., 2020). With this motivation, we assess the impact of anthropogenic and natural fragmentation on species-specific densities and spatial patterns of roe deer genetic diversity in Iran. We first analyze microsatellite loci to provide the genetic population structure of roe deer throughout their range in Iran. We then used an individual-based landscape genetics approach and ecological niche modeling (ENM) to test hypotheses about the effects of landscape attributes (i.e. isolation by distance (IBD), isolation by resistance (IBR), and isolation by environment (IBE) and niche divergence in gene flow. (i.e. population-level niche comparisons).

2. Materials and methods

2.1. Study area

Our study area includes the relict forests of the temperate Hyrcania (18,000 km^2) and the Arasbaran forests (1600 km^2) in Iran (Fig. 1). The Hyrcanian forests extend from the Talysh Mountains in Azerbaijan Province in western to northeastern Iran (Soofi et al., 2018). The Hyrcanian Forests are a biodiversity hotspot and have recently been nominated as a World Heritage Site (Ahmadi et al., 2020). These forests are home to various native mammal species such as the Persian leopard (*Panthera pardus tulliana),* brown bear (*Ursus arctos*), gray wolf (*Canis lupus*), bezoar goat (*Capra aegagrus*), Caspian red deer (*Cervus elaphus maral*) and roe deer (*Capreolus Capreolus*) (Soofi et al., 2018; Shokri et al., 2021).

Elevationranges from 0 to 2800 m. Although these forests are rich in biodiversity, they are under severe anthropogenic pressures, including cattle grazing and the expansion of road networks and farmland (Sofi et al., 2018). The forests consist mainly of oriental beech (*Fagus orientalis*), oaks (*Quercus castaneifolia* and *Quercus macranthera*), sessile oak (*Quercus petraea*), hornbeam (*Carpinus betulus*), Caspian honey locust (*Gleditsia caspica*), ironwood (*Parrotia persica*) and velvet maple (Acer *velutinum*) (Sagheb-Talebi et al., 2014). The Arasbaran forests are located in northwestern Iran and are part of the Lesser Caucasus biodiversity hotspot (Sagheb-Talebi et al., 2014).

2.2. Roe deer genetic sampling

We opportunistically extracted DNA from deer feces, tissues, bones, and antlers throughout northern Iran (Fig. 1). Fecal sampling was placed in test tubes containing 96–99% ethanol and stored at −4 °C (Khosravi et al., 2017). Tissue, bone, and antler samples were provided by the Iranian Ministry of Environment (DOE). From 111 total specimens, 63 had a successful extraction (6 feces, 35 tissues, 9 bones, and 13 antlers). All geographic coordinates of the samples were recorded by the GPS (Geographic position system).

2.3. DNA extraction and PCR protocols

The samples (tissues, bones, and antlers) were successfully extracted using the phenol–chloroform extraction method (Chan et al., 2001). The Genomic DNA fecal samples were isolated using DNA Stool Mini-Kit (Yekta-Tajhiz Azma, Iran). For tissue samples, 50–100 mg were separated and used directly for extraction. For bone and antler, the samples were powdered in liquid nitrogen and 200 mg of each sample was transferred to a sterilized vial for extraction we considered 30 microsatellite polymorphic loci, of which only 12 were amplified for our analysis. Specifically, we optimized the polymerase chain reaction (PCR) conditions for each primer separately through a total volume of 15 μL over 10 ng/L DNA template, 25 mM MgCl2, 0.25 M forward and reverse primers, 40 mM dNTPs, and 5 units of Fermenta's Taq polymerase.

We ran the PCR reactions for 35 cycles based on the following setup: Initial denaturation was performed at 94 °C for 1 min. We then tested 35 cycles with different ranges including 94 °C (4 min), 57–60 °C (45 sec), and 72 °C (2 min). Finally, the extension step was performed at 72 °C (4 min). After amplification of the desired sequences, the PCR products were run on a 4% Metaphor gel in a horizontal electrophoresis setup.

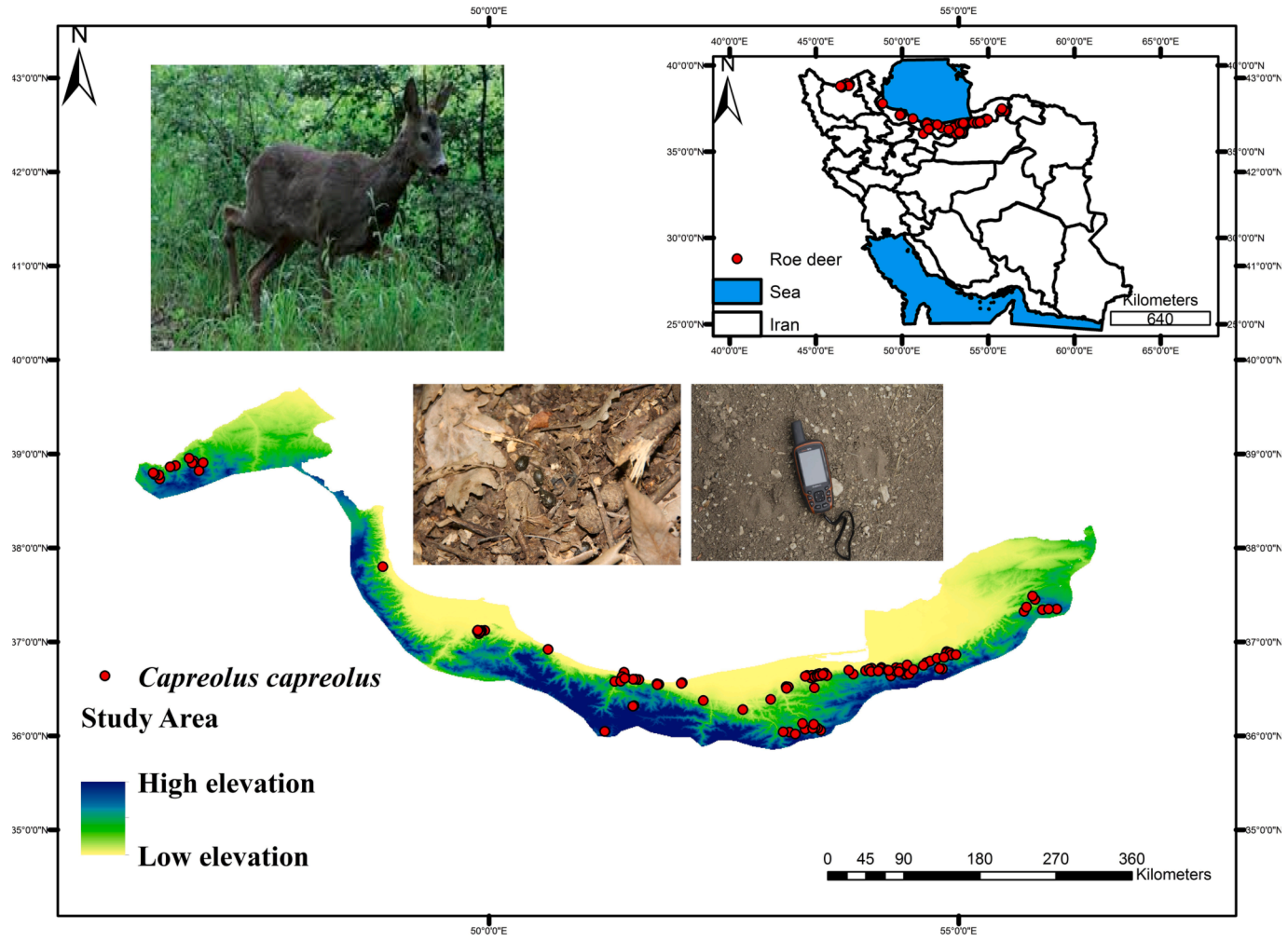

Fig. 1. The map shows the investigation area and the localities of the roe deer specimens (n = 66) in northern Iran, (red circles showed the occurrences of roe deer in the study area).

2.4. Genetic diversity analyses

We calculated the mean number of alleles per loci, expected and observed heterozygosity, and the polymorphism information content (PIC) using GenAlEx software v.6.5 (Peakall et al., 2006). We also applied the Weir-Cockerham method to measure the inbreeding coefficient (FIS) and fixation index (FST) for each locus as well as for each population using the match test in GenAlEx. In addition, we calculated gene flow rates within and between roe deer populations using the Analysis of Molecular Variance (AMOVA) test in GENEPOP and ARLEQUIN v. 3.5 (Raymond, 1995; Excoffier and Lischer, 2010).

2.5. Population differentiation analysis

We used the STRUCTURE software v. 2.1 (Pritchard et al., 2000) to examine the genetic clusters of deer with K values from 1 to 10, 10 replicates with 100,000 iterations of the Markov Chain Monte Carlo (MCMC) and a burn-in of 30,000 were listed. The number of clusters was estimated using K and Ln (Pr) (Pritchard et al., 2000). We compared our results with the R (version 4.1.0) package adegenet (Jombart, 2008). For each variable described in the drag surface construction, we calculated the effective distances between the 63 individuals using the gdistance package in R. We then ran a univariate linear mixed effects model using the maximum likelihood population effects method.

2.6. Landscape genetic analysis

For landscape genetics, the susceptibility patterns of genetic variations were assessed using three hypotheses: (1) isolation by distance, (2) isolation by environment, (3) isolation by resistance and individual landscape genetic analysis (Ashrafzadeh et al., 2018). We used the Mantel test to look for evidence of genetic distance between IBD, IBR, and IBE matrices.

2.6.1. Isolation by environment

To assess IBE, we conducted an analysis of environmental differentiation between pairs of individuals using a set of 19 bioclimatic variables. These variables were obtained from the WorldClim database, which we downloaded at a resolution of 1 km (Fick et al., 2005; https://www.worldclim.org). To define the accessible areas for each species, circular buffers with a radius of 50 km were created around each occurrence point. We considered all available areas for the focal species and incorporated them as background niches (Ashrafzadeh et al., 2018). Subsequently, we extracted the 19 bioclimatic variables using the buffers as a mask layer (Ashrafzadeh et al., 2018). Furthermore, we performed a Principal Component Analysis (PCA) with varimax rotation. Principal components (PCs) with eigenvalues >1 were selected, as they were deemed suitable for our objectives. Consequently, four PCs that met this criterion accounted for 95% of the total variance observed in the 19 bioclimatic variables. To quantify the environmental dissimilarity between each pair of points, we employed the Euclidean distance

method, utilizing the 'dist' function in R 3.2.2 (R Core Team, 2016). The relationship between the genetic distance matrix and the matrix of environmental dissimilarities was evaluated using the Mantel test in the 'ZT' software (Bonnet and Van de Peer, 2002). In order to investigate the influence of the ecological niche on genetic variations, we conducted a genetically-informed ecological niche analysis within the PCA framework proposed by Broennimann et al. (2012). This approach allowed us to assess uncorrelated principal components. To elucidate niche segregations, we compared the position of the Kernel density estimate of sample points (at a resolution of 100 m) against the accessible space for each entity within the brown bear groups (Broennimann et al., 2012). Additionally, for pairwise comparisons between existing groups, we computed a niche overlap index, hereafter referred to as Schoener's D, using the density grids. To ensure that any observed pairwise differences in niche segregation were not due to chance, we performed niche similarity and niche equivalency randomization tests (Warren, Glor & Turelli, 2008), as recommended by Ashrafzadeh et al. (2018). To determine the environmental space available across three roe deer subpopulations (in three provinces), we considered all pixels of the 19 climatic variables within 50-kilometer buffers around each presence point. We used the ecospat package (Di Cola et al., 2017) to compare the roe deer niches in each population. The relationship between the genetic distance matrix and the matrix of Schoener's D values was estimated using the Mantel test in the 'ZT' software.

2.6.2. Isolation by distance (IBD)

We evaluated the IBD in GenAlex version 6.5 using the Mantel test (ESRI, Redlands, CA, USA). We also quantified the pairwise linear genetic distances and the linear Euclidean distances in GenAlex and ArcGIS version 9.3 (ESRI, Redlands, USA), respectively.

2.6.3. Isolation by resistance (IBR)

Species distribution modeling (SDM) was applied to assess the impact of landscape resistance on genetic isolation (i.e. IBR). A habitat suitability map as an indicator of landscape resistance was constructed from several SDM algorithms including: Generalized Boosting Models (GBM), Maximum Entropy (Maxent), Random Forest (RF), Generalized Linear Model (GLM), Multivariate Adaptive Regression Splines (MARS) and artificial neural networks (ANN) and an ensemble model using the biomod2 package in R (R Core Team, 2016). To avoid multicollinearity between predictor variables, we excluded variables if they were greater than the limit of $|r|$ were > 0.7 using Pearson's correlation coefficient test (Dormann et al., 2013; Mahmoodi et al., 2022) (Table 1). To evaluate the performance of the models, the true skill statistics (TSS) and the receiver operating characteristic (ROC), and the area under the curve (AUC) were used (Mahmoodi et al., 2023; Ahmadi et al., 2023). Finally, individual models were weighted based on their AUC values based on the weighted-average technique to perform the ensemble model (Shadloo et al., 2021).

Different scenarios were employed to determine the variables that might be affected by the potential gene flow of roe deer. Species distribution modeling based on three different approaches was applied to produce habitat resistance layers. Firstly, the values were subtracted from 101, and the habitat suitability was classified from 1 to 101 (Hirzel et al., 2006; Wang et al., 2009; Richards-Zawacki, 2009, Wang and Summers, 2010). Then, the habitat suitability map was categorized into four equal categories, including category I: HS < 25; Cat II: $50 >$ HS ≥ 25; CatIII: $75 >$ HS ≥ 50; Cat III: $100 >$ HS ≥ 75. Next, a resistance value was assigned to each habitat suitability class: Cat I: 1000, Cat II: 100, Cat III: 10, and Cat III: 1 (Wang et al., 2009). A threshold-based approach was selected for the sensitivity and specificity test outputs drawn from the ensemble model, which was derived from the third applied approach. According to the selected threshold, the suitability map was classified into two categories of suitable and unsuitable areas, each of which was assigned resistance values of 1 and 1000, respectively (Wang et al., 2009).

To generate a resistance model for the slope variable, 18 scenarios were considered (Epps et al., 2007). Three cut-off points (10%, 20%, and 30%) were used based on the response curves of the variables. For each cut-off point, six grids were produced, and each cell implied the range of different values of resistance for each cell in the slope layer (Epps et al., 2007). For example, the resistance level of 1 for a cell without slopes $>$ 0, the cells' resistance levels (ranging from strong to weak) were weighed as 0.1, 0.3, 0.5, 0.7, 0.01, and 0.05, respectively.

The next scenario was based on land use, it has been shown that roe deer in the Hyrcanian forest avoid areas where anthropogenic activities are high and tend to occur in dense forest areas (Soofi et al., 2018). We reclassified land use layer into two classes: forested areas and non-forested areas in ArcGIS. We then assigned the resistance value of 1 to the forested areas and the resistance value of 1000 to non-forested areas. In addition, we used normalized vegetation index (NDVI) in the analysis. We obtained this index using MODIS satellite images with a resolution of 1 km. Initially, we multiplied NDVI values to 100 and were categorized into two resistance classes: resistance of 1000 (with a value of 0) and resistance of 1 (with values of 0 to 100). Then we categorized NDVI layer into four classes (i.e., 0–25, 25–50, 50–75 and > 75), on which resistance values of 1, 10, 100, and 1000 were assigned, respectively.

The resistance model was obtained based on elevation variable, for which we assumed that altitude could be an indicator of higher diversity and richness of roe deer and that at the moderate elevation, the resistance level might be lower. We further expected that any deviation from that optimal elevation can lead to an increase in the resistance value. Hence, we used an inverse Gaussian function (Eq. (1)) to reclassify the digital elevation model (DEM) layer (Castilho et al., 2011). To do so, we applied 4 different values of maximum resistance (Rmax: 2, 10, 100, and 500), 5 optimal altitudes (Eopt: 200, 800, 1200, 1500, 1800- and 2000-meters elevation ranges), and 3 standard deviation values (ESD: 100, 200, 300). Finally, we computed 94 models based on the elevation variable described in Eq. (1) as

$$R = Rmax + 1 - Rmax * e^{\frac{-(Elev.-Eopt)^2}{2*E_{SD}^2}} \quad (1)$$

where R indicates resistance, Rmax: maximum resistance Eopt: optimum elevations ESD:standard deviations.

To assess the potential impact of the aspect variable on roe deer gene flow, we reformed the heat load index, so that to find the optimum geographical aspect in ArcGIS (McCune and Keon, 2002). The heat load index proposed by McCune and Keon (2002) suggests that the coldest and the warmest points are located in northeastern (45°) with value 0 and the southwestern (225°) with value 1, respectively.The plain areas with −1 cell value were classified as Rmax/2 in the original unclassified file (Castilho et al., 2011). We generated geographical aspect categories of 45°from 0 to 315 (X = 0.5, 1, 2, 4, and 10), based on our heat load index, with optimal aspects (θopt) of 0, 45, 90, 135, 180, 225, 270, and 315. The Rmax value was selected in a similar manner as we did for elevation-based resistance model. Overall, we produced 160 models

Table 1
Environmental variables participating in habitat modeling.

Variable	Unit	Source
Altitude	Meter	https://earthexplorer.usgs.gov/
Slope	Percentage	https://earthexplorer.usgs.gov/
Aspect	Category	DEM
Max Temperature of Warmest Month (BIO5)	°C × 10	Fick and Hijmans, 2017
Annual Precipitation (BIO12)	Millimeter	Fick and Hijmans, 2017
Land cover	Meter	IFRWO*, 2010
Distance to river	Meter	IFRWO*, 2010
Distance to road	Meter	IFRWO*, 2010
Distance to village	Meter	IFRWO*, 2010

using equation (2) (Castilho et al., 2011) expressed as follows:

$$R = \left[\frac{1-\cos(\theta-\theta_{opt})}{2}\right]^{x} Rmax \quad (2)$$

where, R indicates resistance, Rmax is maximum resistance, and θ denotes the optimal aspect.

2.7. The optimal model with a single variable

We examined the univariable resistance model by comparing pairwise genetic and cost differences. To quantify the least-cost path between each pair and generate pairwise matrices, we used the PATHMATRIX tool v. 1.1 used in ArcGIS (Ray, 2005). We also created a grid map with five landscape variables with a cell size of 30 m (Zhu et al., 2010). We also calculated the pairwise genetic distances in GenAlex. In addition, to determine the relationship between matrices, we applied Mantel and partial Mantel tests in the ZT software (Bonnet and Van de Peer, 2002). Finally, standoff isolation was used to control for the standoff effect alone in the partial Mantel test (Castilho et al., 2011).

3. Results

3.1. Microsatellite loci characteristics

The results show that the allele size (range: 75 to 280) of roe deer in the majority of the microsatellite loci examined was consistent with the previous literature (Table 2).

Data for SSR loci polymorphism showed that CSSM41, MCM505 and CSSM39 exhibited the highest diversity and CSSM41, BM302 remained the lowest diversity in the population. However, BMC1009 showed a monomorphic pattern across the populations studied and was therefore excluded from further analysis. Our Shannon Index result also indicated that the majority of the loci examined were suitable for assessing roe deer genetic diversity (Table 3).

3.2. Intra-and-inter population genetic diversity

Our estimated FIs index showed that the Golestan, Arasbaran, and Guilan populations had the highest and lowest genetic diversity among roe deer populations. According to the Fst criteria, our results showed that Golestan and East Azarbaijan (Arasbaran) had the highest and Mazandaran the lowest genetic distance patterns (Fig. 2).

Our cluster analysis revealed a graphical pattern of genetic diversity within and between populations of roe deer (Fig. 3).

Table 3
Summary of indicators of polymorphism of SSR loci in the studied population.

Locus	AlleleNo	Obs-Het	Exp-Het	F_{ST}	R_{ST}	N_m
NVHRT48	4.00	0.32	0.58	0.38	0.38	0.25
CSSM41	5.00	0.39	0.67	0.59	0.25	0.56
BM1818	3.00	0.17	0.50	0.69	0.01	3.05
OarFcB304	4.00	0.26	0.65	0.79	0.03	0.58
BM757	5.00	0.21	0.69	0.75	−0.01	3.76
Roe06	4.00	0.19	0.66	0.84	0.09	3.30
BMC1009	2.00	0.00	0.00	–	–	–
MCM505	5.00	0.58	0.67	0.80	0.13	3.33
CSSM22	4.00	0.14	0.66	0.90	0.15	1.13
BM302	3.00	0.08	0.51	0.88	−0.04	2.54
CSSM39	5.00	0.14	0.62	0.71	−0.06	17.54
MAF70	3.00	0.11	0.50	0.79	−0.01	0.59
Mean	3.92	0.18	0.56	0.57	0.06	0.05

Fst = (Ht − Mean He)/Ht, Nm = [(1/Fst) − 1]/4; Obs-Het: observed heterozygosity; Exp-Het: expected heterozygosity; Rst: Genetic, differentiation by stepwise mutation.

Our microsatellite loci analysis has unequivocally deciphered the genetic diversity, suggesting that a potential genetic linkage appears to be occurring within roe deer populations. It also shows genetic distance and allelic differentiation between populations.

3.3. SSR-based cluster analysis

Our SSR-based cluster analysis revealed high genetic relatedness and gene flow in the study areas (i.e., Golestan, Mazandaran, Gilan, and Arasbaran). Furthermore, our PCA analysis showed a strong genetic correlation between the roe deer populations in the study areas (Fig. 4). The first and second PCs were justified by ~81.09% and ~18.91% of the variance between the studied populations, respectively. However, we did not find a significant genetic distance between the populations, but the genetic distance between the roe deer population in Gilan (western Hyrcanian forests) and the population in East Azerbaijan (Arasbaran forests) was high (Fig. 4). Fig. 4 shows that genetic mixing between populations is high.

3.4. SSR-based structure analysis

Our results further showed that ~0.75% of roe deer genotyping and historical biological background was associated with the population itself, while 25% of the population appeared to have migrated from other populations. The highest migratory rate was observed between Golestan and Mazandaran populations. Most violations have been linked to the

Table 2
Characteristics of microsatellite loci and molecular descriptive statistics in the studied population.

Locus	Size allele	na*	ne*	I*	Obs_Het	Exp_Het*	Nei**	Ave_Het	PIC
NVHRT48	75–99	3.00	2.33	0.92	0.32	0.58	0.57	0.57	0.49
CSSM41	110–118	4.00	2.98	1.20	0.39	0.67	0.66	0.66	0.61
BM1818	240–248	2.00	2.00	0.69	0.17	0.50	0.50	0.50	0.37
OarfcB304	150–200	3.00	2.85	1.07	0.26	0.65	0.65	0.65	0.57
BM757	180–204	4.00	3.14	1.25	0.21	0.69	0.68	0.68	0.63
ROe06	102–114	3.00	2.89	1.08	0.19	0.66	0.65	0.65	0.58
BMC1009	280–280	1.00	1.00	0.00	0.00	0.00	0.00	0.00	
MCM505	112–188	4.00	2.97	1.14	0.16	0.67	0.66	0.66	0.59
CSSM22	188–208	3.00	2.92	1.08	0.14	0.66	0.66	0.66	0.58
BM302	142–148	2.00	2.00	0.69	0.08	0.50	0.50	0.50	0.37
CSSM39	160–168	4.00	2.61	1.07	0.14	0.62	0.62	0.62	0.56
MAF70	122–128	2.00	1.99	0.69	0.11	0.50	0.50	0.50	0.37
Mean		2.92	2.47	0.91	0.18	0.56	0.55	0.55	
St. Dev		1.00	0.63	0.35	0.11	0.19	0.19	0.19	

Na = No. of Different Alleles; Ne = No. of Effective Alleles = 1/(Sum pi^2); I = Shannon's Information Index = −1* Sum (pi * Ln (pi)); Obs-Het = Observed Heterozygosity = No. of Hets/N; Exp-Het = Expected Heterozygosity = 1 − Sum pi^2; F = Fixation Index = (He − Ho)/He = 1 − (Ho/He); Where pi is the frequency of the allele for the population & Sum pi^2 is the sum of the squared population allele frequencies. *P < 0.05; **P < 0.01; ***P < 0.001. No test was done for the loci with less than five alleles because the number of permutation configurations is too low to carry out a test at a 5% level. Nei = measure of the average genetic diversity per locus, HS; Ave_Het = Average He across the populations; PIC = Polymorphic Information Content.

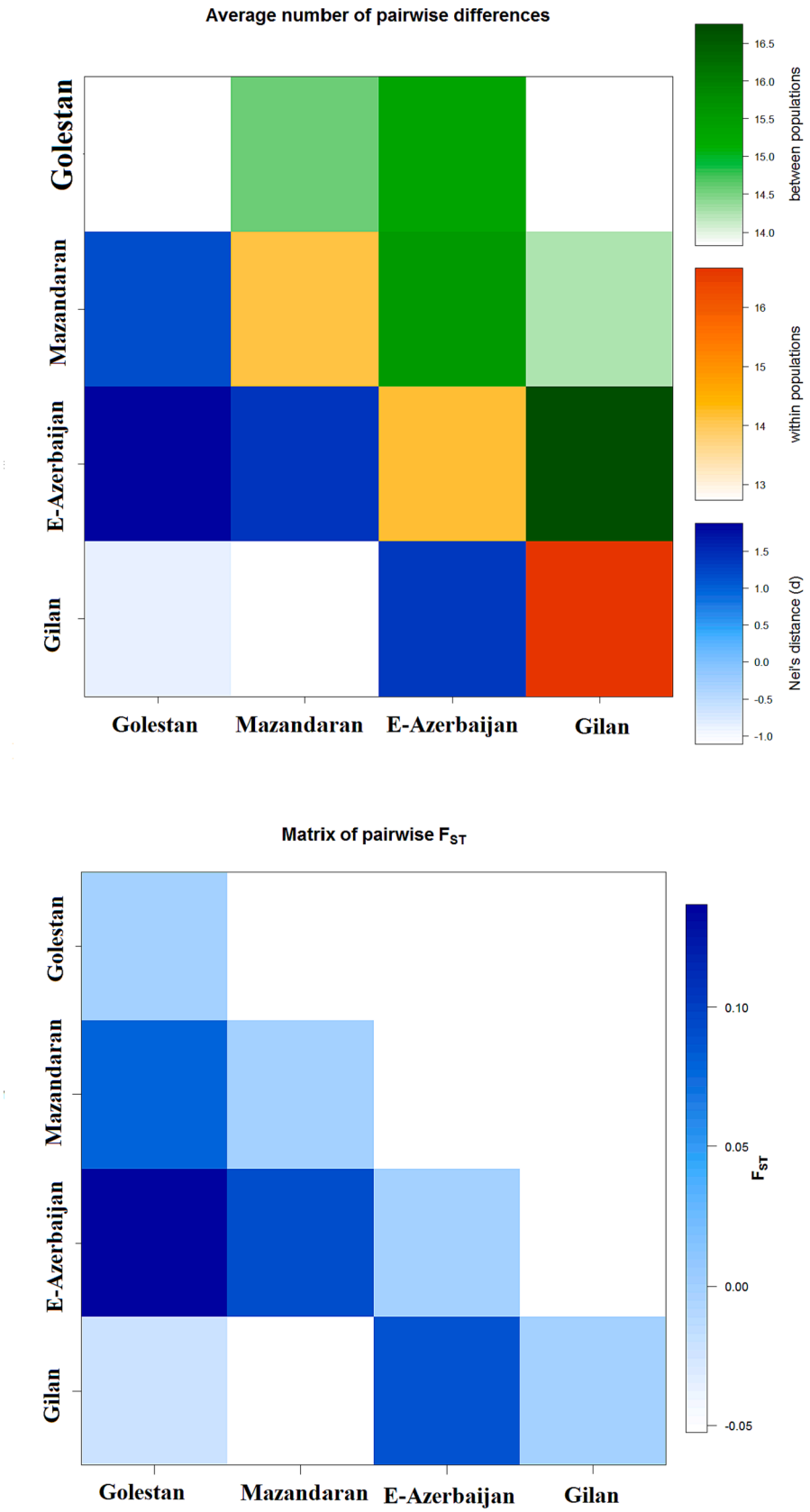

Fig. 2. Illustrates the genetic differentiation within and amongst populations. B – displays the extent of genetic differentiation between populations by FST fixation index).

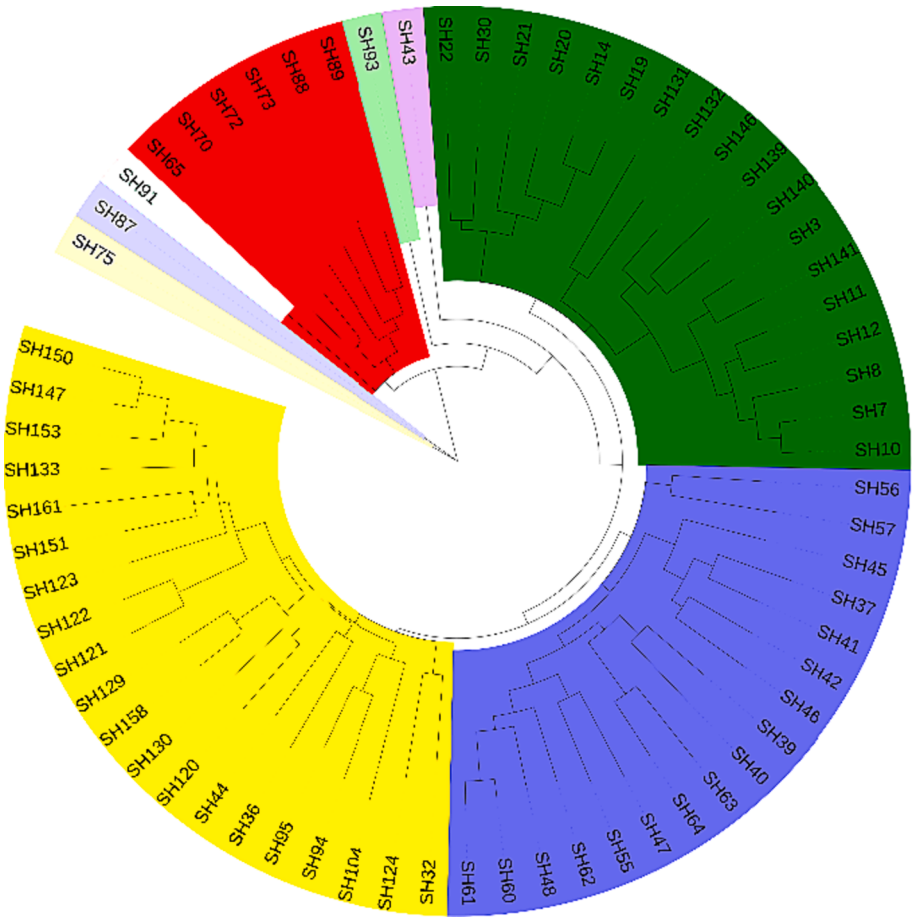

Fig. 3. Diagram of phylogenetic networks between samples using R-based adegenet package.

Mazandaran population. We found a high level of allelic similarity in the populations studied. Only 25% of the genotype per sample population was shared with other populations and 75% were possibly related to the individuals in each population. The maximum numerical value of the K statistic (K = 4) indicates the best standard criterion for classifying the population studied. The Bayesian clustering approach identified four distinct genetic clusters in which only genetic mixing between individuals was high, indicating strong gene flow between individuals (Fig. 5).

The genetic cluster assignments in different groups showed that each population studied retains proportional allelic admixture and associations with other populations (Fig. 6). Taken together, these results indicate that the level of genetic association of individuals between populations was relatively high.

3.5. Isolation by distance, environment and resistance

Our results showed that genetic differentiation and geographic distance correlation were not significant across the study area ($p = 0.122$, $p > 0.05$), while isolation by environmental results ($p = 0.17$, $p > 0.05$) suggested that these Mantel tests based on environmental differences were not significantly associated with genetic differentiation between individuals.

3.6. Niche comparisons at the population level

The population-level ecological niche differentiation test showed that (PCA 79.61% axis = 1.79%) was the highest trend in the climate variable (Fig. 7). We found the highest Schoener's D value (D = 0.07) for MazandaranGolesan, a moderate value for MazandaranArasbaran (D = 0.17), and a very low value for GolestanArasbaran (Fig. 8). Niche equivalence analyses showed that the Golestan and Mazandaran populations did not differ significantly ($p = 0.9$, $p > 0.05$). Likewise, we found no significant ($p = 1$, $P > 0.05$) ecological niche difference for Golestan and Arasbaaran populations and for the Arasbaran-Mazandaran populations ($p = 0.04$, $p < 0.05$). However, our niche similarity analysis between these three clustered populations reveals a significant ($P < 0.05$) similarity pattern. Niche similarity analyses showed that Golestan-Arasbaran populations did not differ significantly ($p = 0.7$, $p > 0.05$) and Arasbaran-Mazandaran and Mazandaran-Golestan populations ($p = 0.009$, $p < 0.05$). (Fig. 9). Isolation by environmental analysis revealed that there was no significant association between genetic distance and niche overlap index between populations, and that separation based on ecological niches does not affect genetic patterns. The results of the univariate analysis indicated that none of the environmental variables were significantly related to genetic distance in the presence of the IBD control factor.

For the comparison of the Golestan and Mazandaran populations, the hypothesis of niche equivalence was rejected ($p = 0.9$, $p > 0.05$); Fig. 9f), Golestan-Arasbaaran populations ($p = 1$, $P > 0.05$); Fig. 9c and Arasbaran-Mazandaran populations ($p = 0.04$, $p < 0.05$); Fig. 9d. However, our niche similarity analysis between these three clustered populations reveals a significant ($P < 0.05$) similarity pattern. Niche similarity analysis showed that the Golestan-Arasbaran populations were not significantly different ($p = 0.7$, $p > 0.05$); Fig. 9l) as well as for Arasbaran-Mazandaran and Mazandaran-Golestan populations ($p = 0.009$, $p < 0.05$); Fig. 9g, h).

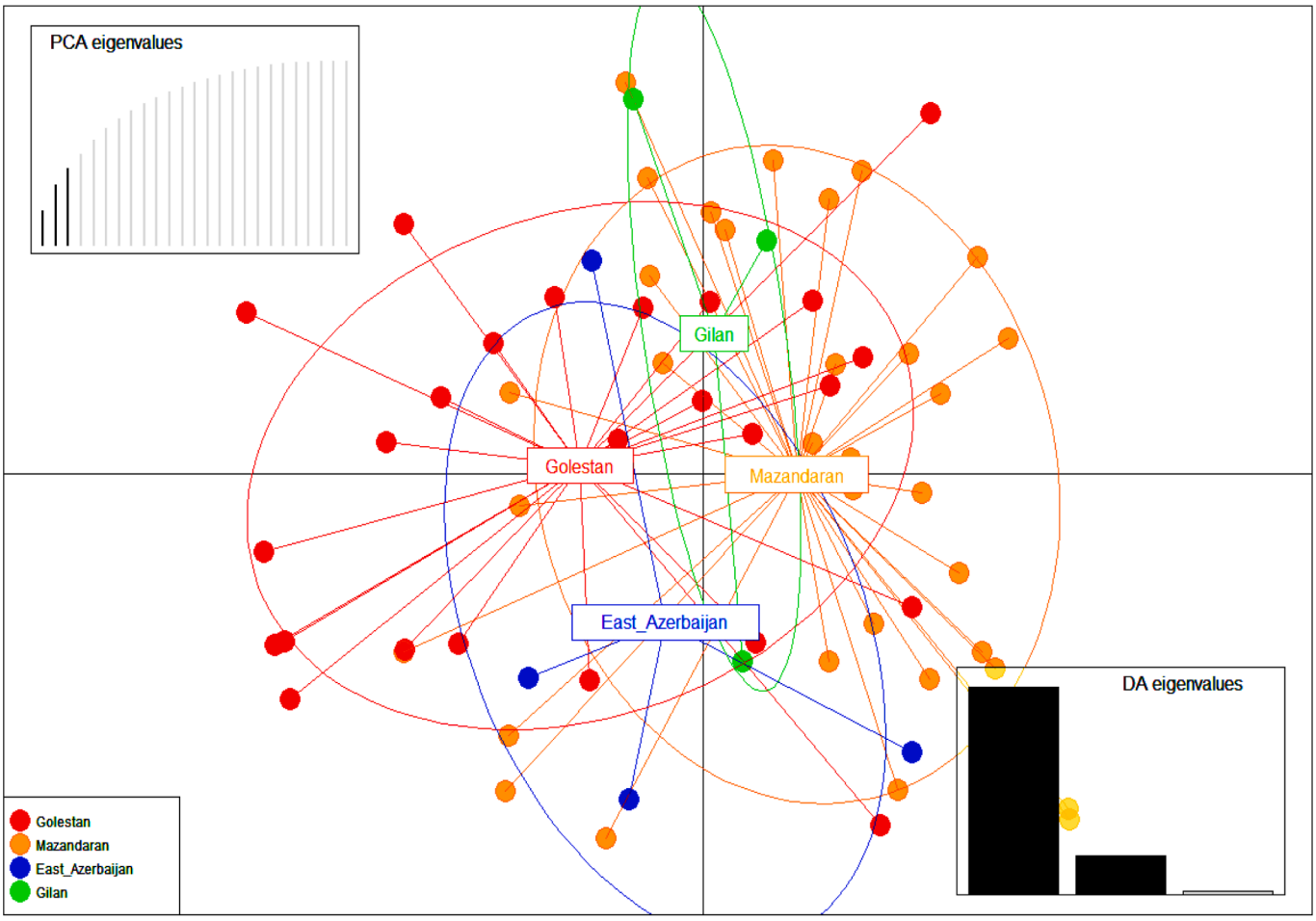

Fig. 4. PCA analysis for the species studied based on the adegenet package in R software Environment for each population.

3.7. Binary habitat suitability

The binary map showed that 14% of the study area was optimal (Fig. 10). Optimum centers are more commonly found at lower elevations and low-lying areas. The results also showed that the RF method performs best among other methods. Overall, this method was the one that provided the most robust solutions. The most important and effective variables in determining wildlife suitable habitat, which accounted for most of the model construction, were elevation and land cover, which were very similar effects. The Maximum Temperature of the Warmest Month also had a greater impact on roe deer distribution. Land cover and Maximum Temperature of the Warmest Month turned out to be the most important factors influencing the distribution of roe deer in the study area (Table 4).

3.8. Isolation by resistance

We found no significant relationship between the matrix of least cost path and the matrix of genetic distance between pairs of sampled individuals ($P > 0.05$, for all scenarios). This suggests that habitat suitability, slope, land cover, and NDVI do not significantly affect gene flow. We found evidence that elevation (range: 1000–1800 m) and north aspect explained variants for optimal gene flow, while landscape variables showed no significant impact on habitat suitability (Table 5).

4. Discussion

4.1. Population genetic diversity and structure

One of the main goals of this study was to investigate the genetic diversity of the roe deer populations in the study area. Analyzes of genetic patterns based on F statistics and Bayesian clustering showed that high gene flow between populations formed a genetically homogeneous group. We found relatively low to moderate genetic diversity and also flat genetic patterning in the remaining roe deer populations. Our results also showed that roe deer populations exhibit moderate genetic variation. The genetic diversity of roe deer in this area is lower than in other regions where the species occurs, including Europe (Markov et al., 2016; Matosiuk et al., 2014; Amiri et al., 2021). Amiri et al. (2021) assessed the genetic diversity and phylogeography of roe deer populations in Iran, with the results showing that roe deer have low genetic diversity. Genetic variation plays a crucial role in enabling populations to adapt to environmental change and persist over time (Frankel, 1974). Studies have shown that populations with higher genetic variation are more likely to survive ecological or evolutionary changes (Quattro & Vrijenhoek, 1989; Leberg and Smith, 1993). Furthermore, even minor changes in genetic variation can have a significant impact on population fitness (Frankham, 1995).

4.2. Landscape genetics approach

In the present study, different IBR scenarios were applied to identify and measure the relationship between roe deer genetic structure and landscape patterns. Our results showed that elevation and aspect affect

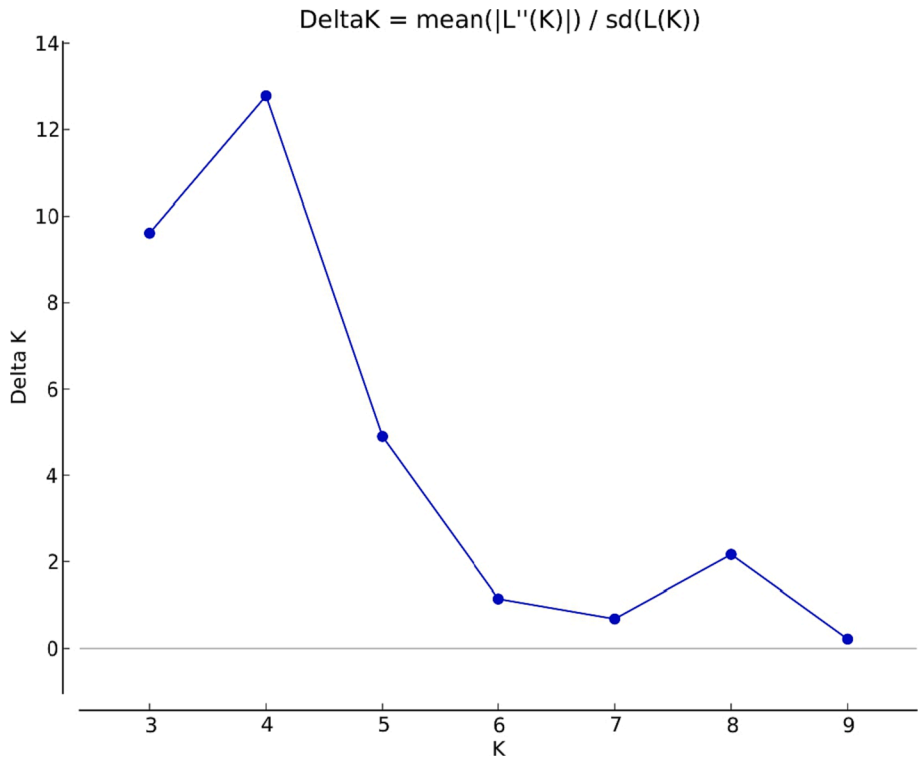

Fig. 5. The most likely number of genetically distinct clusters within roe deer populations in the north and northwestern Iran is estimated based on Delta K (Evanno, Regnaut & Goudet, 2005).

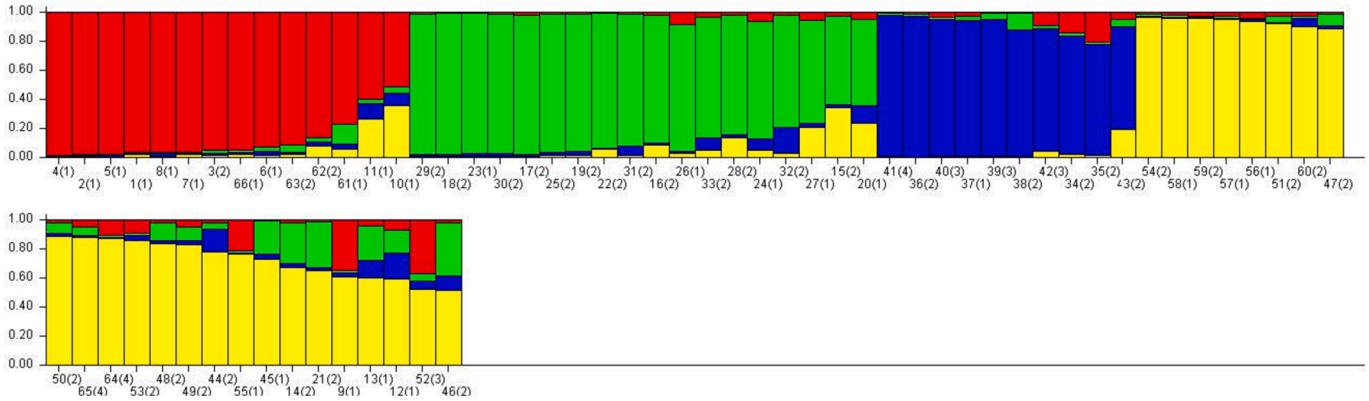

Fig. 6. Each vertical bar represents one individual roe deer. Colors indicate the most likely genetic cluster assignments in different groups identified by STRUCTURE software (red = Golestan; yellow = Arasbaran, green = Mazandaran; and blue = Gilan). Inferred ancestry of individuals: Inferred clusters = Y; Individual (Pop) = X.

the gene flow of roe deer in northern and northwestern Iran. Areas of medium elevation and dense forest cover increase gene flow in the study area. The deer are assigned to the forest type in the middle elevation and avoid the forests of the lower elevation in the study area. Deer prefer mature forests in winter and this has been confirmed for other deer species such as white-tailed deer, mule deer, and mouse deer.

On the other hand, the results showed that virgin forest use increases with increasing snow depth and that snow is an important factor in habitat selection by roe deer and its presence decreases at high elevations and more mature forests are used (Mysterud et al., 1999; Nilsen et al., 2004; Hewison et al., 2001; Bonnot et al., 2013). Elevation and climate variables (such as precipitation and temperature) play important roles in determining feeding times and food access restrictions for deer. In winter, deer approach roads and water sources at low elevations. In winter, species are more likely to be seen by hunters due to less vegetation (Telfer, 1967).

Results showed that the removal of water sources had little effect on deer presence. Mahmoodi's et al. (2020) results showed that water is not a limiting variable for this species in Iran as water is easily accessible and usable. Deer usually choose their habitat near water sources and rivers. Because this species prefers to feed on fresh plants and tree buds (Jasińska et al., 2021). Roe deer swim around the river in the study area and this behavior is consistently observed, although rivers or lakes, even at high mobility, impede gene flow for other mammalian species (Mladenoff et al., 1995). Hepenstrick et al. (2012) showed that rivers have only a moderate impact on gene flow in roe deer. The results of Coulon et al. (2004) showed that while rivers separate urban areas and highways for deer populations, they do not present impenetrable barriers and allow individuals to cross them.

The results showed that artificial barriers such as roads do not make a significant genetic difference between roe deer populations. Roads are considered a barrier to gene flow for small species (Keller et al., 2004),

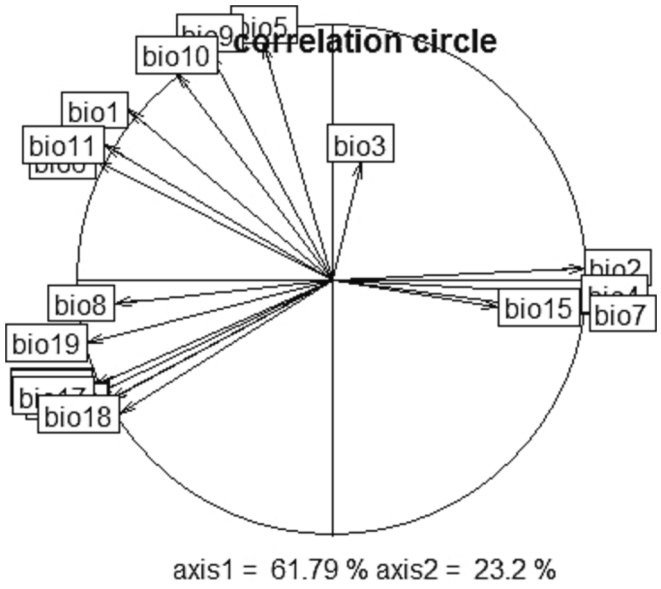

Fig. 7. Results of population-level niche differentiation of Iranian roe deer groups based on the PCA-env method (Broennimann et al., 2012).

but large and highly mobile species are less affected (Kuehn et al., 2007). Although there are important tourist routes in the study area, they must not cause genetic differentiation of the population and must not affect the exchange and movement of roe deer. However, the negative impact of roads on the distribution of mammals has been reported (Roach et al., 2001; Jedrzejewski et al., 2004; Epps et al., 2005; Waller and Servheen, 2005; and Riley et al., 2006). Deer can exhibit resilient behavior and even adapt to human-induced disturbances (Hewison et al., 2001; Bonnot et al., 2013; Jeppesen, 1987). But the potential of roads to create genetic infrastructure should be considered when planning future roads (Jiang et al., 2009; Bonnot, 2013).

The minor effects of land cover and man-made phenomena may be the result of multiple non-interacting processes. It has weak genetic differentiation in the same country appearance. Given the species distribution in the study area, we believe that landscape heterogeneity is not sufficient to influence gene flow. Deer can also move to different habitats. In this study, the land cover map was divided into two parts (forested areas and non-forested areas) and the resistance map was created. According to the Mantel tests, the correlation between genetic distance and the least cost path matrix was not significant. When the study area is large, habitat type has less impact on genetic diversity patterns and does not pose a barrier to gene flow (Burkart et al., 2016). Despite being forested areas, the relationship between roe deer (Coulon et al., 2004) and white-tailed deer (Long et al., 2005) in our study area according to the partial fur test by land cover map classification (forested areas & non-forested areas) does not count as an obstacle for gene flow in the considered deer.

Several scenarios were defined to create the habitat suitability map landform resistance model. The results showed that habitat suitability is not a barrier to gene flow in roe deer. This may be because most areas of the study area are suitable for roe deer. Consequently, there is no relationship between the genetic distance matrix and the least-cost path matrix. The results of Balkenhol et al. (2009) also showed that the relationship between genetic diversity and habitat suitability is less clear at the landscape level. They showed that deer in moderately suitable habitats differed from deer in low and highly suitable habitats.

The results of the niche similarity test show that there is no significant association between population divergence and ecological niche similarity and therefore evolutionary separation events through allopatric adaptation may not have occurred for Arasbaran-Gilan populations and not for Golestan and Mazandaran. Ecological niche models showed that roe deer niches, genetic clusters with different geographic

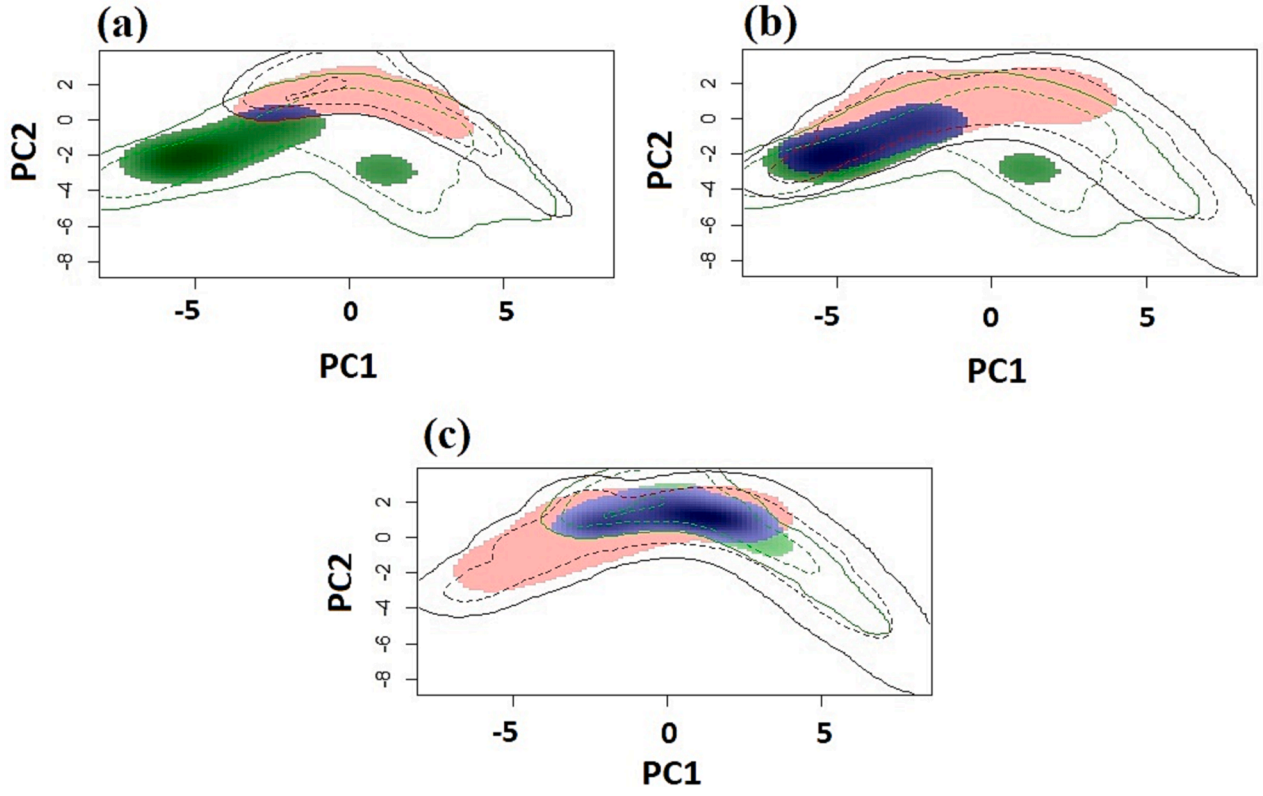

Fig. 8. Results of the population-level niche overlap analysis showed low values of niche overlap between Arasbaran- Golestan (Schoener's D = 0.07; Fig. 8a), Arasbaran-Mazandaran (Schoener's D = 0.39; Fig. 8b) and Golestan – Mazandaran (Schoener's D = 0.17; Fig. 8c.

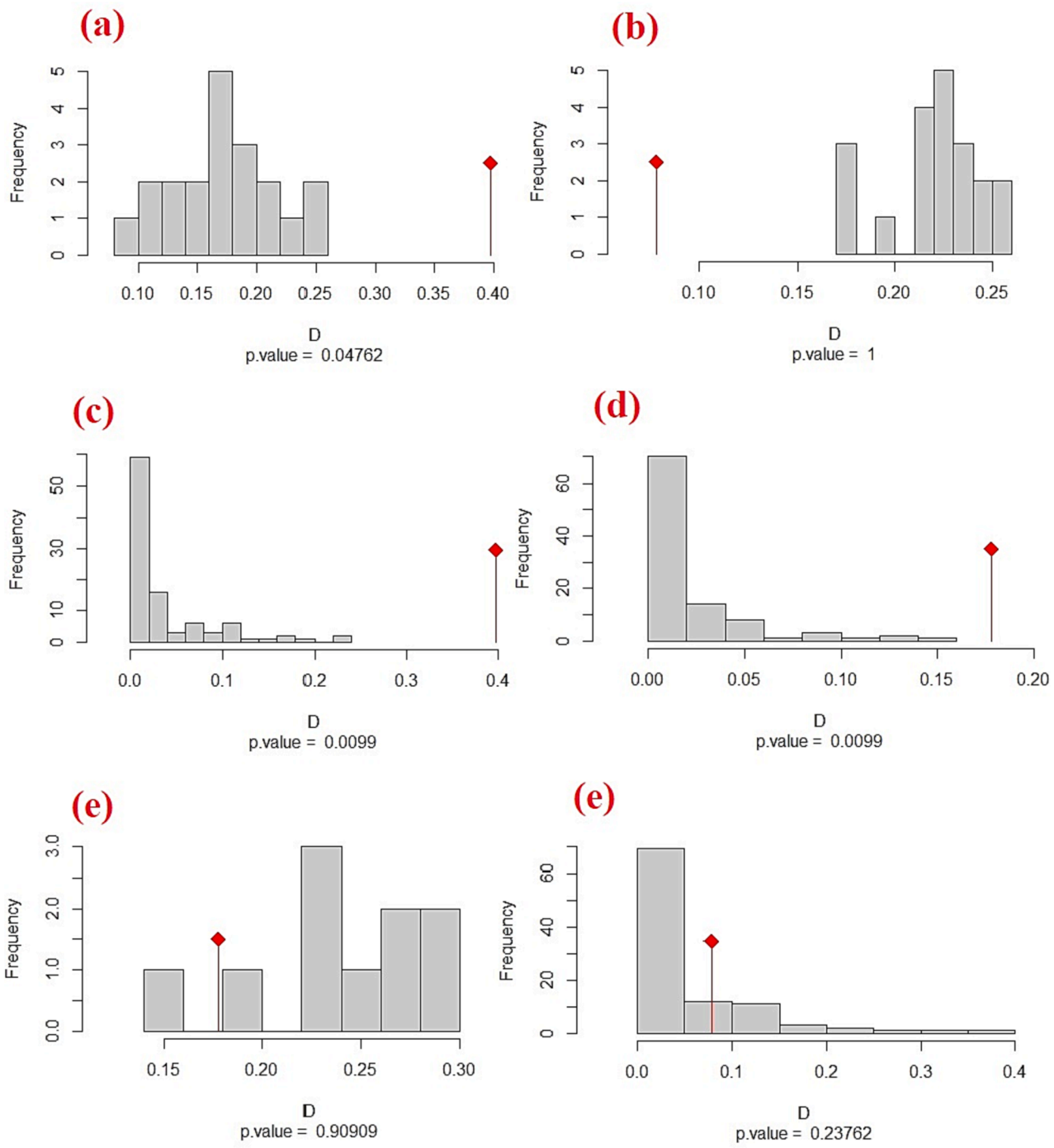

Fig. 9. Result of niche similarity analysis between three clustered populations.

distributions, showed a slight overlap. According to the IBD hypothesis, geographic distance does not affect genetic differentiation and the relationship between a geographic distance matrix and a genetic distance matrix was not significant. which is common in migratory species such as roe deer (Khosravi et al., 2017). The results of Kierepka et al. (2016) showed that geographic distance is an important and influential factor in gene flow in roe deer.

5. Conclusion

This study examined for the first time the effect of several land traits on roe deer gene flow, and the results showed that despite the relatively large extent of the study area, no significant population structure with high dispersibility of roe deer was found. It is possible that the genetic specificity is not related to the current connection or isolation of the study areas, but to some processes that took place many years ago. Our results show that despite the small genetic differences between the populations, future strategies need to be implemented to keep this population under optimal conditions. Our study also suggests how knowledge at the individual level can be used to determine the impact of land fragmentation on migratory (herbivorous) mammalian species. We observed relatively low to moderate genetic diversity and slightly differentiated genetic structure in the roe deer population.

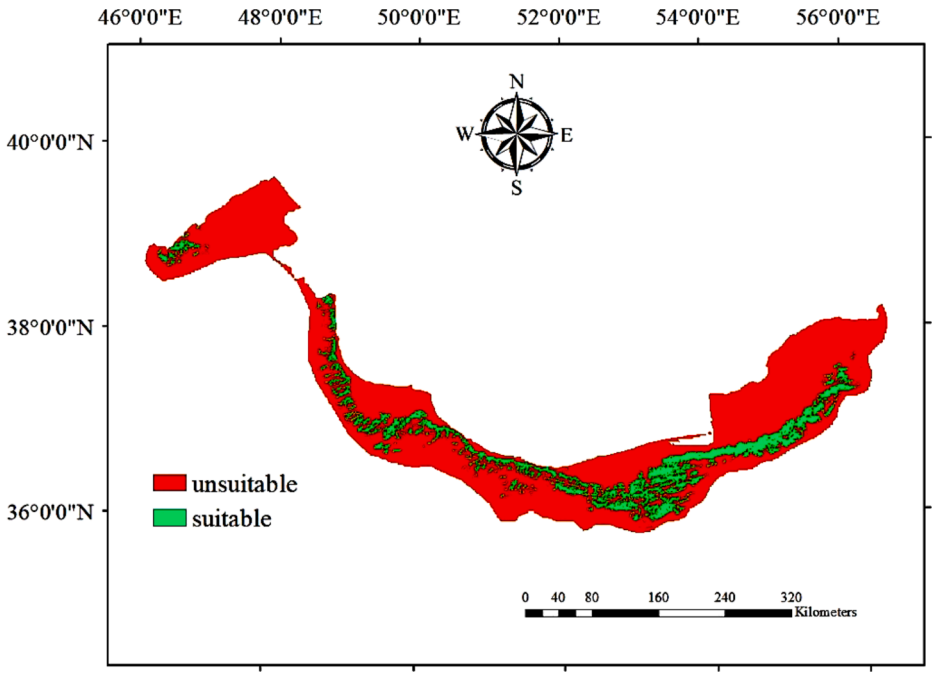

Fig. 10. The binary habitat suitability map for roe deer in the north (Hyrcanian forest) and north western (Arsabaran forests) of Iran.

Table 4
Analysis of variable contribution for the ensemble model fit explaining the distribution of roe deer in North of Iran.

Variable	Ensemble model	GLM	MAXENT	GBM	RF	MARS	ANN
Elevation	29%	31%	30%	21%	27%	13%	22%
Landcover	29%	22%	20%	46%	11%	13%	24%
Max Temperature of Warmest Month (BIO5)	17%	30%	19%	2%	12%	1%	9%
Slope	11%	4%	15%	15%	20%	6%	18%
Annual Precipitation (BIO12)	8%	7%	5%	7%	13%	4%	2%
Aspect	4%	5%	2%	2%	3%	1%	0.4%
Distance from road	1%	0.4%	4%	5%	4%	2%	1%
Distance from river	0.3%	00%	3%	1%	6%	00%	0.4%
Distance from village	0.1%	00%	3%	0.3%	4%	00%	0.4%

Table 5
Result of suitability model and classified it in three different ways for producing resistance layers on gene flow by a single-variable optimum model using mantle and partial mantle and its significance level.

suitability model and classified it in three different ways for producing resistance layers	Partial mantel (r)	p-value
First. Habitat suitability was classified from 1 to 101 by subtracting the habitat suitability value from 10.	0.03	0.18
Second. We classified habitat suitability to four equal categories (class1: HS < 25; class 2: 50 > HS ≥ 25; class 3: 75 > HS ≥ 50; class 4: 100 > HS ≥ 75)	0.04	0.05
Third. The suitability map was classified into suitable and unsuitable categories.	0.02	0.24

CRediT authorship contribution statement

Shirin Mahmoodi: Methodology, Formal analysis, Validation, Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Visualization, Writing – original draft. **Kourosh Ahmadi:** Formal analysis, Software, Writing - review & editing. **Afshin Alizadeh Shabani:** Validation, Resources. **Mehrshad Zeinalabedini:** Validation. **Arash Javanmard:** Validation, Writing – review & editing. **Olyagholi Khalilipour:** Validation, Writing – original draft. **Mohammad Hossein Banabazi:** Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110835.

References

Ahmadi, K., Alavi, S.J., Amiri, G.Z., Hosseini, S.M., Serra-Diaz, J.M., Svenning, J.C., 2020. The potential impact of future climate on the distribution of European yew (Taxus baccata L.) in the Hyrcanian Forest region (Iran). Int. J. Biometeorol. 64, 1451–1462.

Ahmadi, K., Mahmoodi, S., Pal, S.C., Saha, A., Chowdhuri, I., Nguyen, T.T., Socha, J., 2023. Improving species distribution models for dominant trees in climate data-poor forests using high-resolution remote sensing. Ecological Modelling 475, 110190.
Allio, R., Donega, S., Galtier, N., Nabholz, B., 2017. Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. Mol. Biol. Evol. 34 (11), 2762–2772.
Amiri, M., Rezaei, H.R., Naderi, S., Kiabi, B.H., 2021. Genetic diversity and phylogeography of European Roe Deer, Capreolus capreolus, in Iran as inferred from mtDNA genes (Mammalia: Cervidae). Zool. Middle East 67 (2), 95–105.
Ashrafzadeh, M.R., Khosravi, R., Ahmadi, M., Kaboli, M., 2018. Landscape heterogeneity and ecological niche isolation shape the distribution of spatial genetic variation in Iranian brown bears, Ursus arctos (Carnivora: Ursidae). Mamm. Biol. 93 (1), 64–75.
Balkenhol, N., Cushman, S.A., Waits, L.P., Storfer, A.. 2016. Current status, future opportunities, and remaining challenges in landscape genetics [Chapter 14]. In: Balkenhol, Niko; Cushman, Samuel A.
Balkenhol, N., Waits, L.P., Dezzani, R.J., 2009. Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. Ecography 32 (5), 818–830.
Baur, B., Erhardt, A., 1995. Habitat fragmentation and habitat alterations: principal threats to most animal and plant species. GAIA-Ecological Perspectives for Science and Society 4 (4), 221–226.
Benoit, L., Hewison, A.M., Coulon, A., Debeffe, L., Gremillet, D., Ducros, D., Morellet, N., 2020. Accelerating across the landscape: The energetic costs of natal dispersal in a large herbivore. J. Anim. Ecol. 89 (1), 173–185.
Bhargava, A., Fuentes, F.F., 2010. Mutational dynamics of microsatellites. Mol. Biotechnol. 44 (3), 250–266.
Bonnet, E., Van de Peer, Y., 2002. zt: A sofware tool for simple and partial mantel tests. J. Stat. Softw. 7 (10), 1.
Bonnot, N., Morellet, N., Verheyden, H., Cargnelutti, B., Lourtet, B., Klein, F., Hewison, A.M., 2013. Habitat use under predation risk: hunting, roads and human dwellings influence the spatial behaviour of roe deer. Eur. J. Wildl. Res. 59 (2), 185–193.
Broennimann, O., Fitzpatrick, M.C., Pearman, P.B., Petitpierre, B., Pellissier, L., Yoccoz, N.G., Guisan, A., 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Glob. Ecol. Biogeogr. 21 (4), 481–497.
Burkart, S., Gugerli, F., Senn, J., Kuehn, R., Bolliger, J., 2016. Evaluating the functionality of expert-assessed wildlife corridors with genetic data from roe deer. Basic Appl. Ecol. 17 (1), 52–60.
Buschiazzo, E., Gemmell, N.J., 2006. The rise, fall and renaissance of microsatellites in eukaryotic genomes. Bioessays 28 (10), 1040–1050.
Castilho, C.S., Marins-Sá, L.G., Benedet, R.C., Freitas, T.O., 2011. Landscape genetics of mountain lions (Puma concolor) in southern Brazil. Mamm. Biol. 76 (4), 476–483.
Chan, P.K.S., Chan, D.P.C., To, K.F., Yu, M.Y., Cheung, J.L.K., Cheng, A.F., 2001. Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. J. Clin. Pathol. 54 (5), 401–403.
Charlesworth, D., Willis, J.H., 2009. The genetics of inbreeding depression. Nature reviews genetics 10 (11), 783–796.
Chastagner, A., Pion, A., Verheyden, H., Lourtet, B., Cargnelutti, B., Picot, D., Bailly, X., 2017. Host specificity, pathogen exposure, and superinfections impact the distribution of Anaplasma phagocytophilum genotypes in ticks, roe deer, and livestock in a fragmented agricultural landscape. Infect. Genet. Evol. 55, 31–44.
Chistiakov, D.A., Hellemans, B., Volckaert, F.A., 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255 (1–4), 1–29.
Côté, S.D., Rooney, T.P., Tremblay, J.P., Dussault, C., Waller, D.M., 2004. Ecological impacts of deer overabundance. Annu. Rev. Ecol. Evol. Syst. 35, 113–147.
Coulon, A., Cosson, J.-F., Angibault, J.M.A., et al., 2004a. Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Mol. Ecol. 13, 2841–2850.
Coulon, A., Cosson, J.F., Angibult, J.M., Cargnelutti, B., Gala, Morellet, M., Petit, N., Hewison, A.J., 2004b. Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual–based approach. Mol. Ecol. 13, 2841–2850.
Cushman, S.A., 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biological conservation 128 (2), 231–240.
Cushman, S.A., McKelvey, K.S., Hayden, J., Schwartz, M.K., 2006. Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. Am. Nat. 168, 486–489.
Debeffe, L., Morellet, N., Cargnelutti, B., Lourtet, B., Bon, R., Gaillard, J.M., Mark Hewison, A.J., 2012. Condition-dependent natal dispersal in a large herbivore: Heavier animals show a greater propensity to disperse and travel further. J. Anim. Ecol. 81 (6), 1327.
Di Cola, V., Broennimann, O., Petitpierre, B., Breiner, F.T., d'Amen, M., Randin, C., Guisan, A., 2017. ecospat: an R package to support spatial analyses and modeling of species niches and distributions. Ecography 40 (6), 774–787.
Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Lautenbach, S., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36 (1), 27–46.
Ducros, D., Morellet, N., Patin, R., Atmeh, K., Debeffe, L., Cargnelutti, B., Hewison, A.M., 2020. Beyond dispersal versus philopatry? Alternative behavioural tactics of juvenile roe deer in a heterogeneous landscape. Oikos 129 (1), 81–92.
Epps, C.W., Palsbøll, P.J., Wehausen, J.D., et al., 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecol. Lett. 8, 1029–1038.
Epps, C.W., Wehausen, J.D., Bleich, V.C., Torres, S.G., Brashares, J.S., 2007. Optimizing dispersal and corridor models using landscape genetics. J. Appl. Ecol. 44, 714–724.
Excoffier, L., Lischer, H.E., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10 (3), 564–567.
Fahrig, L., 2003. Effects of habitat fragmentation on biodiversity. Annu. Rev. Ecol. Evol. Syst. 34 (1), 487–515.
Fahrig, L., 2007. Non-optimal animal movement in human-altered landscapes. Funct. Ecol. 21 (6), 1003–1015.
Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37 (12), 4302–4315.
Forge, A., Becker, D., Casalotti, S., Edwards, J., Marziano, N., Nevill, G., 2003. Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessement of connexin composition in mammals. J Comp Neurol 467 (2), 207–231.
Frankel, O.H., 1974. Genetic conservation: our evolutionary responsibility. Genetics 78, 53–65.
Frankham, R., 1995. Conservation genetics. Annu. Rev. Genet. 29 (1), 305–327.
Gerlach, G., Musolf, K., 2000. Fragmentation of landscape as a cause for genetic subdivision in bank voles. Conserv. Biol. 14 (4), 1066–1074.
Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Petit, R.J., 2011. Current trends in microsatellite genotyping. Mol. Ecol. Resour. 11 (4), 591–611.
Heilpern, S.A., Weeks, B.C., Naeem, S., 2018. Predicting ecosystem vulnerability to biodiversity loss from community composition. Ecology 99 (5), 1099–1107.
Hepenstrick, D., Thiel, D., Holderegger, R., Gugerli, F., 2012. Genetic dis continuities in roe deer (Capreolus capreolus) coincide with fenced transportation infrastructure. Basic Appl. Ecol. 13, 631–638.
Hepenstricka, D., Thiel, D., Holderegger, R., Gugerlia.F,, 2012. Genetic discontinuities in roe deer (Capreolus capreolus) coincide with fenced transportation infrastructure. Basic Appl. Ecol. 13, 631–638.
Hewison, A.J.M., Vincent, J.P., Joachim, J., Angibault, J.M., Cargnelutti, B., Cibien, C., 2001. The effects of woodland fragmentation and human activity on roe deer distribution in agricultural landscapes. Can. J. Zool.-Revue Canadienne De Zoologie 79 (4), 679–689.
IUCN (International Union for the Conservation of Nature and Natural Resources) (2019) IUCN Red List of Threatened Species. IUCN,Gland. Accessed 10 Oct 2019.
Jasińska, K.D., Jackowiak, M., Gryz, J., Bijak, S., Szyc, K., Krauze-Gryz, D., 2021. Habitat-Related Differences in Winter Presence and Spring—Summer Activity of Roe Deer in Warsaw. Forests 12 (8), 970.
Jedrzejewski, W., Niedzialkowska, M., Nowak, S., Jedrzejewska, B., 2004. Habitat variables associated with wolf (Canis lupus) distribution and abundance in northern Poland. Divers. Distrib. 10, 225–233.
Jeppesen, J.L., 1987. Impact of human disturbance on home range movements and activity of red deer.
Jiang, G., Ma, J., Zhang, M., Stott, P., 2009. Effects of human activities on the spatial distribution of eastern roe deer Capreolus pygargus bedfordi in the Lesser Khingan Mountains, northeastern China. Acta Theriol. 54 (1), 61–76.
Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24 (11), 1403–1405.
Keller, I., Largiader, C.R., 2003. Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. Proc. R. Soc. Lond. Series B: Biol. Sci., 270(1513), 417-423.
Keller, I., Nentwig, W., Largiadèr, C.R., 2004. Recent habitat fragmentation due to roads can lead to significant genetic differenciation in an abundant flightless ground beetle. Mol. Ecol. 13, 2983–2994.
Keller, I., Excoffier, L., Largiader, C.R., 2005. Estimation of effective population size and detection of a recent population decline coinciding with habitat fragmentation in a ground beetle. Journal of Evolutionary Biology 18 (1), 90–100.
Khosravi, R., Hemami, M.R., Silva, T.L., Rezaei, 2017. Effect of landscape features on genetic structure of the goitered gazelle (Gazella subgutturosa) in Central Iran. Conserv. Genet.
Kierepka.E,, Latch.E.K,, 2016. Fine-scale landscape genetics of the American badger (Taxidea taxus): disentangling landscape effects and sampling artifacts in a poorly understood species. Heredity 116, 33–43.
Kuehn, R., Hindenlang, K.E., Holzang, O., Senn, J., Stoeckle, B., Sperisen, C., 2007. Genetic Effect of Transportation Infrastructure on Roe Deer Populations (Capreolus capreolus). J. Hered. 98 (1), 13–22.
Lande, R., 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. Popul. Ecol. 40 (3), 259–269.
Landguth, E.L., Cushman, S.A., Schwartz, M.K., McKelvey, K.S., Murphy, M., Luikart, G., 2010. Quantifying the lag time to detect barriers in landscape genetics. Mol. Ecol. 19 (19), 4179–4191.
Leberg, P.L., Smith, M.H., 1993. Influence of density on growth of white-tailed deer. J. Mammal. 74 (3), 723–731.
Long, E.S., Diefenbach, D.R., Rosenberry, C.S., Wallingford, B.D., Grund, M.D., 2005. Forest cover influences dispersal distance of white-tailed deer. J. Mammal. 86, 623–629.
Long, E.S.E.S., Diefenbach, D.R., Wallingford, B., Rosenberry, C.H., 2010. Influence of roads, rivers, and mountains on natal dispersal of white-tailed deer. J. Wildl. Manag. 74 (6), 1242–1249.
Mahmoodi, S., Ahmadi, K., Heydari, M., Karami, O., Esmailzadeh, O., Heung, B., 2023. Elevational shift of endangered European yew under climate change in Hyrcanian mountain forests: Rethinking conservation-restoration strategies and management. Forest Ecology and Management 529, 120693.

Mahmoodi, S., Alizadeh Shabani, A., Zeinalabedini, M., Khalilipour, O., Ashrafi, S., 2020. Identifying habitat patches and suitability for roe deer, Capreolus capreolus as a protected species in Iran. Caspian J. Environ. Sci. 18 (4), 357–366.
Mahmoodi, S., Heydari, M., Ahmadi, K., Khwarahm, N.R., Karami, O., Almasieh, K., Mosavi, A., 2022. The current and future potential geographical distribution of Nepeta crispa Willd., an endemic, rare and threatened aromatic plant of Iran: Implications for ecological conservation and restoration. Ecological Indicators 137, 108752.
Manel, S., Holderegger, R., 2013. Ten years of landscape genetics. Trends Ecol. Evol. 28, 614–621.
Markov, G., Zvychaynaya, E., Danilkin, A., Kholodova, M., Sugar, L., 2016. Genetic diversity and phylogeography of roe deer (Capreolus capreolus L.). In: Different Biogeographical Regions in Europe. Comptes Rendus De L'Académie Bulgare Des Sciences, p. 69(5).
Martin, J., Vourc'h, G., Bonnot, N., Cargnelutti, B., Chaval, Y., Lourtet, B., ... & Morellet, N. (2018). Temporal shifts in landscape connectivity for an ecosystem engineer, the roe deer, across a multiple-use landscape. Landscape Ecol., 33(6), 937-954.
Matosiuk, M., Borkowska, A., Świsłocka, M., Mirski, P., Borowski, Z., Krysiuk, K., Ratkiewicz, M., 2014. Unexpected population genetic structure of E uropean roe deer in P oland: an invasion of the mt DNA genome from S iberian roe deer. Mol. Ecol. 23 (10), 2559–2572.
McCune, B., Keon, D., 2002. Equations for potential annual direct incident radiation and heat load. J. Veg. Sci. 13 (4), 603–606.
McRae, B.H., Dickson, B.G., 2008. Using circuit theory to model connectivity in ecology, evolution, and conservation. Ecology 89, 2712–2724.
Metzgar, D., Bytof, J., Wills, C., 2000. Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res. 10 (1), 72–80.
Mladenoff, D.J., Sickley, T.A., Haight, R.G., Wydeven, A.P., 1995. A regional landscape analysis and prediction of favorable gray wolf habitat in the northern Great Lakes region. Conserv. Biol. 9, 279–294.
Mysterud, A., Larsen, P.K., Ims, R.A., Ostbye, E., 1999. Habitat selection by roe deer and sheep: does habitat ranking reflect resource availability? Can. J. Zool.-Revue Canadienne De Zoologie 77 (5), 776–783.
Nilsen, E.B., Linnell, J.D.C., Andersen, R., 2004. Individual access to preferred habitat affects fitness components in female roe deer Capreolus capreolus. J. Anim. Ecol. 73 (1), 44–50.
Peakall, R.O.D., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular ecology notes 6 (1), 288–295.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Quattro, J.M., Vrijenhoek, R.C., 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science 245 (4921), 976–978.
Ray, N., 2005. PATHMATRIX: a geographical information system tool to compute effective distances among samples. Mol. Ecol. Notes 5 (1), 177–180.
Reh, W., Seitz, A., 1990. The influence of land use on the genetic structure of populations of the common frog Rana temporaria. Biol. Conserv. 54 (3), 239–249.
Richards-Zawacki, C.L., 2009. Effects of slope and riparian habitat connectivity on gene flow in an endangered Panamanian frog, Atelopus varius. Divers. Distrib. 15 (5), 796–806.
Riley, S.P.D., Pollinger, J.P., Sauvajot, R.M., et al., 2006. A southern California freeway is a physical and social barrier to gene flow in carnivores. Mol. Ecol. 15, 1733–1741.
Roach, J.L., Stapp, P., Van Horne, B., Antolin, M.F., 2001. Genetic structure of a metapopulation of black-tailed prairie dogs. J. Mammal. 82, 946–959.
Sagheb-Talebi, K., Pourhashemi, M., Sajedi, T., 2014. Forests of Iran: A Treasure from the Past, a Hope for the Future. Springer.
Sala, O.E., Stuart Chapin, Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. science 287 (5459), 1770–1774.
Schlaepfer, D.R., Braschler, B., Rusterholz, H.P., Baur, B., 2018. Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: A meta-analysis. Ecosphere 9 (10), e02488.
Sergio, F., Blas, J., Hiraldo, F., 2018. Animal responses to natural disturbance and climate extremes: a review. Global Planet. Change 161, 28–40.
Shadloo, S., Mahmoodi, S., Hosseinzadeh, M.S., Kazemi, S.M., 2021. Prediction of habitat suitability for the desert monitor (Varanus griseus caspius) under the influence of future climate change. J. Arid Environ. 186, 104416.
Shokri, M., Cozzoli, F., Ciotti, M., Gjoni, V., Marrocco, V., Vignes, F., Basset, A., 2021. A new approach to assessing the space use behavior of macroinvertebrates by automated video tracking. Ecol. Evol. 11 (7), 3004–3014.
Soofi, M., Ghoddousi, A., Zeppenfeld, T., Shokri, S., Soufi, M., Jafari, A., Waltert, M., 2018. Livestock grazing in protected areas and its effects on large mammals in the Hyrcanian forest, Iran. Biol. Conserv. 217, 377–382.
Storfer, Andrew T.; Waits, Lisette P., eds. Landscape Genetics: Concepts, Methods, Applications, First Edition. John W Storfer, A., Murphy, M. A., Evans, J. S., Goldberg, C. S., Robinson, S., Spear, S. F., ... & Waits, L. P. (2007). Putting the 'landscape' in landscape genetics. Heredity, 98(3), 128-142.iley and Sons Ltd. p. 247-255., 247-255.
Taylor, P.D., Fahrig, L., Henein, K., Merriam, G., 1993. Connectivity is a vital element of landscape structure. Oikos 571–573.
Team, R., c, 2016. R language definition. R foundation for statistical computing, Vienna, Austria.
Telfer, E.S., 1967. Comparison of moose and deer winter range in Nova Scotia. The Journal of Wildlife Management 418–425.
Waller, J.S., Servheen, C., 2005. Effects of transportation infrastructure on grizzly bears in Northwestern Montana. J. Wildl. Manag. 69, 985–1000.
Wang, I.J., Summers, K., 2010. Genetic structure is correlated with phenotypic divergence rather than geographic isolation for linking landscape and genetic data. Ecography 32, 818–830.
Wang, W., Qiao, Y., Li, S., Pan, W., Yao, M., 2017. Low genetic diversity and strong population structure shaped by anthropogenic habitat fragmentation in a critically endangered primate, Trachypithecus leucocephalus. Heredity 118 (6), 542–553.
Wang, I.J., Savage, W.K., Bradley Shaffer, H., 2009. Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (Ambystoma californiense). Mol. Ecol. 18 (7), 1365–1374.
Warren, D.L., Glor, R.E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evol.: Int. J. Org. Evol. 62 (11), 2868–2883.
Wilson, M.C., Chen, X.Y., Corlett, R.T., Didham, R.K., Ding, P., Holt, R.D., Yu, M., 2016. Habitat fragmentation and biodiversity conservation: key findings and future challenges. Landscape Ecology 31, 219–227.
Zhang, D.X., Hewitt, G.M., 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol. Ecol. 12 (3), 563–584.
Zhu, X., Zhang, P., Lin, X., Shi, Y., 2010. Active learning from stream data using optimal weight classifier ensemble. IEEE Trans. Syst., Man, Cybernet., Part B (Cybernetics) 40 (6), 1607–1621.