Genetic diversity and spatial genetic structure of Caucasian apple (Malus orientalis Uglitzk.) populations based on microsatellite markers for conservation strategy

Hamid Bina
Kharazmi University
Farrokh Ghahremaninejad
fgh@khu.ac.ir

Kharazmi University
Shahin Zarre
University of Tehran

Research Article

Keywords: Microsatellite marker, Conservation unit, Genetic diversity, M. orientalis

Posted Date: May 13th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6546353/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Ecology and Evolution on November 10th, 2025. See the published version at https://doi.org/10.1186/s12862-025-02474-9.

Abstract

In Eurasia, *M. orientalis* Uglitzk. (Caucases apple) is a tree with important ecological and economic (fruit) benefits. We measured eight quantitative morphological traits, none of which showed significant differences among the investigated Caucasian apple populations. In this research, we used 26 microsatellite (SSR) markers to investigate genetic diversity and define unit conservation in the Caucases apple. The mean values of genetic diversity,allelic richness (Ar), private allele (Ap), expected heterozygosity (H_E) and observed heterozygosity (H_O) were 1.74, 0.21, 0.65 and 0.76, respectively. In the regions studied, three major genetic clusters and two significant genetic barriers were discovered. Our gene flow findings revealed that there is little connection between *M. orientalis* population in the Caucasus indicating habitat fragmentation. Two regions in the Hyrcanian forest and one in the Zagros forest were identified as having the highest priority for conserving the genetic diversity of the Caucases apple.

Introduction

As a Southwest Asian nation, Iran exhibits remarkable climatic, topographic, and soil diversity, fostering exceptional biodiversity. The country harbors approximately 8,000 flowering plant species (distributed across 167 families and 1,200 genera), with roughly 1,700 being endemic (1). These species thrive across four distinct ecological zones, Hyrcanian, Zagros, Irano-Turanian, and Khalij-Omanim, each characterized by unique physiographical and climatic conditions. Among these, Iran's primary forest ecosystems are concentrated in the Hyrcanian and Zagros regions (2).

The Hyrcanian forests of Iran is a biodiversity hotspot in northern Iran, support a rich assemblage of temperate flora, including *Fagus orientalis* Lipsky (oriental beech), *Carpinus betulus* L. (hornbeam), and the relict *Parrotia persica* (DC.) C.A.Mey. (Persian ironwood). This region also hosts valuable fruit-bearing species such as *Punica granatum* L. (pomegranate), *Betula pendula* Roth (Birch tree) and wild members of the *M. orientalis*, which enhance genetic diversity and ecosystem resilience (2–4). The understory features shrubs like *Berberis integerrima* Franch. and *Ruscus hyrcanus* Woronow, while the endangered *Taxus baccata* L. (English yew) persists in shaded ravines. However, invasive species, overharvesting of *Malus* and other wild fruit trees, and land-use changes threaten these unique plant communities (2, 3, 5, 6).

The Zagros woodlands, Iran’s largest oak-dominated ecosystem, are characterized by drought-adapted species such as *Quercus brantii* Lindl (Persian oak) and *Pistacia atlantica* Desf. (wild pistachio). The *Malus* genus, particularly *M. orientalis*, thrives in these semi-arid highlands alongside wild pear (*Pyrus syriaca* Boiss.) and almond (*Prunus dulcis* (Mill.) D.A.Webb). Steppe vegetation, including *Astragalus* spp. and *Salvia* spp., dominates the understory, while remnants of *Crataegus* L. (hawthorn) and *Celtis* L. (nettle tree) mark degraded forest edges (Heshmati, 2007; Zarre et al., *2024*). Despite their ecological adaptability, Zagros species face severe pressures from charcoal production, overgrazing, and climate-induced aridification, which disproportionately affect wild fruit trees like *Malus* and endemic oaks (2, 7, 8).

The genus *Malus* Mill (Rosaceae) includes 25 to 47 species, which are among the most economically important crops worldwide and are widespread in North America, East Asia, Europe, and the Caucasus (9). Caucases wild apples play an important role in the local diet of rural and peri-urban communities (4, 10, 11). This species has been cultivated in the Caucasus region since ancient times primarily for the exceptional nutritional value of its fruits (more than 4000 years ago) (12, 13). The Caucasian apple is one of the ancestors of the Mediterranean cultivar apple (10, 14, 15). Gene flow along the Silk Road facilitated their spread from Asia to Europe, with *M. orientalis* serving as a key ancestor of Caucasian cultivar. Wild *Malus* species are ecologically vital, providing food for wildlife and serving as genetic reservoirs for breeding stress-resistant apple varieties. Their conservation is critical to maintaining both ecosystem stability and agricultural resilience (8). The habitat of this species in Iran is located across the Trade Silk Route in two areas: Hyrcanian forest and Zagros forest (16–18). These mountainous regions differ in slope, elevation, vegetation, and ecological conditions; however, both are Middle Eastern hotspots of plant diversity (11, 19–21).

Habitat fragmentation of Caucasian apple populations in Iran poses significant challenges for biological conservation, evolutionary studies, and genetic diversity assessments. This fragmentation primarily leads to population isolation, restricting gene flow among wild Iranian apple populations. Such isolation can result in reduced genetic diversity, increased inbreeding, and potential loss of adaptive potential over time (10, 22, 23). The protection of habitats against the negative effects of fragmentation helps increase the ability of native plants to adapt to reduce the extinction rates of natural populations (24). Gene flow can enable the exchange of variability between distant populations and, by increasing dispersal, can reduce the rate of extinction of species (12, 25–29). To select candidate populations with the highest priority for conservation, molecular markers are often used to understand spatial

genetic structure, which allows the identification of natural populations for conservation (30–33). Knowledge of the spatial genetic structure of species can help in understanding the geographic and dispersal patterns of a species over time and space (34). In many studies focused on such aims, the genetic diversity pattern of a species is defined by analyzing its demographic structure and estimating the gene flow, genetic drift, Nei's distance, and migration pathways of the species (35–38). The comparison of differentiation in particular populations is helpful for determining conservation units and inferring the nature of selection in geographic regions (39–42). Researchers have used various molecular markers like ISSR, RFLP and SSR to illustrate the spatial genetic structure and demographic history of species. These markers can provide accurate information about the evolution of natural stands and help select wild populations with high conservation priority (43–45). Microsatellites or simple sequence repeats (SSRs) are among the most powerful molecular markers that can be used for the identification of conservation units and the study of genetic diversity. Compared with other markers, microsatellites are characterized by high information content and versatility as molecular tools for germplasm characterization (46–49).

In the present study, we applied SSR markers to characterize microsatellite loci in the Caucasus apple to 1) describe the spatial genetic structure of populations across the Silk Trade Route, with a particular focus on Iranian populations; 2) assess gene flow and genetic diversity; and 3) identify a conservation unit to conserve the genetic diversity of Caucasian apple.

Material and Methods

Sampling

The data used in this study were obtained from our previously published research on the genetic diversity and domestication of apple (*M. orientalis*) in Iran and the Caucasus (8). The Caucasus apple samples we used were collected from two main regions of Iran: the Hyrcanian forest, including western Hyrcanian (Asalem region: 5 samples), central Hyrcanian (Tilak region: 14 samples, Sngdehsari region: 9 samples, Shit region: 4 samples, Vatna region: 8 samples, Gaznsara region: 16 samples, Abesk region: 9 samples), and eastern Hyrcanian (Vatna region: 14 samples, Arsam region: 4 samples, Gorgan region: 14 samples, Dorak region: 9 samples, Toskestan region: 10 samples, Siamarzkoh region: 9 samples, Sheshab region: 7 samples)—and the Zagros forest (Nozhyan: 8 samples, Droud: 4 samples, Sepdkoh: 10 samples, Marivan: 6 samples, Sagez: 2 samples, Bufloo: 5 samples), totaling 167 samples. All plant samples were morphologically identified by Dr. Farrokh Ghahremaninejad, a specialist in botany at the Department of Plant Sciences, Faculty of Biological Sciences, Kharazmi University. Voucher specimens have been deposited at the Kharazmi University Herbarium (herbarium code: T) under the deposition numbers 25760 to 25927. The habitats of Malus orientalis in Iran are not located in protected areas or national parks; therefore, no specific permission was required for sampling in these regions.

DNA Extraction

Genomic DNA was isolated from dried leaf samples of Caucasian apple using the NucleoSpin Plant II kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. For microsatellite analysis, performed multiplex PCR amplification using a Qiagen multiplex PCR kit with 26 SSR markers, following established protocols (8, 15, 50). Control genotypes were cross-validated against the 2013 reference dataset. Only multilocus genotypes with < 20% missing data were included in subsequent analyses. The reliability of these markers for population genetics studies has been well documented in prior research (15).

In this study, parameters of genetic diversity for each microsatellite locus were calculated via FSTAT v2.9.3 (51). ADZE software was employed to estimate the population genetic diversity, including allelic richness (Ar) and private allele (Ap), using standardized sample sizes of ADZE = 2 (52). The number of different alleles (Na), number of effective alleles (Ne), expected heterozygosity (He) and observed heterozygosity (Ho), within-population inbreeding coefficient FIS and Hardy–Weinberg equilibrium were calculated with GENEPOP 4.2 (53, 54). The inverse distance weighted (IDW) interpolation function implemented in the Geographic Information System (GIS) software ArcGIS 9.3 (55) was used to estimate the geographic patterns of H_O and H_E for all 20 populations of *the* Caucasus apple. We explored the relationships among clusters via principal component analysis (PCA) with the *adegenet* package in the R environment (56). Analysis of molecular variance (AMOVA) was performed via GENALEX v6.5 (57) to infer the genetic differentiation between populations from Hyrcanian forest and Zagros forest. Barrier analysis, which is based on the (58) maximum difference algorithm, was conducted to define significant genetic breaks and spatial differences between populations. Genetic barriers were assessed via BARRIER 2.2 (59).

Genetic Structure and Gene Flow Analysis

We assessed the genetic structure of the Caucasus apple via STRUCTURE v2.3.4 software (60). This tool was used to examine range-wide genetic clustering among populations of Caucasian apple. Ten independent runs were performed for each K from 2–10 with 200,000 burn-in steps followed by 500,000 Markov chain Monte Carlo (MCMC) steps. The STRUCTURE output was analyzed and visualized by using StructureSelector (61). To infer historical gene flow (Nm) patterns, MIGRATE-N v3.6 (62) was used to estimate the effective population sizes (θ) and mutation-scaled immigration (M) among the groups identified by STRUCTURE. Additionally, we used the Bayesian method implemented in the software BayesAss (BA3) to estimate current gene flow between populations (63). Estimates via this method are based on migration over the last few generations. We selected the best run for allele frequencies and posterior probability densities of inbreeding coefficients with different random seeds among three independent runs. The Bayesian deviance was calculated to determine the best run via the script of (64), which was applied in R (65). To obtain a correct acceptance rate, the values of the parameters ranged between 20% and 40% (66). The inbreeding coefficient was set at 0.35, and the migration rate was set at 0.75 for the final analysis. For allele frequencies, the default setting of mixing parameters was used. The analysis was conducted with 10^6 burn-ins and 10^7 MCMC iterations; every 1,000th iteration was sampled. The results were visualized via QGIS 2.18 'Las Palmas' (67). CIRCUITSCAPE software (McRae & Beier, 2007) was used to evaluate the influence of topographic complexity as a resistance factor affecting gene flow among populations. This software models populations as nodes connected by resistors, based on the principles of electrical circuit theory and the connectivity-resistance approach. While altitude alone may not fully capture topographic complexity, we used a digital elevation model (DEM) to derive a resistance surface for the habitats of *Malus orientalis* in Iran. To statistically validate this resistance model, we compared the CIRCUITSCAPE-derived resistance distances with a null model of isolation-by-distance (IBD) using Mantel tests (68).

Unit of conservation selection

To determine the conservation priority of the Caucasus apple, we used reserve selection analysis via DIVA-GIS software (www.diva-gis.org). The reserve selection analysis algorithm, which is based on allele frequency in each population, determines the minimum number of geographic units necessary to maintain genetic diversity (69). The populations with the highest allelic richness are given first priority for conservation, and the priorities of other populations are selected on the basis of the first population. After the first priority of populations for conservation, new alleles were found in the next priority populations that were not observable in the previous populations (69).

Morphological parameters studied

Eight quantitative morphological traits were used in this study (leaf area, leaf angle with petiole, leaf length, petiole length, maximum leaf length, maximum leaf width at 0.1 of leaf length, maximum leaf width at 0.9 of leaf length, and stomatal morphology) (70, 71). Statistical parameters and AMOVA were computed via SPSS software (72), and PCA for morphology was performed via the Clustvis website (73).

Results

Genetic diversity of Caucasian Apple populations

This study identified 26 loci with 455 alleles for wild apples. All the loci were polymorphic for the population of Caucasian apples. The range of alleles per locus ranged from 7 (CH02c03b) to 29 (CH04e03), and the average number of alleles was 17.5 (Table S1). The average number of alleles per population was 5.22, with values ranging from 2.07 (Droud region) to 7.5 (Vatna region). In this study, the mean gene flow (Nm) was calculated to be 0.6 (Table S2).

The Vaz and Sangdeh regions of the Hyrcanian forest have the highest H_O and H_E values, whereas the Nozhyan and Droud regions of the Zagros forest have the lowest H_O and H_E values. Overall, the observed heterozygosity in the Zagros forest populations was lower than that in the Hyrcanian forest populations (Table 1). The FIS parameter for Caucasian wild apple in Iran was estimated to be in the range of -0.83–0.11, but the mean for populations from Zagros forest was negative (Table 1). Overall, Caucasian apple populations in

the Hyrcanian forest showed greater mean allelic richness compared to those in the Zagros forest. However, the mean number of private alleles was higher in Zagros forest populations (Fig. 2).

Table 1
) Estimated genetic diversity parameters for *M. orientalis* in Iran on the basis of genotyping samples

	Pop	Province	Latitude (X)	Longitude (Y)	N	Na	Ne	I	H_E	H_O	PPL (%)	FIS
Hyrcanian Forest	Asalem	Gilan	37.75	48.86	5	5.38	4.29	1.48	0.75	0.81	100	0.07
	Abesk	Mazandaran	36.29	52.62	9	6.50	4.49	1.60	0.81	0.8	100	0
	Sangdeh Sari	Mazandaran	36.1	53.28	9	6.38	4.34	1.56	0.73	0.83	100	0.11
	Ganasara	Mazandaran	36.33	52.09	16	7.42	4.69	1.61	0.76	0.76	100	0
	Shit	Mazandaran	36.54	53.37	4	3.57	2.86	1.09	0.75	0.78	100	0.03
	Tilak	Mazandaran	36.07	53.61	14	6.92	4.32	1.56	0.75	0.69	100	-0.08
	Vaz	Mazandaran	36.31	52.94	8	5.19	3.72	1.38	0.85	0.46	100	-0.83
	Gorgan	Gorgan	36.71	54.3	14	6.15	4.00	1.42	0.82	0.45	100	-0.81
	Vatna	Gorgan	36.66	53.99	14	7.50	4.67	1.64	0.67	0.7	100	0.03
	Sheshab	Gorgan	36.9	55.13	7	5.80	4.27	1.50	0.76	0.75	100	-0.01
	Arsam	Gorgan	36.67	54.66	4	2.80	2.34	0.87	0.73	0.71	100	-0.02
	Tokestan	Gorgan	36.76	54.53	10	5.80	3.68	1.43	0.7	0.74	100	0.05
	Dorak	Gorgan	36.75	54.38	9	5.96	4.50	1.54	0.7	0.76	100	0.081
	Siamarzkoh	Gorgan	36.79	55.1	9	6.23	4.18	1.55	0.7	0.74	100	0.05
	Average					5.82	4.02	1.45	0.74	0.71	100	-0.09
Zagros Forest	Marivan	Kordestan	35.4	46.34	6	4.84	3.70	1.38	0.72	0.73	100	0
	Saghez	Kordestan	36.46	46.6	2	2.80	2.63	0.95	0.79	0.73	100	-0.08
	Buflo	Kordestan	36.25	46.23	5	4.57	3.69	1.34	0.7	0.58	100	-0.2
	Sepedkoh	Lorestan	33.95	48.49	10	4.88	3.34	1.29	0.72	0.74	100	0.02
	Nozian	Lorestan	33.23	48.58	8	3.69	2.89	1.11	0.65	0.69	96	0.06
	Droud	Lorestan	33.24	33.24	4	2.07	1.94	0.65	0.83	0.44	88	-
	Average					3.81	3.03	1.12	0.73	0.65	97.3	-0.04
Total					**167**							
Mean						5.25	3.74	1.35	0.74	0.69	99.2	-0.08

N = numner of samples, Na = Number of Alleles, Ne = Effective Number of Alleles, I = Shannon's Information Index, H_e= Expected Heterozygosity, H_o= Observed Heterozygosity, PPL%= Polymorphic Loci Percentage, FIS = Inbreeding Coefficient

According to the pairwise FST analysis, most apple populations from the Hyrcanian and Zagros forests showed very low genetic differentiation, with FST values ranging from 0.005 to 0.30 (Fig. 2b, Table S2). Statistical significance of pairwise FST values was tested using 10,000 permutations in Arlequin. Although the FST between Mrivan and Vatna was very low genetic differentiation (FST = 0.005). Moderate levels of genetic differentiation was observed between some western and central populations of the Hyrcanian forest, with FST values above 0.25 ($P < 0.001$), indicating moderate genetic structure among these regions (Fig. 3b, Table S2).

On the basis of our findings from the AMOVA, the majority of variance (80%) was discovered within samples, with only 4% and 8% of variance found within groups and populations within groups, respectively (Table 2).

Table 2
The results of the AMOVA test in two main regions of Caucus apples in this study

Source of changes	Degrees of freedom	Sum of squares	Components of variance	Percentage of Variance
Between Hyrcanian forest and Zagros forest	1	74.71	0.45	4
Between populations from Hyrcanian forest and Zagros forest	18	454059	0.87	8
Among samples	147	1573.85	0.88	8
within samples	167	1493	8.94	80
Total	333	3596.15	11.15	100

Population structure and genetic differentiation

The STRUCTURE results revealed that the Caucasus apple samples exhibited a high level of heterozygosity and indicated five genetically distinct clusters corresponding to five main geographic regions, with the clearest separation observed at K = 5. This K value showed the best geographical pattern for the apple populations, and five distinct clusters were identified, each associated with a main geographic region, with no new groups appearing in the STRUCTURE analysis after K = 5 (Fig. 3a). At K = 3, populations from Nozhyan, Droud, and Sepedkoh (purple group) were separated from all others, while apple populations from Marivan, Saghez, and Buflo clustered with the Hyrcanian populations (blue group). At K = 4, the Zagros populations split into two subgroups (red and purple), and most Hyrcanian populations remained in the blue group, except those from Asalem, Abesk, and Toskestan. K = 3 was supported as the optimal number of clusters based on the ΔK method (Fig. S4), although K = 5 showed the clearest geographic pattern. STRUCTURE bar plots for K = 3 to K = 6 are shown in Fig. 3a.

We used Monmonier's maximum difference algorithm to find potential genetic barriers between populations of Caucasian apples to complement our analysis and detect potential genetic discontinuity. Among Caucasians, there are two statistically significant genetic barriers (bootstrap support 90%). The first is in the forest of Hyrcanin, and the second is in the forest of Zagros. This caused apple populations from the Sheshab, Siamarzkoh, and Arsam regions in the Hyrcanina forest to be separated from other apple populations and caused apple populations from the Nozhyan region in the Zagros forest to be separated from other apple populations (Fig. 4).

PCA

Results of principal component analysis (PCA) for all populations in this study illustrated that PC2 and PC3 both explain 5.71 percent and 4.37 percent of the variance, respectively. The populations of the Zagros forest were divided into two groups via PCA; however, populations from northern Zagros overlapped with those from the central part of the Hyrcanian forest. The populations from the west of the Hyrcanian forest have mixed with the populations from the central area of the forest the most (Fig. 5).

Gene flow between the habitats of M. orientalis

To estimate gene flow, stands of the Caucasus apple were classified by geographical region. Our findings illustrate some gene flow between provinces. Gorgan populations were the predominant source of exogenous allelic variants, and strong gene flow was observed from Gorgan into Mazandaran, Gilan, and Kordestan populations (Ne respectively from Golestan into Mazandaran, Gilan and Kordestan provinces: 0.172, 0.185, 0.47). We observed minor gene flow from Gilan's population into Lorestan's population (Ne = 0.05) as well as minor gene flow from Kordestan's population into Mazandaran's population (Ne = 0.01). Little or no gene flow between Zagros region populations (Lorestan Province and Kordestan Province (Ne = 0.01) was detected, suggesting that *M. orientalis* in Zagros are isolated from each other (Fig. 3c). This result is confirmed by CIRCUITSCAPE analysis, which reveals the existence of a strong topographic barrier between populations from the Zagros Mountains. In contrast, for Hyrcanian forest populations, some admixture in populations was apparent. Lowlands near the Caspian Sea allow undisturbed gene flow between Hyrcanian populations. Interestingly, the connection between Zagros and Gorgan is better than that between Zagros and the western part of the Hyrcanian region, which is confirmed both by MIGRATE-N and CIRCUITSCAPE (Fig. 4).

Identification conservation units of Caucasus apple

The minimum number of geographic units required to preserve the genetic diversity of the Caucasus apple in the two main regions—the Hyrcanian forest and the Zagros forest was analyzed. Our findings indicate that in the Hyrcanian forest, the Toskestan, Siamarzkoh, Abesk, and Asalem regions are designated as the first priority for conservation, while the Sangdehsari and Vaz regions are the second priority.In the Zagros forest, the Sepedkoh region is the first priority for conservation, whereas the Bufloo, Sgez, and Nozhyan regions are the second priority. Additional areas, marked in orange and green (Fig. 1), have lower conservation priority.

Morphology of Caucasus apples

A bar chart of the seven characteristics of the leaves of Iranian wild apple is shown in Figure S1. The leaf angle parameter widely used to describe the morphology of plants (74) was the most variable parameter in this study. The maximum variance of the analyzed statistics was observed for the population of Siamarzkoh for leaf length and leaf area. The measurement of the maximum leaf width at 0.1 of the leaf length and the maximum leaf width at 0.9 of the leaf length revealed that the Vatna region has the maximum variance.

Analysis of variance (ANOVA) and PCA of morphological traits

The analysis of variance of the length of the petiole revealed significant differences among the Buffalo region and other regions in this study. On the basis of leaf width, population of Buflo has been signed with all regions except thepopulation of SangdehSari. The population of Tuskestan and population of Sheshab region from Hyrcanian forest and the Grin region from Zagros forest have been clustered with other regions on the basis of the maximum length of the leaf. Analysis of variance of the maximum leaf width at 0.1 and maximum leaf width at 0.9 length revealed significant differences among the population of Sheshab, population of Tuskestan, and population of Vatna regions from Hyrcanian forest and other regions. The leaf areas of the Grin region and Vatna region were significantly different from those of the other Zagros forest regions (Fig. S2). Fig. S3B summarizes the principal component analysis results of the morphological data for the wild apple plants. We used seven principal components that explained the variation between Hyrcanian forest and Zagros forest. These principal components explained approximately 14.2% and 67.6% of the variation (PC1 and PC2), respectively, and, on the basis of the studied morphological traits, we did not observe any pattern of differences between the Hyrcanian forest and Zagros forest (Fig. S3A).

Discussion

Several analyses of the spatial genetic structure of the Caucasus apple were previously performed and described in the literature (10, 11, 75, 76). However, the present study is the first large-scale genetic analysis of stands of this species from Iran, across the natural range from the Hyrcanian forest to the Zagros, using a large set of SSRs (26 SSR markers). Our findings confirmed that the gene pool of the Caucasus apple moved from East Asia to Europe across the Trade Silk Road.

Genetic diversity within populations of M. orientalis

According to our results, many populations of *M. orientalis* in the Hyrcanian forest (Asalem and Gorgan regions), as well as in the Zagros forest (Droud and Nozhyan regions), are genetically unique. High genetic diversity among Iranian populations across the natural range of species in the Zagros and Hyrcanian forests is confirmed by high dissimilarity between populations and limited dispersal between them (77). Previous research has suggested genetic similarities between stands in Iran and Central Asia. Using molecular markers, proven that discovered that Iranian wild apples in Hyrcanian forests are closely related to apple populations in Central Asia. These results could indicate that apples could migrate across the northern part of the Silk Road (11).

In conservation genetics, allelic richness is the most important parameter, and it can be used to forecast historical bottleneck populations (78–80). The average allelic richness value in Iran (6.2 ± 0.04) is lower than that in Europe and China (75). A decrease in allelic richness resulted in a reduction in the effective population size, increasing the vulnerability of these populations to various unfavorable conditions (80, 81). Another important parameter is expected heterozygosity, which is a good indicator of genetic diversity (82). The mean H_E for Caucasus apple in Iran was 0.65, which was lower than the H_E for *M. orientalis* in Europe and China (0.80), indicating that population growth in Iran is not uniform (83, 84). However, it is easy to explain why the Caucasus apple is found in two main regions in Iran: the Hyrcanian forest and the Zagros forest, both of which have different climatic conditions and vegetation, which can lead to nonuniform growth (85). The FIS parameter predicts future generations' genetic diversity, and a negative value indicates that regeneration could fail (86). The lower value of this parameter for Iranian wild apple (-0.81 ± 0.02) than for European wild apple (0.04 ± 0.06) suggests that the regeneration of Iranian populations can be disrupted. The gene pool of

various *Malus* species has been strongly influenced by human impact since ancient times because apple fruits were already important crops in the era of the Sumerians (87). Thus, it is difficult to recognize whether natural populations of *M. orientalis* were shaped more by natural processes or by long-term human influence.

Gene flow

Gene flow and migration have a significant impact on adaptation and may lead to population homogeneity (88). In the prevuous study about spatila genetic structure of *M. orientalis* concluded that the geographical location of a population has no effect on the genetic structure of wild apples (14), but we found a strong link between genetic structure and geographic region in this study. The results of the gene flow analysis revealed strong migration from the eastern part of the Hyrcanian forest to populations in the western part of this region and, with lower intensity, to the northern part of the Zagros forest. According to (76), Iran has been one of the main regions through which various taxa from the *Malus* genus moved from Central Asia to Europe across the Trade Silk Road. Our results support this theory and suggest that Iran served as a link between East Asia and Europe in the transportation of genetic variability in the Caucasus apple (76, 89)

The topography of the central part of Iran creates an enormous barrier for gene flow between the Hyrcanian and Zagros populations. Additionally, the northern and southern parts of the Zagros range are poorly connected; the Migrate-n results show that admixture in Lorestan is very low. The situation on the coast of the Caspian Sea is completely different gene flow along coastal lowlands is theoretically undisturbed, which allows for the exchange of genetic variability between Hyrcanian stands. Thus, northern populations are genetically similar, as indicated by the STRUCTURE and PCA results. The Caucasus apple is one of the minor ancestors of the European Caucasus apple (14). Long periods of cultivation and crossing between various taxa of *Malus* could have affected natural populations of ancestral species as well; however, it is difficult to quantify this impact.

Spatial genetic structure of M. orientalis

Gene affinity and exchange between populations can be determined by analyzing the spatial genetic structure of populations (24). The whole Iranian range of the species was divided into three clusters via STRUCTURE analysis, which differs from the results of previous studies (10, 11, 90). One of these variations may be the response of Iranian wild apples in the Hyrcanian and Zagros forests to population size and dispersal (91). In contrast to previous studies, our findings reveal a strong spatial genetic structure between wild apple populations in the Hyrcanian and Zagros forests (10, 75). We hypothesize that the differences between Iranian wild apples are due to two gene pools with similar geographic origins. Interestingly, the population from Gilan is similar to Zagros stands; this particular population is quite far from other Hyrcanian stands; however, the topography of Iran hinders the natural flow of genes from Gilan to the Kordistan region. It is possible that the similarity of the gene pool in these two regions is connected with human impact. Stands from Lorestan differ strongly from both Hyrcanian and northern Zagros populations. The isolation of this area is supported by the results of migration analysis, as well as CIRCUITSCAPE and PCA. The distinctiveness of this area, which is difficult to assess due to topographic barriers, may indicate that a unique gene pool exists, which should be protected with high priority.

Although STRUCTURE analysis revealed broad-scale clustering patterns, Monmonier's algorithm identified localized genetic barriers in the Hyrcanian and Zagros forests. This difference is not unexpected, as STRUCTURE detects genome-wide patterns assuming Hardy Weinberg and linkage equilibrium, while Monmonier's method identifies sharp spatial genetic discontinuities regardless of such assumptions (Manel et al., 2003; Chen et al., 2007; Prates et al., 2016). The genetic barrier around Nozhyan aligns with a distinct STRUCTURE cluster at K = 3, supporting its genetic uniqueness. Other barriers, particularly in the Hyrcanian forest, may represent incipient or incomplete divergence, gene flow reduction, or adaptation to local environments not yet strong enough to form fully distinct STRUCTURE clusters (Storfer et al., 2007; Blair et al., 2012). These results reflect the complementary nature of barrier and clustering analyses in revealing both historical genetic structure and ongoing differentiation processes (Sexton et al., 2014).

Conservation of Caucasian apple

Caucasian apple is one of the main ancestors of cultivated apples in the Caucasian region (Bina et al., 2022). These wild apple species play a crucial role in sustaining ecosystems, since they are an important source of food for wildlife. Additionally, they are valuable sources for apple breeding, helping to develop features like stress resistance against infections and dehydration. Therefore, it is crucial to protect this species' germplasm from serious threats like the negative effects of habitat fragmentation and futher challenges (90).

Future projects such as regeneration and breeding are needed to conserve the genetic diversity of wild plants (92). Asia is the main center of genetic diversity in the genus *Malus*, implying that wild apple habitats in Asia have a large gene pool that needs to be preserved for future generations (93). However, no studies on the genetic diversity of this important species have been reported, and our study is the first in this field. To define a conservation unit for the Caucasian apple gene pool, we used reserve selection analysis. Our findings revealed three high-priority and medium-priority populations for conservation in two main regions. On a local scale, this illustrates the Caucasus region's lack of consideration of *M. orientalis* genetic conservation.

Conclusion

In this study, we discovered two high-priority regions in the Hyrcanin forest for preserving *M. orientalis* genetic diversity, indicating that *M. orientalis* management in the Hyrcanin forest would need additional attention. In the Zagros and Hyrcanin forests, we discovered ageographic barrier between the population with the highest priority and other populations. As a result, corridors between populations are suggested in this situation (94). Finally, on the basis of these results, we can conclude that Iran is one of the key sources of the *M. orientalis* gene pool that would be useful for cultivating apple breeding programs, but it does not have good habitat conditions and would require performance conservation programs.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: All authors have read and approved the final manuscript and consent to its publication in the journal.

Availability of data and materials: The data supporting the findings of this study are available at Zenodo under the DOI: 10.5281/zenodo.6981530.

Competing interests: The authors declare that they have no competing interests.

Funding: This work is based upon research funded by the Iran National Science Foundation (INSF) under project No. 4034387

Authors' contributions: H.B., F.Gh. and Sh.Z. conceived and designed the experiments; F.Gh. and Sh.Z. obtained funding, H.B., F.Gh. and Sh.Z. analysed the data; H.B. wrote the original draft and preparation of the figures; H.B., F.Gh. and Sh.Z. gave critical inputs in final draft and revisions.

Acknowledgements: This work is based upon research funded by the Iran National Science Foundation (INSF) under project No. 4034387

References

1. Pushpangadan P, Nair K, Ahmad M. Biodiversity and medicinal plant wealth of South Asian countries: country reports of Bangladesh, Bhutan, India, Iran, Maldives, Nepal, Pakistan and Sri Lanka. 2004.
2. Heshmati G. Vegetation characteristics of four ecological zones of Iran. Int J Plant Prod. 2007;1(2):25–224.
3. Bina H, Yousefzadeh H, Ali SS, Esmailpour M. Phylogenetic relationships, molecular taxonomy, biogeography of Betula, with emphasis on phylogenetic position of Iranian populations. Tree Genet Genomes. 2016;12:1–17.
4. Yousefzadeh H, Rajaei R, Larsen B, Bina H, Kozlowski G. S-allele frequency and genetic diversity of Malus orientalis Uglitzk along an altitudinal gradient in the Hyrcanian forest. For Syst. 2020;29(2):e015.
5. Jafari SM, Zarre S, Alavipanah SK, Ghahremaninejad F. Exploring the generality of associations between plant functional traits: evidence within ecological groups along an altitudinal gradient in H yrcanian forest. Plant Species Biol. 2014;29(3):E31–9.
6. Jafari SM, Zarre S, Alavipanah SK, Ghahremaninejad F. Functional turnover from lowland to montane forests: evidence from the Hyrcanian forest in northern Iran. IForest-Biogeosciences For. 2015;8(3):359.
7. Akhani H, Djamali M, Ghorbanalizadeh A, Ramezani E. Plant biodiversity of Hyrcanian relict forests, N Iran: an overview of the flora, vegetation, palaeoecology and conservation. Pak J Bot. 2010;42(1):231–58.
8. Bina H, Yousefzadeh H, Venon A, Remoué C, Rousselet A, Falque M et al. Evidence of an additional center of apple domestication in Iran, with contributions from the Caucasian crab apple Malus orientalis Running head: Apple domestication in

the Caucasus. 2021.
9. Zhukovsky PM. Main gene centres of cultivated plants and their wild relatives within the territory of the USSR. Euphytica. 1965;14(2):177–88.
10. Amirchakhmaghi N, Yousefzadeh H, Hosseinpour B, Espahbodi K, Aldaghi M, Cornille A. First insight into genetic diversity and population structure of the Caucasian wild apple (Malus orientalis Uglitzk.) in the Hyrcanian forest (Iran) and its resistance to apple scab and powdery mildew. Genet Resour Crop Evol. 2018;65(4):1255–68.
11. Yousefzadeh H, Khodadost A, Abdollahi H, Ali SS, Kozlowski G, Bina H. Biogeography and phylogenetic relationships of Hyrcanian wild apple using cpDNA and ITS noncoding sequences. Syst Biodivers. 2019.
12. Diamond PA. Consumption externalities and imperfect corrective pricing. Bell J Econ Manag Sci. 1973;526–38.
13. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. Adv Pediatr. 1976;22:349–78.
14. Cornille A, Gladieux P, Smulders MJM, Roldán-Ruiz I, Laurens F, Le Cam B et al. New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet. 2012;8(5).
15. Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P. The domestication and evolutionary ecology of apples. Trends Genet. 2014;30(2):57–65.
16. Khoshbakht K, Hammer K. Savadkouh (Iran)–an evolutionary centre for fruit trees and shrubs. Genet Resour Crop Evol. 2006;53(3):641–51.
17. Büttner R. Malus. Hanelt P Inst Plant Genet Crop Plant Res Eds Mansfelds Encycl Agric Hortic Crops. 2001;471–82.
18. Browicz K, Hippocastanaceae. Flora Iran. 1972;(92.2).
19. Yousefzadeh H, Hosseinzadeh Colagar A, Tabari M, Sattarian A, Assadi M. Utility of ITS region sequence and structure for molecular identification of Tilia species from Hyrcanian forests, Iran. Plant Syst Evol. 2012;298(5):947–61.
20. Bina H, Yousefzadeh H, Ali SS, Esmailpour M. Phylogenetic relationships, molecular taxonomy, biogeography of Betula, with emphasis on phylogenetic position of Iranian populations. Tree Genet Genomes. 2016;12(5).
21. Mittermeier RA, Gil P, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG. Earth's biologically richest and most endangered terrestrial ecoregions. México CEMEX. 1999.
22. Gharghani A, Zamani Z, Talaie A, Fattahi R, Hajnajari H, Oraguzie NC et al. the Role of Iran (Persia) in Apple (Malus × Domestica Borkh.) Domestication, Evolution and Migration Via the Silk Trade Route. Acta Hortic. 2010;(859):229–36.
23. Gharaghani A, Solhjoo S, Oraguzie N. A review of genetic resources of pome fruits in Iran. Genet Resour Crop Evol. 2016;63(1):151–72.
24. Orton RW, Tucker DB, Harrison JS, McBrayer LD. Spatial and temporal patterns of genetic diversity in a fragmented and transient landscape. Evol Ecol. 2020;1–17.
25. Hanski I, Thomas CD. Metapopulation dynamics and conservation: a spatially explicit model applied to butterflies. Biol Conserv. 1994;68(2):167–80.
26. Verboom J, Schotman A, Opdam P, Metz JAJ. European nuthatch metapopulations in a fragmented agricultural landscape. Oikos. 1991;149–56.
27. Wiens JA. Metapopulation dynamics and landscape ecology. Metapopulation biology. Elsevier; 1997. pp. 43–62.
28. Robinson JP, Harris SA, Juniper BE. Taxonomy of the genus Malus mill. (Rosaceae) with emphasis on the cultivated apple, Malus domestica Borkh. Plant Syst Evol. 2001;226(1–2):35–58.
29. Wilcove DS. Protecting biodiversity in multiple-use lands: lessons from the US Forest Service. Trends Ecol Evol. 1989;4(12):385–8.
30. Janick J. The origins-fruit-growing-breeding.pdf. Plant Breeding reviews. 2005. pp. 255–321.
31. Linder B, Newman R, Jones LK, Debernardi S, Young BD, Freemont P, et al. Biochemical analyses of the AF10 protein: the extended LAP/PHD-finger mediates oligomerisation. J Mol Biol. 2000;299(2):369–78.
32. Haig SM. Molecular contributions to conservation. Ecology. 1998;79(2):413–25.
33. Jarvis DI, Hodgkin T. Wild relatives and crop cultivars: detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. Mol Ecol. 1999;8:S159–73.
34. Li HX, Brewer MT. Spatial genetic structure and population dynamics of gummy stem blight fungi within and among watermelon fields in the southeastern United States. Phytopathology. 2016;106(8):900–8.

35. Nei M, Maruyama T, Chakraborty R. The bottleneck effect and genetic variability in populations. Evolution. 1975;1–10.
36. Liu Z, Zeng X, Yang D, Chu G, Yuan Z, Chen S. Applying DNA barcodes for identification of plant species in the family Araliaceae. Gene. 2012;499(1):76–80.
37. Hollatz C, Vilaca ST, Redondo RAF, Marmontel M, Baker CS, Santos FR. The Amazon River system as an ecological barrier driving genetic differentiation of the pink dolphin (Inia geoffrensis). Biol J Linn Soc. 2011;102(4):812–27.
38. Day J, Clark JA, Williamson JE, Brown C, Gillings M. Population genetic analyses reveal female reproductive philopatry in the oviparous Port Jackson shark. Mar Freshw Res. 2019;70(7):986–94.
39. Alcaide M. On the relative roles of selection and genetic drift in shaping MHC variation. Mol Ecol. 2010;19(18):3842–4.
40. CHAPUIS M, Loiseau A, Michalakis Y, Lecoq M, Franc A, Estoup A. Outbreaks, gene flow and effective population size in the migratory locust, Locusta migratoria: a regional-scale comparative survey. Mol Ecol. 2009;18(5):792–800.
41. Quéméré E, Galan M, Cosson J, Klein F, Aulagnier S, Gilot-Fromont E, et al. Immunogenetic heterogeneity in a widespread ungulate: the European roe deer (Capreolus capreolus). Mol Ecol. 2015;24(15):3873–87.
42. Vásquez-Carrillo C, Friesen V, Hall L, Peery MZ. Variation in MHC class II B genes in marbled murrelets: implications for delineating conservation units. Anim Conserv. 2014;17(3):244–55.
43. Petit RJ, El Mousadik A, Pons O. Identifying populations for conservation on the basis of genetic markers. Conserv Biol. 1998;12(4):844–55.
44. Ren H, Zhang Q, Lu H, Liu H, Guo Q, Wang J, et al. Wild plant species with extremely small populations require conservation and reintroduction in China. Ambio. 2012;41(8):913–7.
45. Kulus D. Genetic resources and selected conservation methods of tomato. J Appl Bot Food Qual. 2018;91:135–44.
46. Rakoczy-Trojanowska M, Bolibok H. Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. Cell Mol Biol Lett. 2004;9(2):221–38.
47. Robinson AJ, Love CG, Batley J, Barker G, Edwards D. Simple sequence repeat marker loci discovery using SSR primer. Bioinformatics. 2004;20(9):1475–6.
48. Korzun V. Molecular markers and their application in cereals breeding. In: Proceedings of the workshop Marker assisted selection: A fast track to increase genetic gain in plant and animal breeding? 17–18 October 2003; University of Turin, Italy. 2003. p. 2003.
49. Patocchi A, Frei A, Frey JE, Kellerhals M. Towards improvement of marker assisted selection of apple scab resistant cultivars: Venturia inaequalis virulence surveys and standardization of molecular marker alleles associated with resistance genes. Mol Breed. 2009;24(4):337–47.
50. Patocchi A, Fernández-Fernández F, Evans K, Gobbin D, Rezzonico F, Boudichevskaia A, et al. Development and test of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. Tree Genet Genomes. 2009;5:211–23.
51. Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9. 3. Httpwww2 Unil Chpopgensoftwaresfstat Htm. 2001.
52. Szpiech ZA, Jakobsson M, Rosenberg NA. ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics. 2008;24(21):2498–504.
53. Raymond M, Rousset F. An exact test for population differentiation. Evolution. 1995;49(6):1280–3.
54. Rousset F. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 2008;8(1):103–6.
55. Desktop EA. Release 10. Redlands CA Environ Syst Res Inst. 2011;437:438.
56. Jombart T. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics. 2008;24(11):1403–5.
57. PE PRS. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics. 2012;28(19):2537.
58. Monmonier MS. Maximum-difference barriers: An alternative numerical regionalization method. Geogr Anal. 1973;5(3):245–61.
59. Manni F, Guérard E, Heyer E. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum Biol. 2004;173–90.
60. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155(2):945–59.

61. Li YL, Liu JX, StructureSelector:. A web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour. 2018;18(1):176–7.
62. Beerli P. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics. 2006;22(3):341–5.
63. Wilson GA, Rannala B. Bayesian inference of recent migration rates using multilocus genotypes. Genetics. 2003;163(3):1177–91.
64. Meirmans PG. Nonconvergence in B ayesian estimation of migration rates. Mol Ecol Resour. 2014;14(4):726–33.
65. Team RC. R: A language and environment for statistical computing. 2013.
66. Rannala B, Zhu T, Yang Z. Tail paradox, partial identifiability, and influential priors in Bayesian branch length inference. Mol Biol Evol. 2012;29(1):325–35.
67. QGIS DT. QGIS geographic information system. Open Source Geospatial Found Proj. 2015;1(2):1–10.
68. Legendre P, Fortin M, Borcard D. Should the Mantel test be used in spatial analysis? Methods Ecol Evol. 2015;6(11):1239–47.
69. Rebelo AG, Siegfried WR. Where should nature reserves be located in the Cape Floristic Region, South Africa? Models for the spatial configuration of a reserve network aimed at maximizing the protection of floral diversity. Conserv Biol. 1992;6(2):243–52.
70. Hofer M, Flachowsky H, Hanke MV, Sem??nov V, ??l??vas A, Bandurko I, et al. Assessment of phenotypic variation of Malus orientalis in the North Caucasus region. Genet Resour Crop Evol. 2013;60(4):1463–77.
71. Höfer M, Eldin Ali MAMS, Sellmann J, Peil A. Phenotypic evaluation and characterization of a collection of Malus species. Genet Resour Crop Evol. 2014;61(5):943–64.
72. Kremelberg D. Practical statistics: A quick and easy guide to IBM® SPSS® Statistics, STATA, and other statistical software. SAGE; 2010.
73. Metsalu T, Vilo J, ClustVis. A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. Nucleic Acids Res. 2015;43(W1):W566–70.
74. Loomis RS, Williams WA. Productivity and the morphology of crop stands: patterns with leaves. Physiol Asp Crop Yield. 1969;27–47.
75. Cornille A, Gladieux P, Giraud T. Crop-to-wild gene flow and spatial genetic structure in the closest wild relatives of the cultivated apple. Evol Appl. 2013;6(5):737–48.
76. Talaie A, Fattahi R, Hajnajari H, Oraguzie NC, Wiedow C, Gardiner SE et al. The role of Iran (Persia) in apple (Malus\${extbackslash}times domestica Borkh.) domestication, evolution and migration via the silk trade route. Int Symp Mol Markers Hortic 859. 2009;(2006):229–36.
77. Jüriado I, Liira J, Csencsics D, Widmer I, Adolf C, Kohv K, et al. Dispersal ecology of the endangered woodland lichen Lobaria pulmonaria in managed hemiboreal forest landscape. Biodivers Conserv. 2011;20(8):1803–19.
78. Schoen DJ, Brown AH. Conservation of allelic richness in wild crop relatives is aided by assessment of genetic markers. Proc Natl Acad Sci. 1993;90(22):10623–7.
79. Bataillon TM, David JL, Schoen DJ. Neutral genetic markers and conservation genetics: simulated germplasm collections. Genetics. 1996;144(1):409–17.
80. Nei M. Genetic distance between populations. Am Nat. 1972;106(949):283–92.
81. SIRKKOMAA S. Calculations on the decrease of genetic variation due to the founder effect. Hereditas. 1983;99(1):11–20.
82. Maruyama T, Fuerst PA. Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics. 1985;111(3):675–89.
83. Zouros E, Singh SM, Miles HE. Growth rate in oysters: an overdominant phenotype and its possible explanations. Evolution. 1980;856–67.
84. Koehn RK, Gaffney PM. Genetic heterozygosity and growth rate in Mytilus edulis. Mar Biol. 1984;82(1):1–7.
85. Schaal BA, Levin DA. The demographic genetics of Liatris cylindracea Michx.(Compositae). Am Nat. 1976;110(972):191–206.
86. Belkhir K, Castric V, Bonhomme F. IDENTIX, a software to test for relatedness in a population using permutation methods. Mol Ecol Notes. 2002;2(4):611–4.
87. Spengler RN. Origins of the apple: the role of megafaunal mutualism in the domestication of Malus and rosaceous trees. Front Plant Sci. 2019;10:617.

88. Hartmann FE, de la Rodríguez RC, Gladieux P, Ma WJ, Hood ME, Giraud T. Higher gene flow in sex-related chromosomes than in autosomes during fungal divergence. Mol Biol Evol. 2020;37(3):668–82.
89. Cornille A, Giraud T, Smulders MJM, Roldán-Ruiz I, Gladieux P. The domestication and evolutionary ecology of apples. Trends Genet. 2014;30(2):57–65.
90. Cornille A, Gladieux P, Giraud T. Crop-to-wild gene flow and spatial genetic structure in the closest wild relatives of the cultivated apple. Evol Appl. 2013;6(5):737–48.
91. Liao J, Li Z, Hiebeler DE, El-Bana M, Deckmyn G, Nijs I. Modelling plant population size and extinction thresholds from habitat loss and habitat fragmentation: Effects of neighbouring competition and dispersal strategy. Ecol Model. 2013;268:9–17.
92. Brown AHD, Hardner CM. Sampling the gene pools of forest trees for ex situ conservation. Conserv Genet Princ Pract. 2000;185–96.
93. Forsline PL, Towill LE, Waddell JW, Stushnoff C, Lamboy WF, McFerson JR. Recovery and longevity of cryopreserved dormant apple buds. J Am Soc Hortic Sci. 1998;123(3):365–70.
94. Rosenberg KV, Raphael MG. Effects of forest fragmentation on vertebrates in Douglas-fir forests. 1986.

Figures

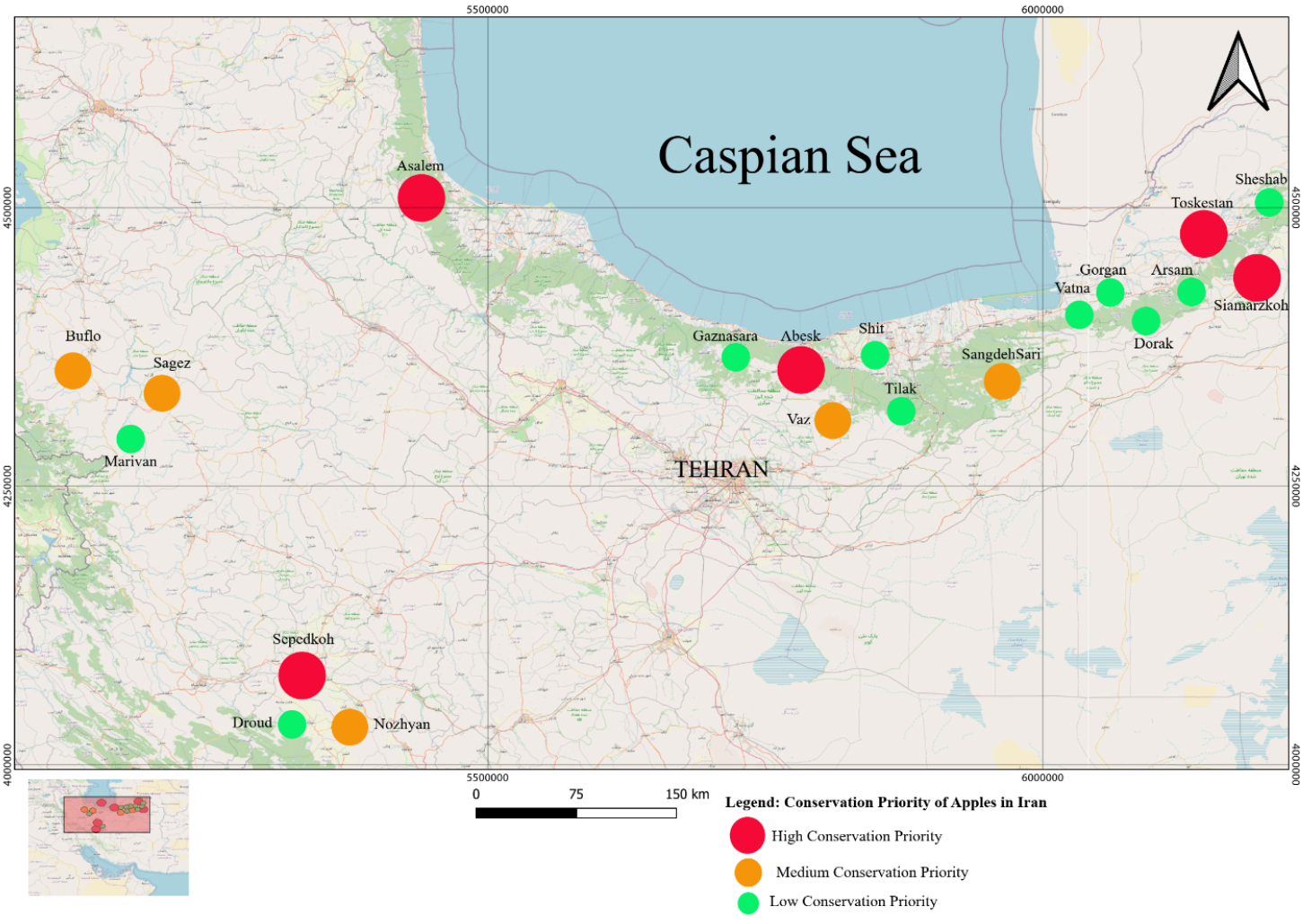

Figure 1

Distribution of *M. orientalis* inIran and conservation priority areas based on DIVA-GIS analysis. The map highlights regions with high, medium, and low conservation priority, considering private alleles and allelic richness.

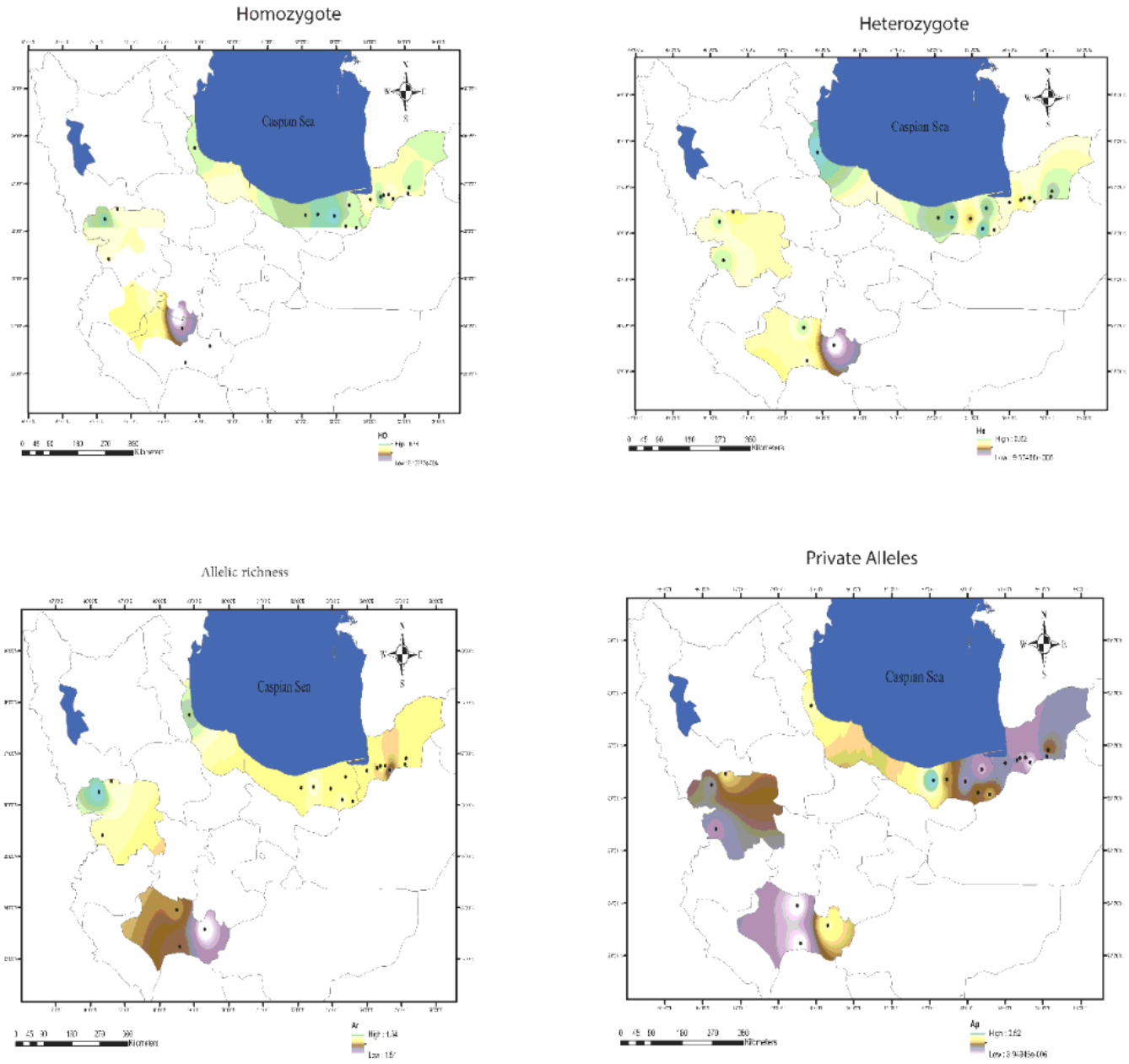

Figure 2

IDW interpolation of allelic richness (Ar) and private allele richness (Ap) for the 20 populations of *M. orientalis* on the basis of 26 SSRs (N=167)

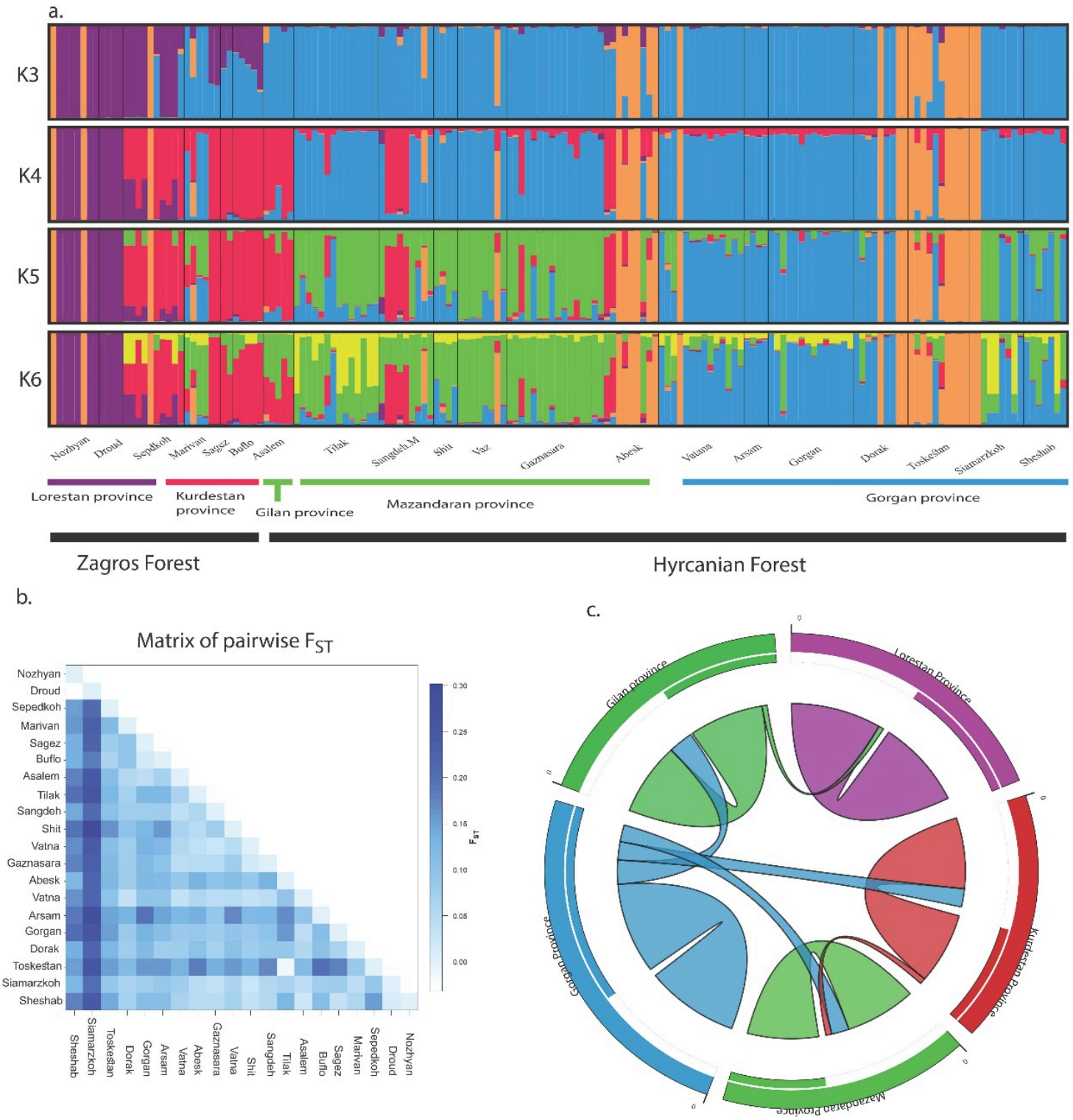

Figure 3

a. Population structure of *M. orientalis* based on STRUCTURE analysis for K3 to K6 **b.** Matrix of pairwise F_{ST} values between sites of *M. orientalis*. C: Gene flow diagrams of the Caucasus apple samples from 5 provinces. The colors correspond to provinces: Gorgan (Blue), Gilan (Green), Lorestan (Purple), Kordestan (Red) and Mazandaran (green). The direction of an arrow represents the direction of gene flow from one region to another. The width of the arrows denotes the relative amount of gene flow within the habitat of the species *M. orientalis* (that is, the wider the arrow is, the greater the degree of gene flow). The 'humps' in the estimates of contemporary gene flow represent gene flow originating from sample sites within provinces. The patterns for each diagram are independent; that is, similar widths of arrows or humps do not represent the same amount of gene flow across each of the diagrams.

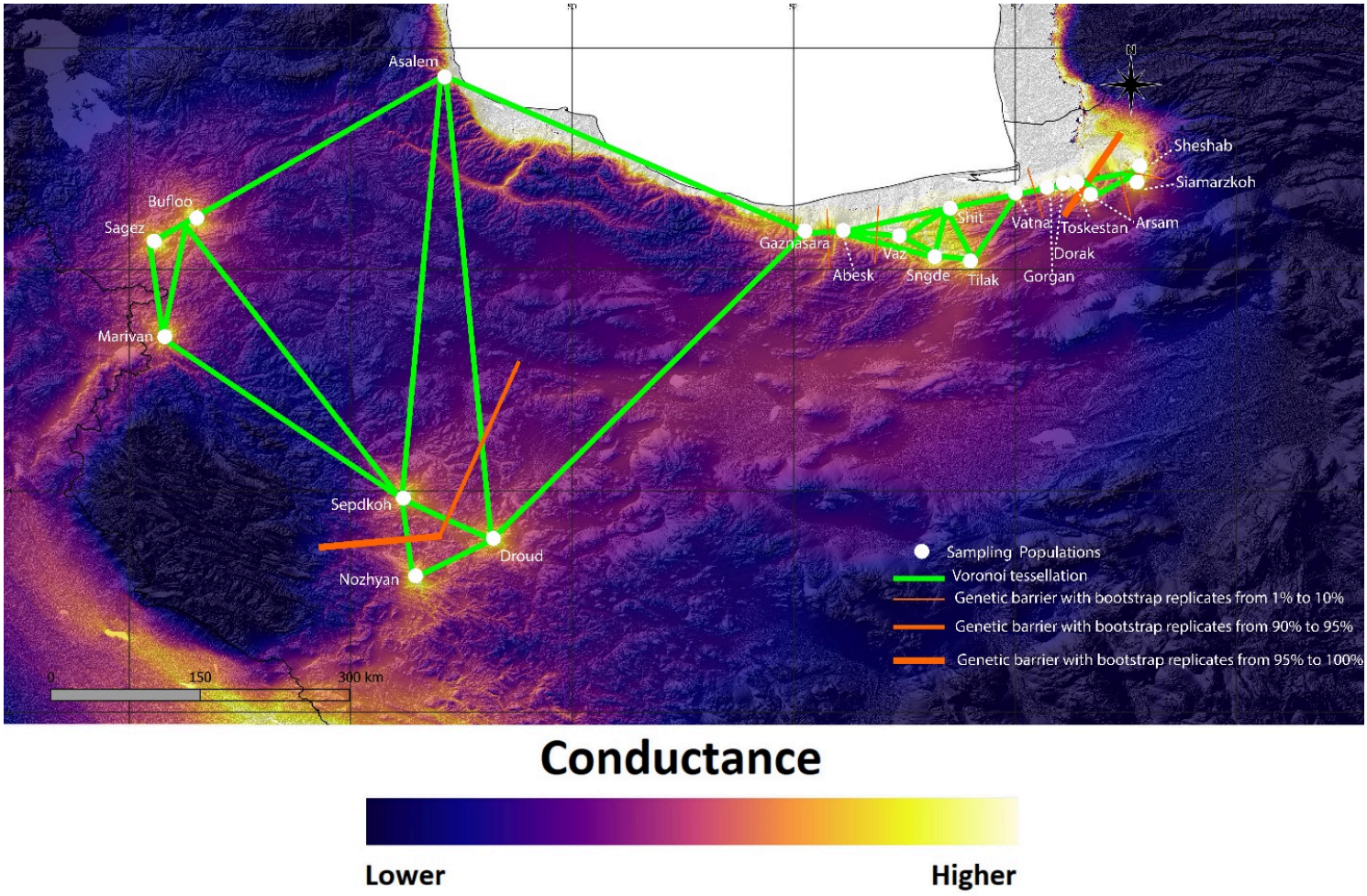

Figure 4

The highest and lowest conductances are shown in yellow and dark blue, respectively, according to the CIRCUITSCAPE results and genetic discontinuities between Caucasian apple populations identified by BARRIER overlaid on a DEM. On the basis of Monmonier's algorithm with 1000 bootstrap replicates, two significant genetic barriers were detected among the 20 populations of the Caucasu apple t. The dotted lines (white) represent the Voronoi tessellation

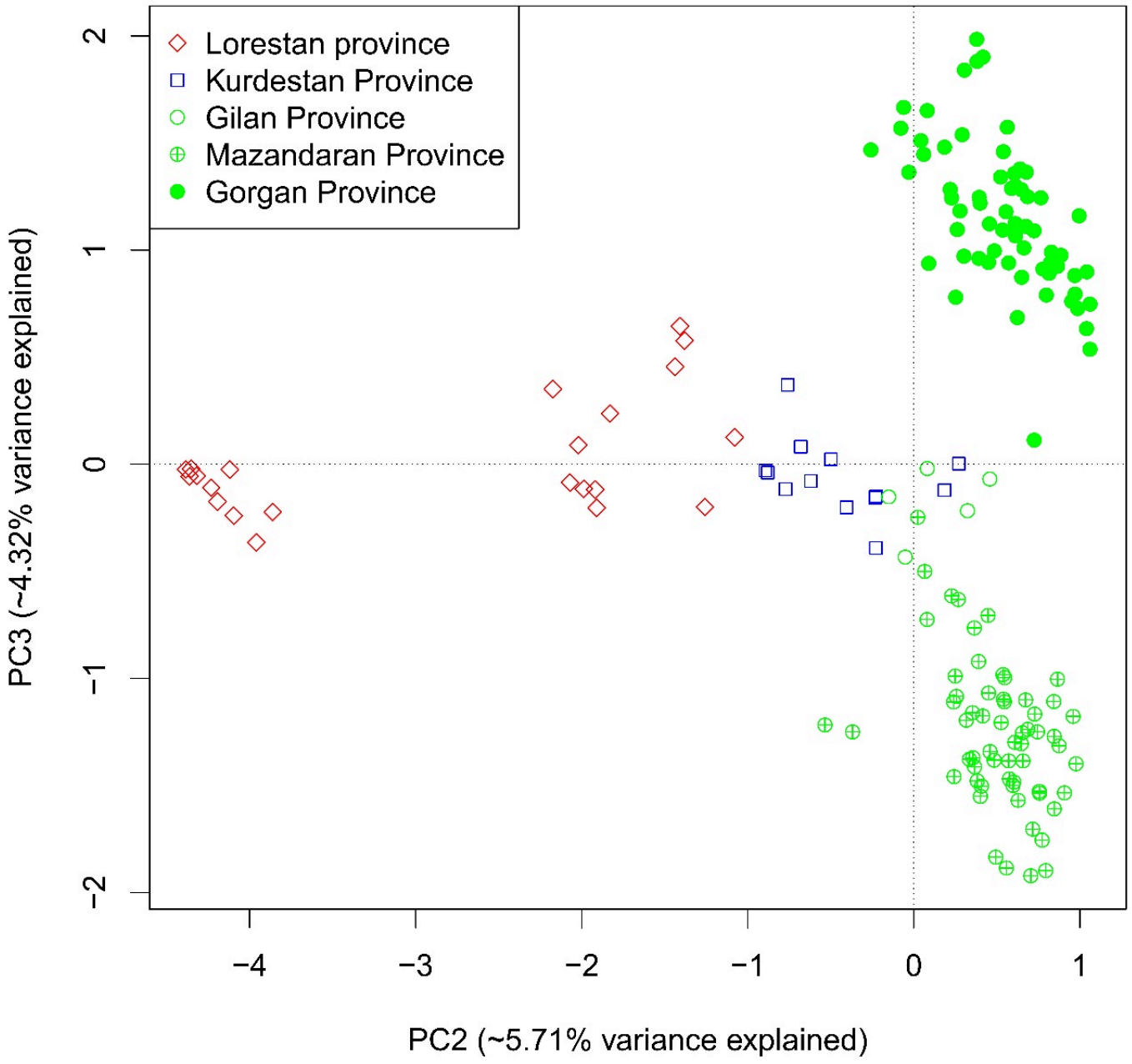

Figure 5

Principal component analysis (PCA) created on the basis of individual genotypes (N=167)