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Transmission of Genetic Variation from the Adult Generation to Naturally Established Seedlings of *Fagus orientalis* in the Hyrcanian Forest

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ABSTRACT

Oriental beech (*Fagus orientalis*) is distributed from Turkey to northern Iran. It is one of the most economically important tree species in this natural distribution range and has been exploited for a long time in the Hyrcanian forest. This study compared genetic variation between adult individuals and recent cohorts of 1–3-year-old seedlings using 14 SSR primers in four pure stands of *F. orientalis* with over 90% canopy cover. The results showed that the expected heterozygosity (He) varied from 0.83 to 0.92 (mean value 0.88), and a significant difference was detected between the expected and observed heterozygosity. In total, 75 private alleles were detected; of these, 56 were rare and had a frequency below 0.05. Pairwise Fst values indicated that seedlings were more similar to each other than to mature trees in the same population. It was found that the populations in each pair (mature trees-seedlings) differed (global average D = 0.35). The average percentage of migrants in the population was 8.83% and varied from 6.64% to 13.41%. The genetic differentiation within the same stands, the genetic differences between adults and seedlings were also significant in some populations, and contemporary gene flow drastically decreased in the next generation. Therefore, the transfer of genetic variation between tree generations is currently strongly affected by anthropogenic influence, at least in the studied beech populations, leading to the high vulnerability of Oriental beech populations to future climate changes.

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Introduction

The gene pool of each forest species is influenced by environmental changes and their consequences, such as range shifts during the last glaciations, pollution, pest attacks, and recent anthropic activities, such as habitat fragmentation and illegal logging (Salehi Shanjani *et al*., 2010; Gougherty *et al*., 2021; Kijowska-Oberc *et al*., 2020). These factors affect gene flow, genetic drift, the mating system, selection, and hence, genetic structure (Finkeldey and Ziehe, 2004; Rajora and Mosseler, 2001; Souza *et al*., 2017; Vekemans and Hardy, 2004). The negative impact of environmental disturbance can include

population declines and even disappearance. High-level genetic diversity guarantees high adaptability to the changing environment, which makes a species less susceptible to extinction (Kramer *et al*., 2010). The ability of forest tree species to withstand biotic and abiotic stresses is dependent on their adaptability, and conservation strategies should be focused on evolutionary information with special attention to within-species genetic diversity (Geburek, 1997; Salehi Shanjani *et al*., 2010). These strategies should ensure the survival of genetic resources for present and future generations (Nonić and Šijačić-Nikolić, 2021; Šijačić-Nikolić *et al*., 2014), which is crucial for maintaining forest sustainability and ecosystem stability (Fageria and Rajora, 2014).

The Hyrcanian forest is a green belt composed of broadleaf and conifer mixed forest, which extends along the southern coasts of the Caspian Sea and covers the northern slopes of the Alborz Mountains. This region, which stretches from sea level to an altitude of 2,800 m, has a total area of 1.85 million ha and comprises 15% of the total Iranian forest (Talebi *et al*., 2014; Shafiei *et al*., 2010; Vajari *et al*., 2012). Among the 80 native woody species in these forests, the Oriental beech (*Fagus orientalis* Lipsky) has the highest ecological and economic value (Pourmajidian *et al*., 2009). This species can achieve up to one meter diameter at breast height and 50 m total height (Talebi *et al*., 2014). It accounts for 30% of the standing volume and 23.6% of the stem number in the Hyrcanian forests in Iran (Rasaneh *et al*., 2001). This widespread tree distributes at an altitude from 600 to 2,000 m above sea level with optimum altitudes from 900 to 1,600 meters above sea level (Sagheb-Talebi and Schütz, 2002). More than 85% of Iranian beech forests are 100 years old, and natural regeneration is seldom achieved in some areas (Salehi Shanjani *et al*., 2010). However, the area of Oriental beech forests in most regions has continuously declined due to human influence, and these forests are comprised of even-aged stands. Beech forests are managed under a shelterwood silvicultural system with unsuitable harvesting methods and without effective protection, which may lead to their further decline (Hosseini *et al*., 2000).

Different kinds of regeneration methods, silvicultural management systems (Hosius *et al*., 2006; Piotti *et al*., 2012), and stand characteristics, such as stand size and tree density (Eckert *et al*., 2010), have a substantial influence on the rate of gene transmission from the parent to the progeny generation in forest stands (Vranckx *et al*., 2014). In the old-growth Oriental beech (Fagus orientalis Lipsky) stands the various causes of gap creation, such as killed trees during the disease, breaking trees during high wind disturbance, and forest management practices altering the post-disturbance structure (Sefidi *et al*., 2011). Forest harvesting and management practices can affect genetic diversity and population structure by affecting demography and several evolutionary processes (Nasiri *et al*., 2023). As reported in studies conducted on stands managed under shelterwood systems, increasing the removal of adult individuals decreases the effective population size of the parental population and tree density. These changes ultimately lead to the reduction of genetic diversity through genetic drift (Finkeldey and Ziehe, 2004) and changes in pollen and seed dispersal patterns (Sork *et al*., 2002). The latter can increase inbreeding in the next generation induced by mating among closely related individuals (Breed *et al*., 2015; El-Kassaby *et al*., 2003; Nasiri *et al*., 2023).

Only a limited number of studies are available on the genetic diversity and structure of *Fagus orientalis* (*e.g*., Bijarpasi *et al*., 2020). However, this species is closely related to European beech (*Fagus sylvatica* L.), which has been intensively investigated. Previous works confirmed the influence of forest management systems (Paffetti *et al*., 2012), habitat fragmentation (Piotti *et al*., 2012), selection and mating systems (Cuguen *et al*., 1988), and isolation of populations during the last glacial period (Kempf and Konnert, 2016) on the genetic diversity and structure of *Fagus sylvatica*. Genetic investigations have indicated the high ratio of intra- vs. interpopulation variability in beech stands (Bresson *et al*., 2011; Hajek *et al*., 2016). Sufficient variability in the intraspecific genetic resources of forest tree species is crucial to cope with current climate change through microevolutionary processes (Alberto *et al*., 2013; Aranda *et al*., 2015). Genetic

differentiation and genetic isolation of Oriental beech due to phenological differences are favorable for the survival of this climax species in temporally and spatially heterogeneous environmental conditions. The present study's objective was to better understand the processes that change genetic diversity across generations in forest stands under different anthropogenic influences. More specifically, this research (1) examined how much genetic diversity is preserved in beech stands between adults and regeneration and (2) measured inbreeding and Within-stand gene flow.

Materials and Methods

Study species, sites, and sampling

For this study, four sites in the Hyrcanian forest were chosen along an east-to-west longitudinal gradient in 2018 (Table 1). At each site, 30 mature trees and 30 seedlings under the selected tree canopy were chosen. In *Fagus*, pollen is dispersed by wind over comparatively great distances; within the population, pollen dispersal distances are between 80 and 184 meters. (Piotti *et al*., 2012). Thus, within each group, the average distance between sampled individuals was at least 50 meters in order to prevent sampling of related family members. Before extracting DNA, leaf samples were taken from each sample (20-30 trees), put into plastic bags with silica gel, and kept at -80°C.

DNA extraction and SSR amplification

DNA was extracted following the methods of Murray and Thompson (1980), with some modifications (Janfaza *et al*., 2017). In total, 240 samples were amplified by 14 SSR markers for genotyping, which are listed in Table 2. Out of the 14 SSR markers, three primers for three SSRs did not amplify any DNA (sfc1143, sfc0289-1 and Fs1-25), and one, sfc0109, produced nonspecific bands. These regions were excluded from further analysis. PCR was conducted based on the methods of Asuka *et al*. (2004) and Pastorelli *et al*. (2003) in a total volume of 10 µl, including 10 ng of DNA template, 0.2 mM dNTPs, 1X PCR buffer, 0.2 mM of each primer, 1 U of Tag DNA polymerase, and 3 mM MgCl2 for each primer (Table 2). PCR products were resolved on an 8% polyacrylamide gel and stained with silver nitrate following the protocol of Merril *et al*. (1981) modified by *Bassam et al*. (1991) to increase sensitivity and reduce the nonspecific background of simple sequence repeats (SSRs). The amplified products' fragment size (SSR variations) was ascertained using the Gel Pro analyzer package 3.9 (Gene, USA). As a size standard, a Gene rulerTM 100 bp plus DNA ladder (Fermentas Company) was employed.

Table 1. *Fagus orientalis* populations sampled in the Hyrcanian forest, their geographical coordinates and other characteristics.

Population	Type	Code	SSe	Lon	Lat	MA	TP	MAT
Salem	Mature	AT	24	48°45′32′′	37°39′04′′	1510	1755	11
	Seedling	AR	24	48°45′57′′	37°38′42′′	1545		
Veysar	Mature	VT	47	51°32′27′′	36°28′25′′	11400	1280	16
	Seedling	VR	47	51°32′20′′	36°28′22′′	1430		
Sangedeh	Mature	ST	22	53°14′02′′	36°02′20′′	1470	1200	12
	Seedling	SR	22	53°14′09′′	36°02′24′′	1480		
Neka	Mature	NT	24	53°24′05′′	36°17′33′′	900	618	15
	Seedling	NR	24	53°24′06′′	36°17′18′′	990		

SS= Sample size, Lon= Longitude, Lat= Latitude, MA= Mean altitude (m), TP= Total precipitation (mm), MAT= Mean annual temperate (°C)

Genetic diversity

The average number of alleles (A), effective number of alleles (Ae), number of private alleles (Ap), expected heterozygosity (Hexp), and observed heterozygosity (Hobs) were all determined using the GENEALEX 6.501 program (Peakall and Smouse, 2006). Using FREENA software, the null allele frequencies for each locus and population were evaluated, along with the global and pairwise FST (with and without ENA corrections), using the Chapuis and Estoup 2007 method.

The significance of a deviation from Hardy–Weinberg equilibrium (HWE) was determined using the *hw.test* function from the package "pegas" in the R environment (Paradis, 2010). The inbreeding coefficient (FIS) was calculated using a Bayesian approach implemented in

INEST software (Chybicki and Burczyk, 2009). This estimation was conducted with 10,000 burn-ins and 500,000 MCMC cycles every 50th update. The Deviance Information Criterion (*DIC*) was used to compare the random mating model ('NB') with the full model ('NFB') to assess whether the heterozygosity level was influenced by inbreeding or by the presence of null alleles. Measures of differentiation for loci and between subpopulations were calculated using the "mmod" package in R (Winter 2012).

Table 2. Microsatellite markers and their repeat types, primer sequences and PCR amplification parameters

Locus name	Repeat sequence	Primer sequence (5′–3′)	AT (°C)	SR (bp)	$Mgcl_2$ (mM)
Sfc0007-2	(AG)24	F: TGTCGCAAACATTGACAAGG R: GTGGATGTGAGGTCGTTGG	60	149-157	1.5
Sfc0036	(TC)23	F: CATGCTTGACTGACTGTAAGTTC R: TCCAGGCCTAAAAACATTTATAG	60	96-142	1.5
Sfc0146	(TC)17	F: TCGATTTCAGACGTGATG R: TCCGCCAATTTGGTATG	55	130-202	1.5
Sfc1063	(CT)13	F: TTTCCAACTACAACTTCATTG R: AGTGCTCGCATCGTATG	60	188-222	1.5
Sfc0305	(GA)24	F: CCAATGGACTTGTTATACCAATC R: GCACCAGTTGCTTACAGAATAG	60	159-203	1.5
FCM5	(AG)10	F: ACTGGGACAAAAAAACAAAA R: GAAGGACCAAGGCACATAAA	60	272-338	1.5
Sf4-46	(TGA)23	F: GCAGTCCTCCACCATTACTA R: TACAACAGCAGGCTATCCAT	60	209-371	1.5
Fs3-04	(GCT)5(GTT)3(GCT)6	F: AGATGCACCACTTCAAATTC R: TCTCCTCAGCAACATACCTC	60	192-204	1.5
Fs1-11	(GA)15	F: TGAATTCAATCATTTGACCATTC R: GGAAGGGTGCTTCAATTTGG	63	98-120	2.5
Fs1-15	(GA)26	F: TCAAACCCAGTAAATTTCTCA R: GCCTCAATGAACTCAAAAAC	60	95-135	2.5
sfc1143	(AG)21	F: TGGCATCCTACTGTAATTTGAC R: ATTCCACCCACCATCTGTC	58	96-136	1.5
sfc0289–1	(AG)8	F: GGAAAGCTTGGTACTATTAGAG R: AAGAGAAGCTTAGTCATGTACAC	60	142–186	1.5
Sfc0109	(GA)27	F: TTGGTGGTCAACATCAC R: TGACCATTAAGTCAACAATC	55	93–175	1.5
Fs1-25	(GA)23	F: GACCCATACCTCTCAGCTTC R: AGAGATCATTGCAACCAAAC	65	80-118	1.5

AT= Annealing temperature, SR= Size range.

Current gene flow between stands was estimated using BAYESASS 3.0 (BA3) software (Wilson and Rannala, 2003), whereas historical migration and mutation-scaled effective population sizes (θ) among the populations were estimated using MIGRATE-N v3.6 software (Beerli and Felsenstein, 1999; Beerli and Palczewski, 2010). Analysis was conducted with 10,000 recorded steps, 1,000,000 sampled parameter values, and a burn-in of 100 steps. A static heating scheme with 4 temperatures was used (chains set at 1, 1.5, 3, and 10^5).

Genetic structure

Structure analysis (Pritchard *et al*., 2000) was used to estimate genetic clusters using the Bayesian approach, whereas DAPC (discriminant analysis of principal components) was used to estimate genetic groups by the non-Bayesian method (Jombart 2008). The Structure procedure included an admixture model, a 100,000 burn-in, 1,000,000 MCMC replications, and ten independent runs for each K with the maximum number of Clusters K= 9. The best K value was chosen using Evanno's delta K method from CLUMPAK software (Kopelman *et al*., 2015). An optimal number of clusters for DAPC analysis was calculated using the *FIND.CLUSTER* function in the *adegenet* package in the R environment (Jombart 2008). The *dapc* function was used to perform the analysis. AMOVA (analysis of molecular variance) was performed using GENEALEX software (Peakall and Smouse, 2006). Principal component analysis (PCOA) was performed to determine Nei's genetic distance using the function *dudi.pca* in package *ade4* in the R environment (Dray and Siberchicot, 2020).

Results

Genetic diversity

In total, 310 alleles were observed at ten nuclear microsatellite loci (Fig. 1, Table 3). The effective number of alleles per locus ranged from 6.51 (FS3-04) to 12.66 (FCM5). The observed heterozygosity ranged from 0.33 (FCM5) to 0.86 (Sfc0036), whereas the expected heterozygosity was between 0.83 (FS3-04) and 0.92 (FCM5). The average frequency of null alleles was 0.159, with the highest value in loci FCM5 (0.308) and the lowest value in loci Sfc0036 (0.035). None of the tested loci conformed to HWE in the analyzed populations, with the exception of Sfc0036 in populations AT and NT. Mature populations had genetic parameter values similar to those of seedling populations (Table 4). Of 310 detected alleles, 201 were common in the mature and seedling populations, 47 alleles were specific to mature populations, and 62 were specific to seedling populations. The expected heterozygosity ranged from 0.86 (population NT) to 0.90 (populations VR and AT), while the observed heterozygosity ranged from 0.38 (VT) to ShT (0.70). Inbreeding coefficient values were rather low (from 0.010 in VR to 0.151 in AR) and were shaped in most populations by the presence of the null alleles (Table 4). In all tested stands, private alleles were observed, with an especially high number in population VR (18 alleles). In total, 75 private alleles were detected; of these, 56 were rare and had a frequency below 0.05. The average global Fst calculated in FREENA software was 0.040 without ENA correction and 0.032 with ENA correction (Supplement 1). Pairwise Fst values ranged from 0.14 (between VT and AT) to 0.065 (between NT and AR) and indicated that seedling populations were more similar to each other than to mature populations (Fig. 2). Population NT was most different from the other stands. Population differentiation parameters were calculated using pairs of stands as an input, with both stands as the subpopulations (Table 5).

Table 3. Genetic parameters of the used loci.

Loci	Size range	Motif length	N	A	Ae	Ap	Null	Hobs	Hexp	Gst	D
FCM5	212-348	2.00	51	18.75	12.66	12	0.308	0.33	0.92	0.04	0.65
Sfc0305	146-198	2.00	27	15.00	10.11	7	0.089	0.74	0.89	0.03	0.40
Sfc0146	114-178	2.00	31	11.63	7.52	13	0.188	0.51	0.85	0.08	0.61
FS3-04	180-240	3.00	21	10.00	6.51	5	0.180	0.49	0.83	0.06	0.41
FS1-11	84-124	2.00	20	12.50	8.35	4	0.134	0.62	0.87	0.03	0.27
FS4-46	171-285	3.00	34	16.75	10.14	7	0.176	0.56	0.90	0.04	0.43
FS1-15	52-136	2.00	38	17.38	10.77	8	0.173	0.57	0.90	0.04	0.45
Sfc1063	150-236	2.00	42	16.63	10.72	11	0.221	0.49	0.90	0.04	0.50
Sfc0007-2	130-172	2.00	21	12.63	9.06	4	0.086	0.78	0.89	0.04	0.41
Sfc0036	86-136	2.00	25	16.50	11.58	4	0.035	0.86	0.91	0.01	0.13
Average			31	14.78	9.74	7.5	0.159	0.59	0.88	0.04	0.43

N= total number of alleles, A= the average number of alleles per population, Ae= number of effective alleles, Ap= number of private alleles, Null= frequency of null alleles, Hobs= observed heterozygosity, Hexp= expected heterozygosity, Gst= Nei's Gst, D= Jost's D.

Table 4. Genetic parameters in the analysed populations.

Population	V		A		SH		N		Average	
	Mature	Seedling	Mature	Seedling	Mature	Seedling	Mature	Seedling	Mature	Seedling
N	30	30	30	30	30	30	30	30	30	30
A	14.1	16	15.4	13.9	14.6	15.4	13.1	15.7	14.3	15.25
Ae	9.42	10.37	10.87	9.31	9.99	10.45	7.97	9.58	9.5625	9.9275
Ap	4	18	10	12	8	9	9	5	7.75	11
Null	0.263	0.161	0.116	0.175	0.112	0.133	0.18	0.133	0.16775	0.1505
Hobs	0.38	0.61	0.69	0.55	0.7	0.66	0.53	0.63	0.575	0.6125
Hexp	0.87	0.9	0.9	0.88	0.89	0.89	0.86	0.89	0.88	0.89
Fis	0.134	0.010*	0.025*	0.151*	0.029	0.025*	0.028*	0.027*	0.054	0.05325

N= total number of individuals, A= the average number of alleles, Ae= number of effective alleles, Ap= number of private alleles, Null= frequency of null alleles, Hobs= observed heterozygosity, Hexp= expected heterozygosity, FIS - fixation index, *= the random mating model was more probable than the full model

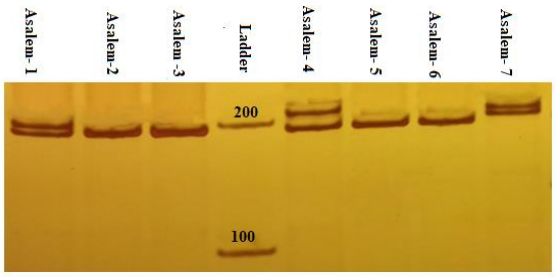

Fig. 1. Polyacrylamide gel for the SSR marker FS3-04 (Sample 1-7 of the population of Asalem; left to right). Coulm 4 is the ladder.

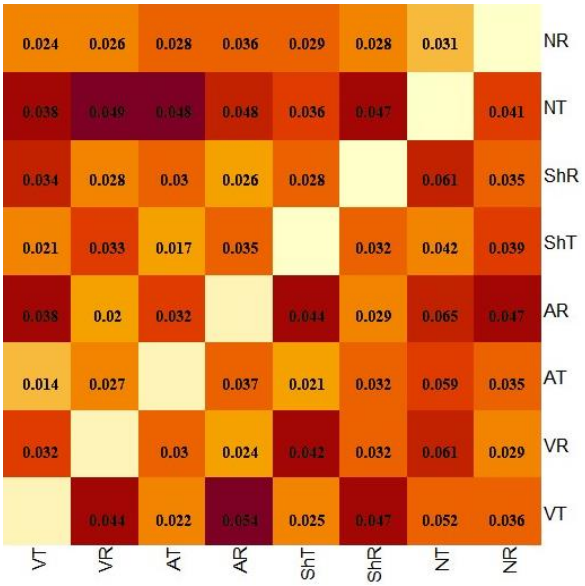

Fig. 2. Values of Fst between tested populations, with ENA correction (above diagonal) and without ENA correction (below diagonal). For explanations of the abbreviations of the populations, see Table 1.

Table 5. Population differentiation measures between pairs of populations.

Stands	Hs	Ht	Gst	G''st	D_het	D_mean
VT-VR	0.90	0.92	0.03	0.51	0.49	0.32
AT-AR	0.90	0.92	0.02	0.44	0.42	0.32
ShT-ShR	0.91	0.93	0.02	0.39	0.37	0.05
NT-NR	0.89	0.91	0.02	0.41	0.38	0.28
Global	0.90	0.94	0.04	0.45	0.43	0.35

Hs-within-population heterozygosity, Ht– expected total-heterozygosity, Gst– Nei's Gst, G''st - Hedrick's G"st; D_het-harmonic mean of Jost's D for each locus; D_mean– average value of Jost's D across loci.

According to the obtained results, the populations in each pair (mature trees-seedlings) were rather different (global average D = 0.35).

The average percentage of current migrants in the population was 8.83% and varied from 6.64% in population VR to 13.41% in population NR. The strongest gene flow was observed from stand NT into population NR, where 7.14% of individuals came from population NT (Fig. 3A). Historical immigration was strongest for population ShR, especially from stand AT (53.1 individuals; Fig. 3B).

Theta was similar in the tested populations and varied between 0.95 in population VT and 2.69 in population NR (Supplement 2).

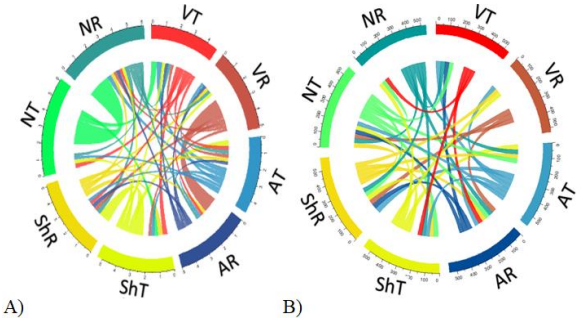

Fig. 3. Results of migration analysis conducted with A) BAYESASS 3.0 software and B) Migrate-N. For explanations of the abbreviations of the populations, see Table 1.

Genetic structure

Structure analysis was performed, and two genetic clusters were identified. The first consisted of mature populations, whereas the second was typical of seedling populations (Fig. 4). One seedling stand, NR, showed a strong admixture from the mature population. The non-Bayesian method used in the DAPC estimated three genetic clusters, with a pattern similar to STRUCTURE – two clusters were typical for mature populations, whereas one was typical for seedling stands. The obtained genetic groups confirmed the results from FREENA and allowed us to clearly distinguish two sets of populations. AMOVA showed that 62% of molecular variance occurs within individuals, 34% among individuals, and 4% among populations (Table 6).

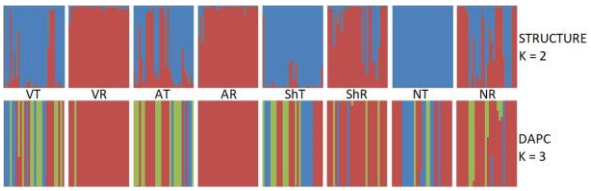

Fig. 4. Results of clustering using STRUCTURE (best K= 2) and DAPC (best K= 3). For explanations of the abbreviations of the populations, see Table 1.

DAPC showed close genetic similarity between adults and regeneration in two populations (SH and N) but clear genetic distance between adults and regeneration in two other populations (V and A; Fig. 5).

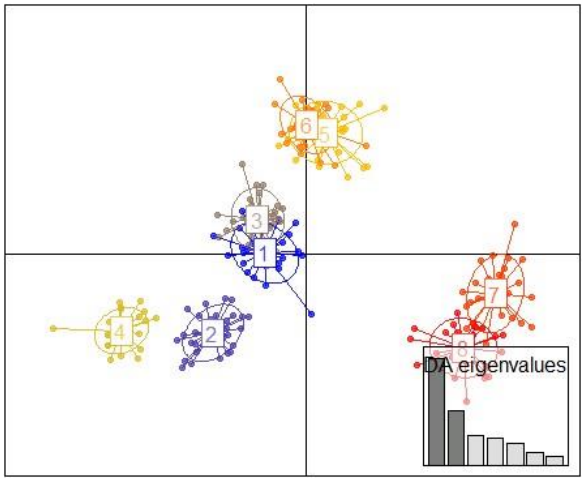

Fig. 5. Results of DAPC Populations: 1-VT, 2-VR, 3-AT, 4-AR, 5-ShT, 6-ShR, 7-NT, and 8-NR; For explanations of the abbreviations of the populations, see Table 1.

Discussion

Assessment of genetic variation among four populations of Oriental beech in the present study showed a relatively high genetic diversity (Ho= 0.61, He = 0.9). Overall, the genetic diversity of beech in the Hyrcanian forest exhibited a tendency toward a heterozygote deficit since the mean value of observed heterozygosity (Ho=0.59) was lower than the expected heterozygosity (He= 0.88) in all studied populations. Similar findings were also presented in other studies of beech, with the following mean values of Ho and He: Ho: 0.606 and He: 0.618 (Müller and Finkeldey 2016), Ho: 0.618 and He: 0.622 (Rajendra et al. 2014), Ho: 0.661 and He: 0.777 (Bilela et al. 2012), and Ho: 0.67 and He: 0.69 (Szasz-Len and Konnert 2018). Large differences between the expected and observed heterozygosity were found for both the offspring cohort and adult generation in all populations under study. If the observed heterozygosity is lower than expected, we must look for reasons to explain this disparity, such as inbreeding and reduced gene flow among populations. Comparison of gene flow from the previous generation (mature trees) to the current generation (seedlings) also indicated a significant reduction in gene flow between populations. The heterozygote deficiency in Oriental beech could be caused by the high rate of inbreeding, the population subdivision (Wahlund effect), and the presence of "null alleles" (nonamplifying alleles). Another possible reason for the high values of inbreeding could be the significant reduction of mother trees due to irregular cutting and silviculture management (Nasiri *et al*., 2023). One or more of these factors may account for a significant deficit of heterozygotes in the studied populations.

Table 6. Analysis of molecular variance (AMOVA) using 10 SSR primers among and within eight stands of 240 *Fagus orientalis* samples.

Source	df	SS	MS	Est. Var.	%
Among Pops	7	119.798	17.114	0.182	4%
Among Indiv	232	1430.450	6.166	1.623	34%
Within Indiv	240	700.500	2.919	2.919	62%
Total	479	2250.748		4.725	100%

The results of the present study provide evidence of clear genetic differentiation between offspring and mature trees in two of the four populations under study. The occurrence of this distinction is probably related to human intervention by altering the forest through fragmentation or intensive exploitation or changing the direction of selection due to pollution or other climatic variations (Savolainen and Kärkkäinen, 1992). Additionally, the distinct genetic structure of the populations may be related to asymmetric migration (fewer migrants than in other stands) and low gene flow from neighboring sites, reducing the number of alleles and genetic diversity and viability as a result of genetic drift (Allendorf *et al*., 2022; Forsdick *et al*., 2017). In line with that concept, compared to *Fagus sylvatica* (Piotti *et al*., 2012) and *Fagus crenata* (Hanaoka *et al*., 2007), less inbreeding depression and migration were found across populations of Oriental beech (with a maximum of 15%). Furthermore, the low average Fst (0.040) found in this study is consistent with Fst values found in Hyrcanian beech forests by other researchers (Salehi Shanjani *et al*., 2010).

The fact that there was only a slight difference in He and Ae between the offspring cohort and the

adult generation supports the findings that genetic drift and inbreeding were minimal in the studied stands, implying that other factors may have influenced the formation of a clear genetic structure between them. In fact, nonoccurrence of inbreeding in the adult generation is expected for this species due to some characteristics of its floral biology (Nielsen and Schaffalitsky-de-Muckadell, 1954). Beech trees are monoecious, wind-pollinated, allogamous, and self-incompatible (Merzeau *et al*., 1994). The position of male and female flowers relative to each other (hercogamy) and the nonsimultaneous anthesis of male and female flowers (protogyny) limit the possibility of self-fertilization. However, in some populations, the higher inbreeding level and significant difference between Ho and He, as well as the significant increase in private allele number, suggest the beginning of processes such as genetic drift, which are usually observed in fragmented populations (Aguilar *et al*., 2019; Wiberg *et al*., 2016). Utilizing different silviculture methods, such as shelterwood cutting, and a great amount of smuggling and human interference for long periods of time leads to a decrease in the density of mature trees in the populations and an increase in the spatial genetic structure by increasing the probability of pollination by spatially proximal relatives (Epperson 2003). Private allele numbers and inbreeding values vary in the stands, most likely due to human intervention. The largest number of private alleles (n = 18) was found in the VR populations, whereas a relatively low genetic diversity (Ho= 0.38, He = 0.87) and the lowest number of private alleles (n = 4) were discovered in the adult stand of this population (VT). Increasing the number of private alleles makes the natural population more capable of use in selective breeding for more adaptability and resistance to the destructive effect of climate change (Sun *et al*., 2016).

Conclusion

The present study is the first to compare genetic diversity among current and future generations of *Fagus orientalis* in the Hyrcanian forest. Allelic diversity and expected heterozygosity were moderately high, although relatively large differences were measured between Ho and He in the populations under study. One of the crucial impacts of forest management practices on forest tree populations is an alteration of the amount and distribution of genetic variation (Sagnard *et al*., 2011). From a large-scale landscape perspective, a relatively similar genetic structure is observed between western and eastern Hyrcanian populations, and populations are moving toward genetic purity. The analysis of gene flow indicated decreased dispersal and suggested a relatively distinct genetic structure between adult trees and their seedlings in certain populations. This confirmed the hypothesis that the human-made landscape drastically affects the genetic pools of *Fagus orientalis* in the Hyrcanian forest and will increase the vulnerability of this species to climate change in the future.

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Author contributions

HY and MN designated the research. HY, NA, and LW wrote the main manuscript text and prepared all figures and tables. TD and GK revised it critically for important intellectual content. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare no competing interests.

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