Altitudinal genetic variations among the *Fagus orientalis* Lipsky populations in Iran

Parvin Salehi Shanjani[1*], Giovanni Giuseppe Vendramin[2], Mohsen Calagari[1]

[1]Research Institute of Forests and Rangelands, P.O. BOX 13185-116, Tehran, I.R. Iran [2]Institute of Plant Genetic, CNR, Via Madonna del Piano, I-50019 Sesto Fiorentino, Firenze, Italy

Abstract
Nuclear simple sequence repeats (nSSRs), together with 16 different enzyme loci, were used to analyze genetic diversity and differentiation among beech (*Fagus orientalis* Lipsky) populations along two altitudinal gradients in Hyrcanian forests of Iran. Both enzymes and nSSR analyses revealed a high level of genetic diversity in natural populations of *F. orientalis*. The genetic diversity, estimated by expected heterozygosity, was 0.19 (by enzymes) and 0.65 (by nSSRs). Genetic variation across both markers did not reveal genetic structuring along altitudinal transects. There was less genetic variation among altitudinal gradients within transects compared to transect sites. Differentiation assays and analysis of molecular variance (AMOVA) indicated that there was a relatively low genetic differentiation among populations, and just 1% and 5% of the genetic variation occurred among populations by nSSR and enzyme data, respectively. Mantel tests showed that there was not a significant correlation between the genetic distances among populations and the distance of elevation. The results of the present study indicate that the relatively low genetic differentiation among *F. orientalis* populations at different elevations was not caused by ecological factors. These patterns suggest that higher rates of gene flow along altitudinal gradients within transects, than between transects; a process that could question altitudinal adaptation.
Keywords**:** *Fagus orientalis* Lipsky; genetic structure; altitudinal gradient; microsatellite; enzyme gene flow

Correspondence to:* **Parvin Salehi Shanjani, Ph.D.
Tel: +98 21 44580276-85; Fax: +98 21 44580223
E-mail: psalehi@rifr-ac.ir, psalehi1@gmail.com

INTRODUCTION

Variation along altitudinal gradients has been studied in a number of plant species for several purposes (e.g. Isik and Kara, 1997; Senjo *et al*., 1999; Sáenz-Romero and Tapia-Olivares, 2003; Jump *et al*., 2006; Sáenz-Romero *et al*., 2006; Truong *et al*., 2007). One of the main objectives with regard to tree species has been to identify suitable populations that can be used in tree breeding programs to provide high-quality wood, for which use of genetic markers is a major approach (Isik and Kara, 1997; Sáenz-Romero *et al*., 2006). Genetic markers (biochemical markers such as allozymes, and more recently, molecular markers such as microsatellites) are traditionally involved in measuring neutral genetic diversity at single loci, often randomly sampled in a genome. In recent years, many studies of genetic variation along altitudinal ranges have also been performed with the aid of neutral molecular markers (Senjo *et al*., 1999; Jump *et al*., 2006; Truong *et al*., 2007). According to these studies, genetic diversity within populations can vary along altitudinal gradients in a number of patterns: 1) Populations at intermediate altitudes have greater diversity than populations at lower and higher altitudes; 2) Higher populations have less diversity than lower populations; 3) Lower populations have lower diversity than higher populations; and 4) Intra-population genetic variation is not affected by altitude (Ohsava *et al*., 2007 a,b; Ohsawa and Ide, 2008). The information obtained in such studies can be very important in several respects. Notably, it can help to assess the distribution, genetic structure and evolution of mountain populations

(Alden and Loopstra, 1987; Jump *et al*., 2006; Truong *et al*., 2007), and provide helpful indicators of appropriate conservation strategies for them (Wen and Hsiao, 2001; Sáenz-Romero and Tapia-Olivares, 2003).

Significant correlations between latitude, longitude, altitude and diversity parameters have also been reported for European beech. Along an altitudinal transect from the base of mount Schauinsland (in Germany) to its top, allele frequencies of three different enzyme systems were found to change (Löchelt and Franke, 1995), suggesting climatic adaptation and man-made selection. Sander *et al*. (2000) studied 13 different enzyme loci along an altitudinal transect on the western slope of mount Vogelsberg in central Germany, and found the highest values for the number of effective alleles, heterozygosity and differentiation in the highest elevated population. In contrast with these results, a previous study of isoenzymatic variation in five *Pinus oocarpa* populations indicated that there was an intense gene flow among them, so that the five populations could be considered as a single panmictic population, with *Fst* not significantly different from zero (Sa´enz-Romero and Tapia-Olivares, 2003). Despite the intense gene flow and the proximal location of the sampled populations (contiguous populations at 100 m altitudinal separation and 4 km average geographic distance), significant differences exist among populations for growth traits (Sa´enz-Romero and Tapia-Olivares, 2003). However, few molecular ecology studies have been reported yet on oriental beech (Salehi shanjani *et al*., 2008, 2004, 2002).

Oriental beech (*Fagus orientalis* Lipsky) is a widespread monoecious and wind-pollinated tree species. Iranian beech forests are located on the northern slopes of Alborz Mountains, Hyrcanian forests, within an altitude of about 600-2000 m above sea level. They assemble a forest strip of 700 km length, located in three provinces of Gilan, Mazandaran and Golestan. Pure and mixed beech (*Fagus orientalis* Lipsky) forests are the most important elements of this ecosystem, making up the richest and the most beautiful forests of Iran. Beech populations tend to be genetically differentiated along altitudinal gradients in response to natural selection. In general, populations from mild environments at low altitudes have larger growth potential, longer period of shoot elongation, and less resistance to frost damage, than populations which originate from colder environments at higher altitudes. High altitude resources show, therefore, lesser growth potential, shorter periods of shoot elongation and more resistance to frost damage (Marvie-Mohadjer, 1976).

The objective of the present study was to evaluate the genetic variation within and between populations of *F. orientalis* at different altitudes. More specifically, we tested whether the genetic diversity of *F. orientalis* populations differs according to altitude. Furthermore, we determined other factores affecting the genetic diversity of these populations.

MATERIALS AND METHODS

Samples: Beech (*Fagus orientalis* Lipsky) samples were collected from six natural populations in two sites distributed along two altitudinal gradients on northern slopes of Alborz Mountains (Table 1). In each population, beech twigs with dormant buds were sampled from 50 nonadjacent individuals (to avoid the sampling of related trees), chosen at random over a 3-4 ha area in a homogeneous environment. The twigs were stored at -18°C as well as were put in water and stored in dark at +4°C until DNA and enzyme extraction, respectively.

Enzyme analysis: Enzymes were extracted (using 0.1 M Tris-HCl buffer pH 7) from dormant buds and cortical tissues of each individual, and were separated by means of starch electrophoresis. Protein separation and staining procedures were performed as described by Merzeau *et al*. (1989) Menadion Reductase (MNR-A), Isocitrate Dehydrogenase (IDH-A), Malate Dehydrogenase (MDH-A-B-C), Phosphoglucose Isomerase (PGI-A-B), Phosphoglucomutase (PGM-A), Müller-Starck and Starke (1993) Shikimate Dehydrogenase (SDH-A), 6-phosphogluconate dyhydrogenase (6PGDH-A), Leucine Aminopeptidase (LAP-A) and Glutamate Oxaloacetate Transaminase (GOT-A-B), and Thiébaut *et al*. (1982) Peroxidase (PX-A-B).

SSR analysis: DNA from bud material was extracted using a DNeasy Plant mini Kit (Qiagen). All trees were

Table 1. Site characteristics of the studied beech populations.

Region	Pop. code	Altitude(m)	Latitude(E)	Longitude(N)
Gorgan	G-1900	1900	36º45'	54º04'
	G-1400	1400	36º41'	54º05'
	G-600	600	36º42'	54º06'
Sangdeh	S-1900	1900	36º00'	53º12'
	S-1400	1400	36º03'	53º14'
	S-900	900	36º06'	53º16'

Table 2. Characteristics of the six polymorphic nuclear microsatellite markers used to analyze genetic diversity in the Iranian beech populations.

Microsatellite locus	Repeat	Observed number of alleles	Annealing temp. (°C)
FS1-15	(GA)26	26	60
FS1-03	(GA)18	18	60
FS1-11	(GA)15	17	63
FS3-04	(GCT)5(GTT)3(GCT)6	6	60
FS4-46	(TGA)23	41	60
FCM5	(AG)10	36	60

genotyped using six primer pairs of microsatellite loci (FS1-15, FS1-03, FS1-11, FS3-04, FS4-46 and FCM5, Table 2), described in Pastorelli *et al*. (2003). All six microsatellites showed mendelian inheritance in controlled crosses of *F. sylvatica*, and are mapped to different linkage groups (Scalfi *et al*., 2004). PCR amplification was performed as described in Pastorelli *et al.* (2003). Amplification products were electrophoresed and visualized using an Amersham ALF Express automatic sequencer.

Data analysis: GENAlEX 6 computer program developed by Peakal and Smouse (2006) was used to analyze allelic data of both marker systems. Population genetic parameters such as mean number of alleles per locus (*Na*), effective number of alleles (*ne*), number of rare alleles (*Nr*), observed heterozygosity (*Ho*), and expected heterozygosity (*He*) (Nei, 1978) were calculated for each population. An estimator of Wright's *Fst* was calculated to assess population differentiation. Gene flow rate was estimated indirectly from the proportion of total diversity found among populations (*Fst*, Wright, 1931, 1951). Wright's fixation index (*Fis*), averaged over all loci, was calculated and deviation from Hardy-Weinberg expectations were determined using GENEPOP 3.3. A paired t-test was performed to test differences between the genetic diversity estimates in the populations. Genetic distances were estimated according to Nei (1978). To examine the relationship between the genetic distance and the geographic distance, a Mantel test on the matrix of *Fst* values and that of the geographic distances was performed (1000 permutations) using GENEPOP 3.3. The results of the analysis were visualized by plotting the *Fst* values against the distances. Genetic distances were estimated according to Nei (1978) and the resulting similarity matrix was subjected to Principal Component Analysis (PCoA). Mantel test (Gower, 1966) was used to assess correlation between the calculated distance matrices and the test statistic tested for significance against 999 random permutations.

RESULTS

Genetic diversity: A total of 45 alleles were found within the 16 polymorphic enzyme gene loci, which corresponds to 2.81 alleles per locus. The number of alleles found at the loci varied as two (PX-A, PX-B, LAP-A, MNR-A, MDH-C), three (LAP-B, GOT-B, IDH-A, MCH-A, MCH-B, PGI-A, PGM-A), four (GOT-A, SKD-A, 6PGDH-A), or five (PGI-B). Fluctuation patterns were different for different allele frequencies in populations of each transect (Table 3). In transect Gorgen, the maximum value for mean number of alleles per locus was obtained in the lowest elevated population; whereas in transect Sangdeh, it was seen in the highest elevated population (Table 4). Differences in genetic parameter measures between and within transect sites were very small and not significant. No clear trend of diversity was found for differences among populations within both transect sites.

The banding patterns obtained by each primer pair were in accordance with single locus variation. Therefore, we refer to the sequence amplified by each primer pair as a locus, and each variant as an allele. Accordingly, a total of 114 alleles were detected at the six microsatellite loci evaluated. The number of alleles

Table 3. Allele Frequencies of sixteen enzyme loci by populations.

Locus	Allele	G-1900	G-1400	G-600	S-1900	S-1400	S-900
PX-A	1	0.125	0.146	0.217	0.177	0.149	0.163
	2	0.875	0.854	0.783	0.823	0.851	0.837
PX-B	1	0.906	0.885	0.840	0.830	0.822	0.848
	2	0.094	0.115	0.160	0.170	0.178	0.152
LAP-A	1	0.969	0.844	0.958	0.927	0.948	0.927
	2	0.031	0.156	0.042	0.073	0.052	0.073
LAP-B	1	0.906	0.896	0.896	0.913	0.854	0.865
	2	0.083	0.104	0.104	0.088	0.146	0.135
	3	0.010	0.000	0.000	0.000	0.000	0.000
GOT-A	1	0.000	0.000	0.000	0.000	0.011	0.000
	2	0.826	0.844	0.906	0.813	0.702	0.865
	3	0.174	0.146	0.094	0.188	0.287	0.125
	4	0.000	0.010	0.000	0.000	0.000	0.010
GOT-B	1	0.000	0.000	0.000	0.031	0.000	0.000
	2	1.000	0.979	1.000	0.969	1.000	1.000
	3	0.000	0.021	0.000	0.000	0.000	0.000
MNR-A	1	0.344	0.167	0.174	0.198	0.125	0.208
	2	0.656	0.833	0.826	0.802	0.875	0.792
IDH-A	1	0.000	0.010	0.010	0.052	0.010	0.042
	2	1.000	0.990	0.990	0.938	0.990	0.958
	3	0.000	0.000	0.000	0.010	0.000	0.000
M3H-A	1	0.000	0.000	0.000	0.010	0.000	0.000
	2	0.573	0.708	0.596	0.521	0.510	0.490
	3	0.427	0.292	0.404	0.469	0.490	0.510
M3H-B	1	0.052	0.115	0.052	0.074	0.042	0.073
	2	0.844	0.833	0.896	0.862	0.865	0.865
	3	0.104	0.052	0.052	0.064	0.094	0.063
MDH-C	1	0.010	0.010	0.010	0.000	0.000	0.000
	2	0.990	0.990	0.990	1.000	1.000	1.000
PGI-A	1	0.000	0.000	0.010	0.000	0.000	0.000
	2	1.000	1.000	0.990	1.000	1.000	0.990
	3	0.000	0.000	0.000	0.000	0.000	0.010
PGI-B	1	0.000	0.010	0.021	0.000	0.052	0.000
	2	0.885	0.938	0.927	0.979	0.917	0.990
	3	0.000	0.010	0.000	0.000	0.000	0.000
	4	0.115	0.042	0.052	0.021	0.021	0.010
	5	0.000	0.000	0.000	0.000	0.010	0.000
PGM-A	1	0.063	0.010	0.073	0.031	0.021	0.031
	2	0.938	0.990	0.917	0.969	0.979	0.969
	3	0.000	0.000	0.010	0.000	0.000	0.000
SK4-A	1	0.000	0.000	0.000	0.010	0.000	0.000
	2	0.979	0.958	0.969	0.938	0.948	0.958
	3	0.021	0.031	0.021	0.042	0.000	0.031
	4	0.000	0.010	0.010	0.010	0.052	0.010
6PGDH-A	1	0.074	0.010	0.052	0.033	0.010	0.011
	2	0.511	0.490	0.479	0.598	0.552	0.660
	3	0.415	0.469	0.458	0.370	0.438	0.330
	4	0.000	0.031	0.010	0.000	0.000	0.000

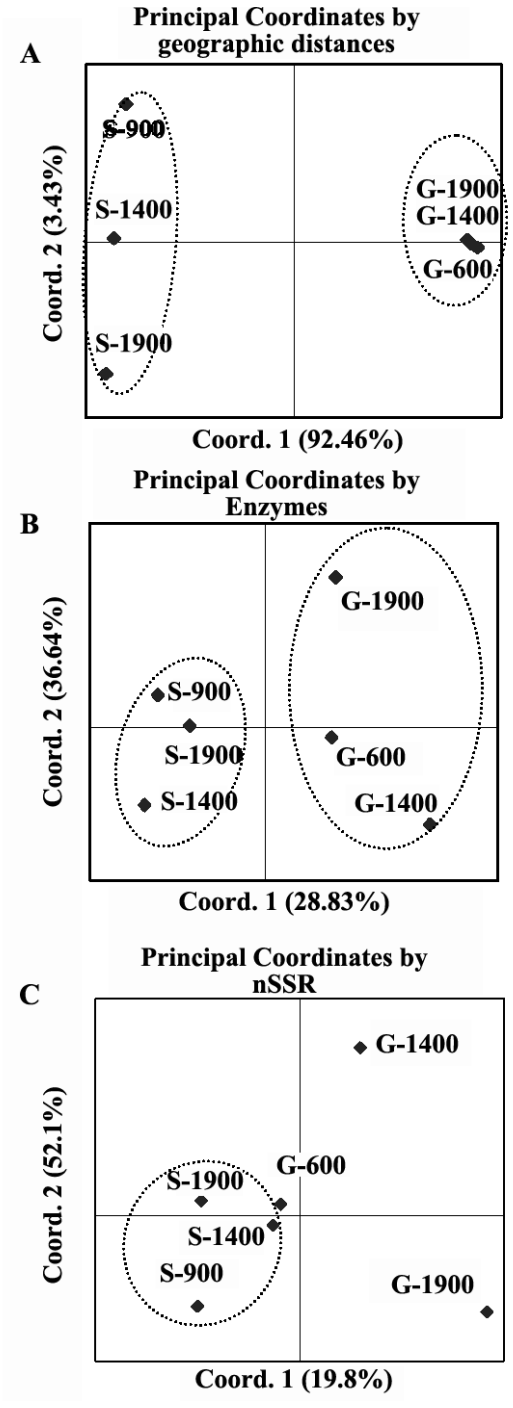

Figure 1. A: Two-dimensional representation of principal coordinate analysis for geographic distances between four populations, B: Nei's genetic distances between four gene pools by enzymes and C: microsatellites.

per locus ranged from 5 (FS3-05) to 31 (FS4-46), with an average of 19 alleles per locus (Table 4). Rare alleles (defined as alleles with a frequency less than 1%) were identified at six loci, which had at least one allele unique to an accession. Sangdeh populations had higher number of alleles and rare alleles. According to the genetic diversity parameters, we found that the genetic variation of beech in site Sangdeh increased with the drop of elevations, i.e., level of genetic variation for the population at 900 m elevation (population S-900) was the highest, and decreased at 1400 m (population S-1400) and 1900 m (population S-1900) elevations. However, there is no clear trend for differences in the diversity measures among different elevations within site Gorgan.

Across all analyzed genotypes of beech, the mean values for *H*e indicate a considerable amount of genetic variation. Furthermore, *H*e values were different between the two marker systems (Table 4). The SSRs showed a 3-fold higher *H*e values than the enzymes (0.65 and 0.19, respectively), which would be expected due to the highly polymorphic nature of the markers. Therefore, there was no association between the proportion of polymorphic loci and the total number of scored loci. The highest ratios for polymorphic bands per loci were observed in nSSR, being two times higher than for Enzyme. For the SSR assays, the *H*e values were different, ranging from 0.580 to 0.702; whereas the enzyme assays generated close *H*e values for the same entries, i.e. from 0.183 to 0.197, indicating suitability of SSRs for population diversity studies (Table 4). A comparison of populations from different elevations in the two transects based on both markers revealed a slightly lower diversity in Gorgan populations.

The mean effective number of alleles (*ne*) varied little from population to population based on both marker systems. Throughout all scored enzyme loci, the maximum *ne* values in each transect were calculated for populations G-1900 and S-1900, the highest elevated populations in each transect, while the mean *ne* values for lower elevated populations were rather smaller (Table 4). Similarly, the highest mean values of Shannon's index (*I*) and expected heterozygosity (*He*) were obtained for populations G-1900 and S-1900. Contrary to Enzyme, nSSR data showed the highest mean values of *ne*, *I* and *He* for the lowest elevated populations, G-600 and S-900.

Genetic structure among the populations: Similarity among the six populations was assessed by principal coordinate analysis, performed on geographic parameters. The populations of the two transects were clearly separated. For description of the differentiation pattern, genetic distances between populations were calculated using Nei´s unbiased estimator. PCoA

Table 4. Genetic variability at sixteen enzyme and six microsatellite loci in six beech populations; by mean number of alleles per locus (*Na*), effective number of alleles (*Ne*), number of rare alleles (*Nr*), Shannon's index (*I*), and observed (*Ho*) and expected (*He*) heterozygosity.

	Enzymes					Microsatellites				
	Na	*Ne*	*I*	*Nr*	*He*	*Na*	*Ne*	*I*	*Nr*	*He*
G-1900	2.000	1.316	0.321	1.000	0.192	7.667	3.474	1.359	3.000	0.624
G-1400	2.375	1.283	0.327	2.000	0.187	8.500	3.287	1.274	2.000	0.580
G-600	2.313	1.294	0.324	2.000	0.186	9.833	4.431	1.600	7.000	0.689
S-1900	2.250	1.305	0.339	4.000	0.197	11.667	4.647	1.583	7.000	0.643
S-1400	2.125	1.301	0.324	2.000	0.194	10.833	4.665	1.609	5.000	0.660
S-900	2.125	1.277	0.311	1.000	0.183	11.667	5.024	1.757	10.000	0.702

Table 5. Mantel test between matrix of Nei's unbiased genetic distances for both markers and matrixes of divergence of ecological factors for different elevations and distances in beech populations.

		Geographic distance		Elevation	
		R^2	p	R^2	p
Gorgan	Enzyme genetic distances	0.885	0.190	0.789	0.2
	nSSR genetic distances	0.663	0.37	0.017	0.7
Sangdeh	Enzyme genetic distances	0.336	0.35	0.651	0.27
	nSSR genetic distances	0.136	0.47	0.079	0.54

R^2: Correlation coefficient, P: Level of significance

was also carried out using the same genetic distances data set (Fig. 1). Enzyme data showed that on the basis of the first principal coordinate, which accounted for 28.8% of the total variation, Gorgan populations were clearly separated from Sangdeh populations. Based on nSSR data, the first principal coordinate, which accounted for 19.8% of the total variation, separated Sangdeh populations; however Gorgan populations were not located in a cluster.

Correlation coefficients among pairwise genetic distance matrices generated by the different marker systems were calculated using Mantel test. Enzymes and nSSRs showed significant correlation in Gorgan trnsecct (Fig. 2).

Table 5 shows relationships between the ecological factors of divergence for different elevations and genetic distances in beech populations. As shown, no significant correlation was found between Nei's unbiased genetic distances for both marker systems, and elevation-distance among populations. This indicates that elevation did not have an impact on the genetic differentiation of *F. orientalis* populations.

The AMOVA results implied that only 1% and 5% (by enzyme and nSSR data, respectively) of the genetic variation occurred among populations and most of the variation (i.e. 98% and 92% by enzyme and nSSR data, respectively) occurred within populations (Table 6). This is in accordance with *Fst* (1.5% and 4.6% by enzyme and nSSR data, respectively) based on Nei's gene diversity index. Table 7 also shows that Nm based on *Fst* by enzyme data (16.32) was higher than Nm by nSSR data (5.19), which indicates that the gene flow estimation among populations by enzyme data was higher.

DISCUSSION

The results determined by enzyme and nSSR markers show that there was a relative high genetic diversity in beech populations of the Hyrcanian forests of Iran. The high intra-population genetic variability may be a consequence of adaptation to a highly heterogeneous and stressful environment. Theoretically, spatiotemporal

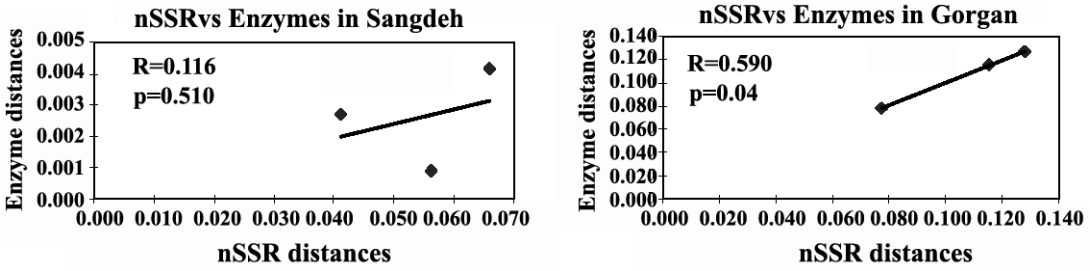

Figure 2. Scatter plot of pairwise enzyme and SSR-based distances in Gorgan (right) and Sangdeh (left).

Table 6. Analysis of molecular variance (AMOVA).

Source	df	MS	Variation	Prob.
Enzymes	1			
Among transects	4	10.435	1%	0.001
Among Pops/transects	282	18.306	1%	0.031
Within Pops	287	886.294	98%	0.001
Total		915.035		
nSSRs				
Among transects	1	30.791	2%	0.001
Among Pops/transects	4	15.485	5%	0.001
Within Pops	278	4.229	92%	0.001
Total	283	50.505		

Table 7. Pairwise estimation of genetic differentiation (*Fst*) and gene flow (*Nm*=migrants per generation) for the studied populations in two transects.

	Enzyme		nSSR	
	Fst	*Nm*	*Fst*	*Nm*
In Gorgan	0.012	20.463	0.041	5.840
Pairwise comparison				
G-1900 - G-1400	0.011	21.636	0.034	7.000
G-1900 - G-600	0.007	34.661	0.034	7.106
G-1400 - G-600	0.008	32.036	0.023	10.396
In Sangdeh	0.009	29.149	0.018	13.522
Pairwise comparison				
S-1900 - S-1400	0.007	34.527	0.012	20.656
S-1900 - S-900	0.003	85.589	0.013	19.648
S-1400 - S-900	0.008	30.056	0.016	15.685
In Species	0.015	16.319	0.046	5.186

variations of diversifying selection can maintain genetic polymorphism (Hedrick *et al*., 1976; Nevo and Beiles, 1988). Many studies have shown strong relationships between levels of genetic polymorphism and degree of environmental heterogeneity and stress (Hedrick, 1986; Nevo, 2001). However, in this study, it was not clear as to what proportion of polymorphic loci were maintained by environmental heterogeneity.

Climate conditions are key factors to determine the distribution of plant species. The climate exerts selec-

tive pressure and has the potential to cause strong directional selection in natural populations including beech forests (Hedrick, 2000; Joshi *et al*., 2001; Jump and Peñuelas, 2007). Temperature plays the most important role as a selective factor for population differentiation (Fang and Lechowicz, 2006). Differentiation among beech populations tends to follow an altitudinal cline along which populations from lower altitudes generally have larger growth potential than populations from higher altitudes. This pattern is likely resulted from differential selection pressures along the altitudinal gradient: Populations from lower altitudes tend to be adapted to the milder climates under which selection has favored high growth potential, while populations from high altitudes display a lower growth potential and greater cold tolerance (Marvie-Mohadjer, 1976). In contrast, there was no clear relationship in our study between genetic diversity and altitude in *F. orientalis* populations. However, using Amplified Fragment Length Polymorphism (AFLP) analysis, Jump *et al*. (2006) reported that, at its upper and lower altitudinal limits, *F. sylvatica* is exceptionally differentiated at a particular locus. Differentiation at this locus is significantly greater than expected assuming selective neutrality, suggesting that a region of the *F. sylvatica* genome is strongly subject to natural selective pressures operating between upper and lower limits of the species' distribution (Jump *et al*., 2006). Highly heterogeneous environmental conditions imposed by altitudinal gradients are likely to affect the neutral sites closely linked to the site under selection (Zhang *et al*., 2006). In agreement with our results, Truong *et al*. (2007) found no change in the genetic diversity of *Betula pubescens* ssp. *tortuosa* with increasing elevation, since the heterozygosity was similar in all populations they examined, but they found a large number of migrants per generation (*N*m), *c.* 50. Thus, they concluded that high levels of gene flow compensated for possible similarity in genetic diversity at different elevations. It could be therefore inferred that extensive gene flow can sometimes homogenize the distribution of genetic diversity along altitudinal gradients. Puglisi *et al*. (1999) also examined the genetic diversity of *Pinus sylvestris* populations by means of electrophoretic enzyme analysis; however, they failed to detect any altitude-related trends. The authors suggested that this failure may have been due to the limited number of populations they sampled along the gradient, and the short distances between them. However, similar results could have been obtained because of other factors, such as extensive gene flow, even if more populations had been examined.

Both enzyme and nSSR markers revealed that *F. orientalis* populations held more genetic variation within rather than between populations. Little genetic differentiation was detected among populations using both markers. According to Hamrick and Godt (1989), reproductive biology is the most important factor in determining the genetic structure of plant populations. They showed that outcrossing plant species tend to exhibit between 10% and 20% genetic variation among populations, while selfing species exhibit on average 50% variation. Studies on the biology of flowering and pollination in *F. orientalis* indicate it as an outcrosser (Tursunov *et al*., 1989). However, Hamrick and Godt (1996) pointed out that life history traits alone only explain a relatively low amount of the variation in genetic structure. The high intra-population variability and genetic homogeneity across populations could have arisen by high levels of gene flow.

From a population genetics perspective, climatic differences along the altitudinal gradient apparently represent a selective pressure strong enough to partially override the effects of gene flow among populations. However, the genetic variation of *F. orientalis* in each altitudinal transect did not vary regularly with elevations. In other words, the pattern of altitudinal divergence observed among the six microsatellite and sixteen enzyme loci indicated that diversifying selection has not driven adaptive differentiation in the face of extremely high levels of gene flow (by wind, and by animal and human vectors). Apparently the swamping action of rampant gene flow has been compensated by differential climatic selective forces along the altitudinal gradient. Consequently, the selectively neutral isozymes and nSSRs are not capable of expressing the genetic differentiation among populations that can be detected by quantitative analyses. All loci exhibited uniformly low *Fst* values. Using Wright's (1943) infinite-island approximation, $Fst = 1 / (1 + 4Nm)$, the weighted mean values of *Fst* translated into an estimate of *Nm* are 5.186 and 16.319 for microsatellites and enzymes, respectively. Kimura and Weiss (1964) showed that when $Nm = 4$, the homogenizing effect of gene flow is sufficient to prevent stochastic differentiation of allele frequencies. Under such conditions, local adaptation may be constrained by high levels of gene flow that produces a spatial averaging of fitness variation among different altitudes; it is likely that the selective forces due to altitude are not strong enough to significantly differentiate the studied populations in

terms of microsatellite and enzyme loci.

Further studies are required to reveal whether there are other factors to cause genetic variation in *F. orientalis*. Although *F. orientalis* had not been listed as a top conservation plant in Iran, it is an important economic tree species endemic to Iran. Therefore, the conservation and further reasonable utilization of the germplasm resources of this species is an urgent task. Our results demonstrate that the divergence of elevation and microenvironments have no obvious effect on the genetic diversity and genetic structure of *F. orientalis* in Hyrcanian forests. Consequently, major attention should be paid to the sustainable conservation of the wild populations of *F. orientalis* at different elevations, when strategies for breeding and germplasm conservation are being implemented in future programs.

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