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Isozyme Variability of *Fagus orientalis* Lipsky in Beech Populations

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ABSTRACT

Beech is one of the most abundant and economically important species in the North of Iran. Isozyme diversity of *Fagus orientalis* Lipsky was investigated in 14 Iranian beech populations originating from the major part of distribution range of this tree in Hyrcanian zone (Southern coasts of Caspian sea). Enzyme variations of beech populations was studied using 16 isozyme Loci at 10 systems including Menadion reductase (MNR), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), Phosphoglucoisomerase (PGI), Phosphoglucomutase (PGM), Shikimate dehydrogenase (SKDH), 6-Phosphogluconate dyhydrogenase (6-PGD), Peroxidase (PX), Leucine aminopeptidase (LAP) and Glutamate oxaloacetate transaminase (GOT) by starch gel electrophoresis. Among 55 observed alleles, 30 rare alleles (less than 5% of the allelic frequency) and 22 specific area alleles (observed in less than five populations) were detected. Results indicated that most of rare alleles are area specific. Population Neka at elevation of 900 m.a.s.l., kheirud at elevation of 600 m.a.s.l. and population Asalem at elevation of 600 m.a.s.l. showed the highest number of area specific alleles. However, population Gorgan at elevation of 2000 m.a.s.l. showed the least area specific allels. In spite of important ecological differences among the studied populations (from far East to far West and from lowest to highest limitation of beech forests in Iran) no unequivocal patterns of geographical trends could be identified.

Key Words: *Fagus orientalis* Lipsky; Beech; Population; Iran; Isozyme; Electrophoresis

INTRODUCTION

Isozyme markers represent electrophoretically detectable forms of enzymatic proteins visualized by substrate-specific staining. Isozymes are alternative enzyme forms encoded by different alleles at the same locus, which can be used as genetic markers. Examining Isozyme variations, which result from changes in protein coding DNA sequences, has been the most common technique in plant population biology (Tanksely, 1983; Wendel & Weeden, 1989; Comps *et al*., 2001). Since the invention of starch gel electrophoresis (Smithies, 1955), the histochemical visualization of enzymes on gels (Hunter & Markert, 1957) and the classic studies of Harris (1966), Hubby and Lewontin (1966), and Lewontin and Hubby (1966), a major revolution in understanding micro evolutionary and Macro evolutionary processes has occurred (Wang & Szmidt, 2001).

Isozyme analysis has several advantages (Bergmann, 1991; Longauer, 1996) as compared not only with metric characters (morphological and physiological ones), but also with other genetic markers; especially isozymes are mostly co-dominant with a simple Mendelian inheritance in most loci, so that the frequency of individual alleles is directly counted. Besides, isozymes can be resolved for most plant species regardless of habitat, size or longevity (that means, isozyme patterns of most enzyme systems are independent of environmental variations and many isozyme patterns are ontogenetically stable) (Wang *et al*., 2000).

The application of genetic markers to investigate genetic diversity of beech populations started later than in conifers. Paganelli *et al*. (1973) as first group, studied the stability and variation of dehydeogenases expression in *Fagus sylvatica* L. Kim (1980) identified the first enzyme gene loci by studying zymogram of acid phosphatase (ACP) and leucine aminopeptidase (LAP) in parent trees and their offsprings. Thiébaut *et al*. (1982) established the formal genetics of three new markers in dormant buds: two in peroxidases (PER) and one in glutamate oxaloacetate transaminases (GOT). Müller-Starck (1985) used several markers to determine genetic differences between tolerant and sensitive beeches in an environmental stress adult forest stand. Merzea *et al*. (1989) analyzed the genetic control of isozyme systems, malate dehydrogenase (MDH), superoxid dismutase (SOD), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI) in offspring of a heterozygous mother. Müller-Starck and Starke (1993) studied inheritance patterns of the enzyme gene loci (20 loci) in progenies from controlled crossings and single trees.

In this research by selection of some beech stands covering a large part of the distribution range of oriental beech in North of Iran, isozyme diversity of 10 enzymes in the stand and regional levels were studied. The main aim is to gather information useful to establish gene reserves in the most valuable regions of beech natural range.

MATERIALS AND METHODS

Population characteristics and sampling. Beech forests are located on the northern slopes of Alborz mountains, within an altitude of about 600-2000 m above sea level. They have formed a forest strip with 600 km length that is located in three provinces of Gilan, Mazandaran and Golestan. Along the northern slope of Alborz montains, 14 autochthonous beech populations (*Fagus orientalis* Lipsky) aged between 80 and 160 years were investigated. It was decided to select five locations along the distribution area of beech (in Hyrcanian zone) from East to West (Gorgan, Neka, Sangdeh, Kheirud, and Asalem) and to establish three investigation stations in each regions (low, middle and high altitude of beech distribution range) to cover most of the geographical range of beech in Iran (Table I, Fig. 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.

Fig. 1. Distribution of studied regions

Table I. Site characteristics of the studied beech populations

Region	Altitude (m)	Abbreviation	Latitude N	Longitude E
Gorgan	2000	G-2000	36' 45"	54' 07"
"	1400	G-1400	36' 41"	54' 05"
"	600	G-600	36' 42"	54' 06"
Neka	1400	N-1400	36' 22"	53' 33"
"	900	N-900	36' 29"	53' 27"
Sangdeh	1900	S-1900	36' 00"	53' 12"
"	1400	S-1400	36' 03"	53' 14"
"	900	S-900	36' 06"	53' 16"
Kheirud	2000	K-2000	36' 28"	51' 40"
"	1200	K-1200	36' 32"	51' 39"
"	600	K-600	36' 35"	51' 33"
Asalem	1900	A-1900	37' 38"	48' 46"
"	1200	A-1200	37' 38"	48' 48"
"	600	A-600	37' 41"	48' 48"

Electrophoresis. 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 described by Merzeau *et al.* (1989) (menadion reductase, isocitrate dehydrogenase, malate dehydrogenase, phosphoglucose isomerase, phosphoglucomutase), Müller-Starck and Starke (1993) (shikimate dehydrogenase, 6-phosphogluconate dyhydrogenase, leucine aminopeptidase and glutamate oxaloacetate transaminase) and Thiébaut *et al.* (1982) (Peroxidase).

Data analysis. Differences of allelic frequencies among populations were tested using the probability test (Raymond & Rousset, 1995a). Subsequently, a global test across loci was calculated using Fisher's method (Rousset & Raymond, 1995b).

RESULTS

Polymorphism of employed isozyme systems. In peroxidase, the interpretation of zymogram followed Thiébaut *et al.* (1982). Peroxidase (PX) is coded by three gene loci and according to observation on the gel; three zones of activity were appeared, of which two zones with a sufficient and stable activity were quantitized as locus PX-A and PX-B. The expression of third zone was season-dependent that was not included in this study. The enzymes produced by both loci are monomer. Two alleles with relative mobilities 100, 105 in PX-A and three alleles with Rm of 26, 39 and 52 were observed in PX-B (Fig. 2). At locus PX-A, allele differences between populations were significant. Such, in population level, the frequency of allele A, varied from 0.111 to 0.319 and the highest frequency at Kheirud (0.28) was more than twice that of the lowest in Asalem (Table II). Allele and genotype differences between populations at locus PX-B was significant and from three observed alleles at locus PX-B, the allele C was only observed at the Gorgan-2000 and Neka-900. Besides frequency of two other alleles (A, B) in these two populations were more different than others.

The polymorphism of leucine aminopeptidase was interpreted following Müller-Starck and Starke (1993). Leucin aminopeptidase is encoded by two enzyme loci (LAP-A and LAP-B). At both loci four allelic variants were observed with Rm of 106, 100, 97 and 94 at locus LAP-A; and Rm 102, 100, 98 and 96 at locus LAP-B (Fig. 3). The enzyme structure produced by both loci is monomer. Allelic differences at both loci were observed in the rare alleles, A and D (Table II).

The variation of glutamate-oxaloacetate transaminase (synonymous: aspartate aminoteransferase) was interpreted according to Müller-Starck and Starke (1993). Isozymes of glutamate-oxaloacetate transaminase were attributed to two loci, GOT-A and GOT-B, comprising four alleles at both

Table II. Allelic frequencies in studied populations (* Probability test)

	G	G	G	N	N	S	S	S	Kh	Kh	Kh	A	A	A	P*
	2000	1400	600	1400	900	1900	1400	900	2000	1200	600	1900	1200	600	Value
PX-A															
A	0.125	0.146	0.217	0.217	0.188	0.188	0.149	0.163	0.219	0.319	0.292	0.125	0.133	0.111	
B	0.875	0.854	0.783	0.783	0.813	0.813	0.851	0.837	0.781	0.681	0.708	0.875	0.867	0.889	0.0035
PX-B															
A	0.448	0.885	0.840	0.811	0.542	0.826	0.822	0.848	0.844	0.717	0.789	0.813	0.615	0.670	
B	0.531	0.115	0.160	0.189	0.417	0.174	0.178	0.152	0.156	0.223	0.211	0.187	0.385	0.330	
C	0.021	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LAP-A															
A	0.000	0.000	0.000	0.042	0.010	0.000	0.000	0.000	0.021	0.000	0.000	0.042	0.031	0.000	
B	0.969	0.844	0.958	0.854	0.844	0.927	0.948	0.927	0.823	0.915	0.917	0.854	0.875	0.854	
C	0.031	0.156	0.042	0.104	0.125	0.073	0.052	0.073	0.156	0.085	0.073	0.104	0.083	0.146	
D	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.000	0.0005
LAP-B															
A	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.010	0.000	0.000	0.021	0.000	0.000	
B	0.906	0.896	0.896	0.646	0.813	0.913	0.854	0.865	0.792	0.628	0.729	0.771	0.802	0.771	
C	0.083	0.104	0.104	0.354	0.177	0.087	0.146	0.135	0.198	0.372	0.260	0.198	0.198	0.229	
D	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.0000
GOT-A															
A	0.000	0.000	0.000	0.000	0.021	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
B	0.826	0.844	0.906	0.857	0.792	0.813	0.702	0.865	0.936	0.698	0.750	0.938	0.875	0.865	
C	0.174	0.146	0.094	0.149	0.188	0.188	0.287	0.125	0.064	0.302	0.229	0.063	0.125	0.135	
D	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.021	0.000	0.000	0.000	0.0000
GOT-B															
A’	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
A	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.010	0.010	0.010	0.021	
B	1.000	0.979	1.000	0.979	1.000	0.969	1.000	1.000	1.000	1.000	0.979	0.990	0.990	0.979	
C	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.0228
MNR-A															
A	0.344	0.167	0.174	0.170	0.208	0.198	0.125	0.208	0.198	0.177	0.229	0.250	0.333	0.293	
B	0.656	0.833	0.826	0.830	0.781	0.802	0.875	0.792	0.802	0.823	0.771	0.750	0.667	0.707	
C	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0159
IDH-A															
A	0.000	0.010	0.010	0.021	0.042	0.052	0.010	0.042	0.010	0.000	0.031	0.000	0.052	0.010	
B	1.000	0.990	0.990	0.979	0.948	0.938	0.990	0.958	0.990	1.000	0.969	1.000	0.948	0.990	
C	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0297
MDH-A															
B	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	
C	0.573	0.708	0.596	0.490	0.573	0.521	0.510	0.490	0.424	0.594	0.677	0.594	0.604	0.594	
E	0.427	0.292	0.404	0.510	0.427	0.469	0.490	0.510	0.576	0.406	0.313	0.406	0.396	0.396	
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.0004
MDH-B															
A	0.052	0.115	0.052	0.104	0.042	0.074	0.042	0.073	0.000	0.021	0.021	0.021	0.010	0.010	
C	0.844	0.833	0.896	0.813	0.896	0.862	0.865	0.865	0.946	0.927	0.917	0.927	0.938	0.948	
D	0.104	0.052	0.052	0.083	0.063	0.064	0.094	0.063	0.054	0.052	0.063	0.052	0.062	0.042	0.0031
MDH-C															
A	0.010	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.021	0.010	0.010	0.000	
B	0.990	0.990	0.990	1.000	1.000	1.000	1.000	1.000	1.000	0.990	0.979	0.990	0.990	1.000	0.9147
PGI-A															
A	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	
B	1.000	1.000	0.990	0.990	0.989	1.000	1.000	0.990	1.000	0.927	1.000	1.000	1.000	0.990	
C	0.000	0.000	0.000	0.010	0.011	0.000	0.000	0.010	0.000	0.073	0.000	0.000	0.000	0.000	0.0000
PGI-B															
A’	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.021	0.000	0.000	0.000	0.000	
A	0.000	0.021	0.021	0.000	0.010	0.000	0.052	0.000	0.010	0.000	0.010	0.000	0.000	0.000	
B	0.883	0.927	0.927	0.958	0.948	0.979	0.917	0.990	0.979	0.979	0.969	1.000	1.000	1.000	
C	0.000	0.000	0.000	0.010	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
D	0.115	0.052	0.052	0.031	0.021	0.021	0.021	0.010	0.000	0.000	0.021	0.000	0.000	0.000	0.0000
PGM-A															
A	0.063	0.073	0.073	0.073	0.073	0.031	0.021	0.031	0.031	0.010	0.010	0.010	0.031	0.010	
B	0.938	0.917	0.917	0.927	0.927	0.969	0.979	0.969	0.969	0.990	0.990	0.990	0.958	0.990	
C	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.0598
SKDH-A															
A	0.000	0.000	0.000	0.010	0.011	0.010	0.000	0.000	0.010	0.000	0.000	0.000	0.031	0.000	
B	0.979	0.969	0.969	0.885	0.894	0.938	0.948	0.958	0.896	0.948	1.000	0.948	0.906	0.659	
C	0.021	0.021	0.021	0.042	0.032	0.042	0.000	0.031	0.073	0.031	0.000	0.031	0.052	0.261	
D	0.000	0.010	0.010	0.063	0.064	0.010	0.052	0.010	0.010	0.021	0.000	0.021	0.010	0.080	0.0000
6PGD-A															
A	0.074	0.052	0.052	0.000	0.031	0.033	0.010	0.011	0.000	0.021	0.031	0.021	0.043	0.021	
B	0.511	0.479	0.479	0.500	0.563	0.598	0.552	0.660	0.593	0.564	0.510	0.564	0.424	0.646	
C	0.415	0.458	0.458	0.500	0.406	0.370	0.438	0.330	0.384	0.415	0.427	0.415	0.489	0.333	
D	0.000	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.031	0.044	0.000	0.000	0.0037

Table III. Frequency and distribution of area specific (As) and rare (Ar) alleles alleles according to populations

	G-2000	G-1400	G-600	N-1400	N-900	S-1900	S-1400	S-900	K-2000	K-1200	K-600	A-1900	A-1200	A-600
Ar	3	7	7	7	15	8	7	7	9	5	14	10	9	8
As	2	4	4	5	11	4	3	2	6	2	8	5	4	3

loci with Rm of 105, 100, 95 and 90 at locus GOT-A and 54, 36, 18 and 02 at GOT-B (Fig. 4). The enzymes produced by both loci are dimetric. At locus GOT-A, although, significant difference was observed at non-frequent allele C (from 0.063 to 0.372) between populations, but in regional level allelic frequencies were not different. At locus GOT-B, allelic differences between populations were not observed, so that frequencies of allele B was 98-100% (Table II).

Menadione reductae is encoded by one locus, MNR-A, comprising three alleles with Rm of 126, 100, and 74 (Fig. 5). The enzyme structure is tetrameric and its interpretation followed by Merzeau *et al*. (1989). Significant differences ($\alpha \leq 5$ %) of allelic frequencies were observed at the eastern and western borders with center. Thus, the highest frequency of allele A at borders was more than twice of lowest frequency in center of Hyrcanian forests (Table II).

The interpretation of isocitrate dehydrogenase followed in principle by Merzeau *et al*. (1989). Isozymes of isocitrate dehydrogenase are coded by two gene loci of which locus IDH-A with three alleles was quantitized. According to observation on the gel, two zones of activity were observed, but the second zone (IDH-B) was stained faintly and this did not allow a reliable scoring. Three alleles had relative mobilities 116, 100 and 84 (Fig. 6). The enzyme structure is dimeric. Difference between frequencies of frequent allele (B) in all populations was not significant (Table II).

The interpretation of malat dehydrogenase followed in

Fig. 2. Observed genotypes of peroxidase in studied beech forests

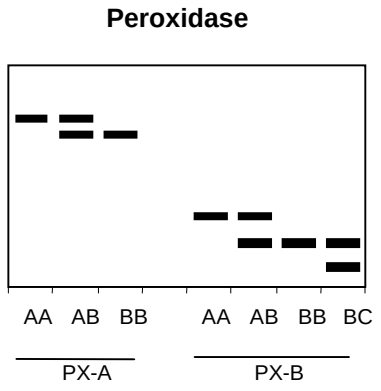


Fig. 4. Observed genotypes of glutamate oxaloacetate transaminase in studied beech forests

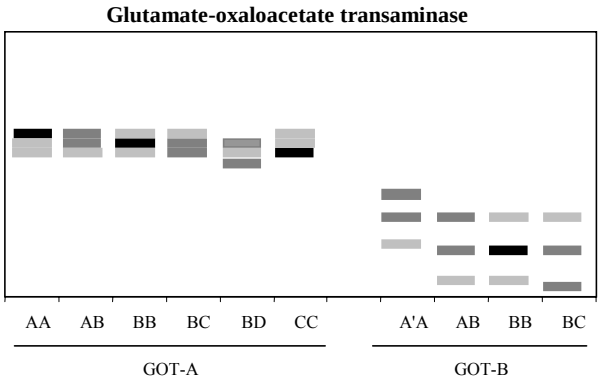


Fig. 3. Observed genotypes of *Leucine aminopeptidase* in studied beech forests

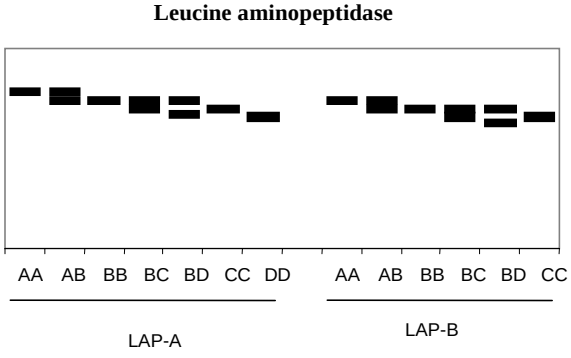


Fig. 5. Observed genotypes of menadion reductase in studied beech forests

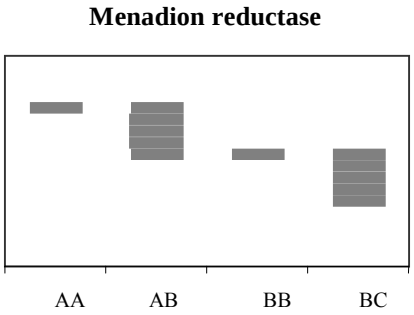


Fig. 6. Observed genotypes of isocitrate dehydrogenase in studied beech forests

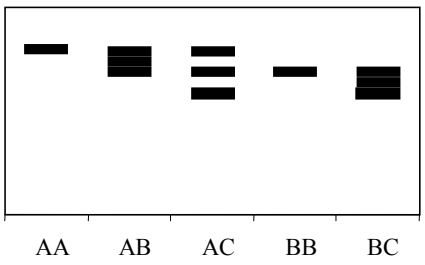


Fig. 7. Observed genotypes of malate dehydrogenase in studied beech forests

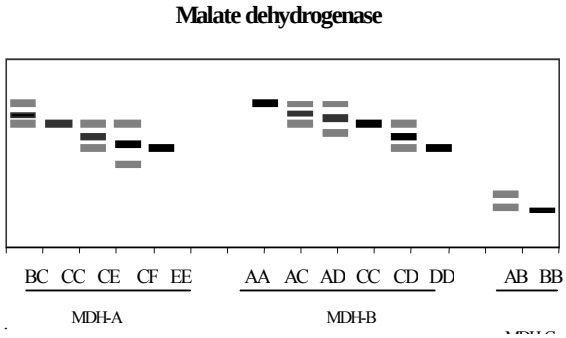


Fig. 8. Observed genotypes of phosphoglucose isomerase in studied beech forests

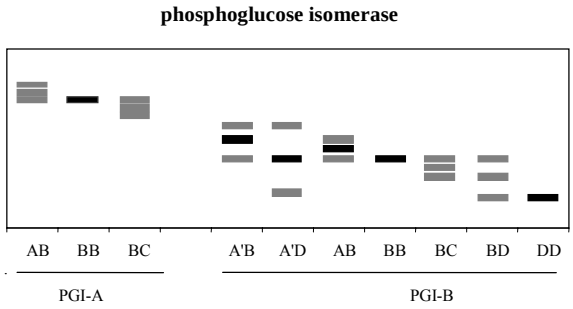


Fig. 9. Observed genotypes of phosphoglucomutase in studied beech forests.

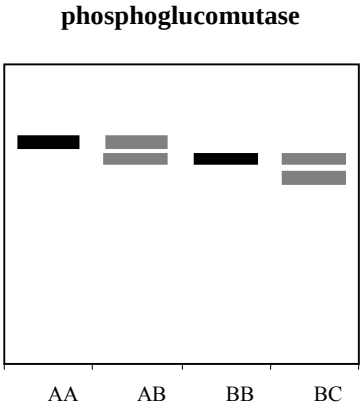


Fig. 10. Observed genotypes of shikimate dehydrogenase in studied beech forests

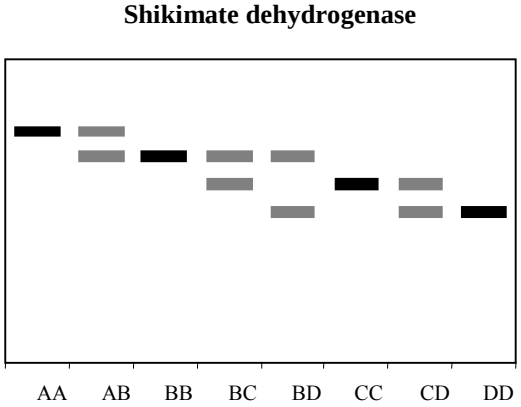
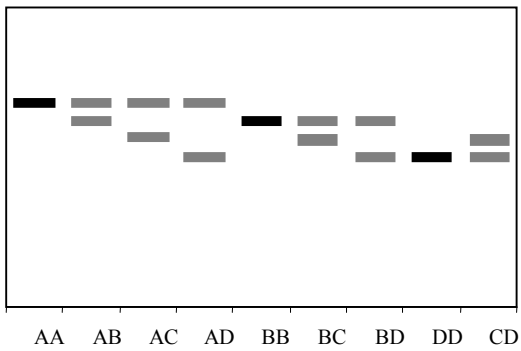


Fig. 11. Observed genotypes of 6-phosphogluconate dehydrogenase in studied beech forests.



principle by Merzeau *et al.* (1989). Malat dehydrogenase is encoded by three enzyme loci (MDH-A, MDH-B and MDH-C). At loci MDH-A[1], MDH-B and MDH-C were found 3, 4 and 2 allelic variants, respectively (Fig. 7). The phenotypic expression of locus MDH-A is very similar to MDH-B and enzymes produced by these loci are dimeric. Whereas, the enzyme produced by locus MDH-C is monomeric. At locus MDH-A, the lowest frequency of allele D and the highest frequency of allele C were observed in border populations, while in locus MDH-B a clinal tendency from east to west and west to east were found in alleles C, A, respectively. At locus MDH-C, in fact, four variants exist. However, the migration velocities of the first and the second allele, as well as the third and forth allele are very similar, so that they were pooled and only two alleles were interpreted. At locus MDH-C, the highest allele frequency belongs to allele B (Table II).

Two loci coding for the enzyme phosphoglucose isomerase (PGI-A, PGI-B) were found, whereby 3 and 5 allele variants were observed at PGI-A and PGI-B, respectively (Fig. 8). The enzyme produced by both loci is diameric and interpretation of second zone followed by Merzeau *et al.* (1989). At locus PGI-A, no difference between frequencies of the frequent allele B was found. At locus PGI-B, a clinal tendency from East to West in allele B and West to East at allele D was observed (Table II).

The variation of phosphoglucomutase was interpreted in principle according to Merzeau *et al*. (1989). Phosphoglocomutase was encoded by one locus (PGM-A), comprising three alleles with Rm of 112, 100 and 94 (Fig. 9). The enzyme structure is monomeric. Except of allele C that was observed only in the two border populations of Hyrcanian forests (Neka-900, Asalem-1200), allelic differences between populations were not found.

Shikimate dehydrogenase was encoded by one locus SKDH-A which possesses four alleles with Rm of 114, 100, 86 and 72 (Fig. 10). The interpretation of the polymorphism of shikimate dehydrogenase followed by Müller-Starck and Starke (1993). The enzyme structure is monomeric. Significant differences in allelic frequencies were not observed at the regional level, but differences in allele frequencies between populations were important. Frequency of allele B varied from 0.659 to 1.0 and allele C was represented from 0.0 to 0.261 (Table II).

6-phosphogluconate dehydrogenase is coded by three loci, of which locus 6PGD-A with four alleles with Rm of 110, 90 and 80 (Fig. 11). The enzyme structure is dimeric. The most differences in allelic frequencies were found at two rare alleles A, D, so that frequency of allele A was high in border populations and allele D was not observed in central populations of beech forests.

Allelic frequencies and distribution. Table II presents mean allelic frequencies in individual populations, regions and whole area. Heterogeneity of allelic frequencies among populations was revealed in 14 of the surveyed loci. That means the frequencies of the most loci are significantly different between the groups of trees sampled at different populations. Because of the modest differentiation and rare polymorphisms, small sizes of tested classes were not enough to conclude on the heterogeneity of allelic frequencies in MDH-A and PGM-A. Five loci out of the 16 found for 10 enzymes system were monomorphic in 1-8 populations (SKDH-A in one population, PGI-B in three, MDH-C and GOT-B in seven and PGI-A in eight populations). Except of loci PX-A, MDH-A and 6PGD-A that showed two frequent alleles in some populations, at each locus, one allele generally appeared more frequently than the others. Six loci (GOT-B, IDH-A, MDH-C, PGI-A, -B and PGM-A) generally showed a low degree of polymorphism and even if the number of alleles per locus in these systems was three or four, the frequency of one allele was usually high, and all other alleles were rare or completely absent from some populations. On the other hand, loci like PX-A, PX-B, MDH-A and MDH-B showed quite high polymorphism in almost all populations. The locus of PGI-A showed moderate variation in eastern and central Hyrcanian zone, but complete fixation in Asalem. However, Got-B presented complete fixation in the most population except of Asalem.

The number of alleles found at 16 loci varied from two (PX-A and MDH-C) to three (PX-B, MNR-A, IDH-A, PGI-A and PGM-A) and four (LAP-A, LAP-B, GOT-A, GOT-B, MDH-A, MDH-B, SKDH-A and 6PGD-A) and five (PGI-B) (Table II). In some loci, the number of alleles per locus differs among populations but in other cases it is constant. For example, number of alleles at locus SKDH-A was from one (in Kheirud-600) to two (in Gorgan-2000) and three (in Kheirud-600) and four (in Neka-900). However, at locus PX-A there are two alleles in all populations.

Within regions, allelic variants varied between different altitudes. There was not found any special tendency, so that the highest number of allelic variants in Gorgan and Asalem were found at optimum range of beech distribution (1200-1400 m above sea level); in Sangdeh at highest altitude (1900 m); and in Kheirud at lowest (600 m). In frequent allele of some loci, slight geographical trends could be observed. For example, frequencies of allele PGI-B/B increased and the frequency of the allele SKDH-A/B decreased from east to west.

Twenty seven of the 55 alleles studied were present in all populations, but there were several alleles identified, which occur only in some parts of the distribution range. Quite few of them are regionally specific in the narrow sense of this term, i.e. occuring exclusively in one population (GOT-B/A' in Gorgan-1400; MNR-A/C in Neka-900; MDH-A/F in Kheirud-600; and PGM-A/C in

[1]MDH-A as first locus, is completely monomorphic in all western and Central populations of European beech, that is why most authors omitted it from the evaluation. However, in Balkan populations as well as in oriental beech, this locus becomes polymorphic (Vyšnỳ, 1997).

Gorgan 600). We considered regionally specific (in a wider sense) those alleles, which occur in less than 50% of regions, provided their distribution area was more or less continuous. Following this criterion, 22 alleles from 13 loci (Table III) appear to be regionally specific. Distribution of these 22 area-specific alleles in populations varied from east to west of beech forests in Iran, that can located in some groups:

- Occurrence in East toward Center (e.g. PX-B/C)
- Occurrence in Center (e.g. Got-A/A)
- Occurrence in West toward Center (e.g. MDH-A/B)
- Occurrence in borders (e.g. PGI-A/A)
- Occurrence in regions far from sea (e.g. 6PGD-A/D)
- Presence only in one region (PGI-B/C)
- Absence only in one region (SKDH-A/A)

The mosaic distribution of some alleles (e.g. GOT-A/D) does not allow conclusion about their area specificity.

However, these alleles are generally rare (usually less than 5%), so that their "specificity" for a particular region must be regarded with a caution, since their frequencies are in most cases much lower than the probability-based limits for losing an allele during sampling. Nevertheless, they were evaluated because of their significance for explaning the phylogenetic relationships.

In this study, rare alleles where presented by allelic variants with frequencies less than 5%. In total, there were observed 30 alleles at 15 loci that their occurance varied from one population (GOT-B/A') to nine populations (IDH-A/A) (Table III). The number of rare alleles in populations varied from 3 in Gorgan-2000 to 15 in Neka 900 (as regeneration compartment) and 14 in Kheirud-600 (as natural population).

DISCUSSION

Allelic profiles. Several rare and extremely rare alleles were observed in different parts of the distribution range. As pointed out by Bergman *et al*. (1990), they play a minor role in the adaptive potential of the population, but they are of interest from the point of view of possible future environment changes.

In most cases, the number and frequencies of the observed alleles (including the rare ones) are in concordance with the findings of the other authors in oriental and European beech (Paule & Gömöry, 1997; Vyšnỳ, *et al*., 1997; Gömöry *et al*., 1999, 1998a, b).

For peroxidases, two loci were found with 2 and 3 alleles, respectively. With few exceptions, the most frequent ones were PX-A/B and PX-B/B, whereas the PX-B/C allele was quite rare. The same pattern was found in oriental and European beech woods in Europe by Barrière *et al*. (1987), Comps *et al*. (1987, 1990, 1991), Gömöry *et al*. (1992), Larsen (1995), Merzeau (1991), Thièbaut *et al*. (1986), Vyšnỳ (1997) and others.

For *Leucine aminopeptidase*, two loci were observed, with four alleles each. Two frequent alleles and two rare one (the slowest and fastest) were found. This finding were quite in contrast with other authors (Kim, 1980; Turok, 1993, 1996; Müller-Starck & Ziehe, 1991; Starke & Müller-Starck, 1992; Gömöry *et al*., 1992; Vyšnỳ, 1997) for this enzyme, which was found to be one locus and the second one (slowly migrating one) was not with enough intensively stained to allow a reliable scoring. Konnert (1995) found a fifth allele (the slowest) for LAP-A, which was extremely rare, in the European beeches from Bavarian. Despite an extensive sampling by others (Kim, 1980; Turok, 1993, 1996; Müller-Starck & Ziehe, 1991; Starke & Müller-Starck, 1992; Gömöry *et al*., 1992; Vyšnỳ, 1997), this allele has not been identified anywhere. On the other hand, Rossi *et al*. (1991) found only two alleles in LAP-A.

Two loci apparently control the variation of glutamate-oxaloacetate transaminases in beech. Although the first locus, GOT-A, has been widely used by French authors (Comps *et al*., 1990, 1991; Merzeau *et al*., 1994; Thiébaut *et al*., 1986), many authors did not interpret it (Gömöry *et al*., 1992; Vyšnỳ, 1997). In this study, four alleles were found for both loci that in all populations, in GOT-A two frequent alleles and two rare alleles, and in GOT-B one frequent alleles and three rare alleles were observed that the fastest rare allele in GOT-B has not reported anywhere else. These results are contrast with other findings, so that in GOT-B Gömöry *et al*. (1992) and Vyšnỳ (1997) found two alleles almost equally represented in the western part of the range and one extremely rare allele occurring in the East. However, Konnert (1995) found this allele also in Bavarian populations, whereas Turok (1996) reports a slower rare allele in North Rhine-Westphalia.

From seven observed alleles in menadione reductase, we observed three alleles that two of them are frequent and one is extremely rare (MNR-A/C), whereas this allele seems to be missing in France and Italy (Belleti & Lanteri, 1996; Demesure, 1991; Gömöry *et al*., 1992).

For isocitrate dehydrogenase, we interpreted one locus with three alleles that the slowest allele IDH-A/C is quite rare. This allelic profile is in concordance with the results from western Europe (Belleti & Lanteri, 1996; Demesure, 1991; Merzeau, 1991; Gömöry *et al*. 1992; Rossi *et al*. 1991; Thiébaut *et al*. 1986). Vyšnỳ (1997) found a fast migrating allele (IDH-A/A') that was presented only in one population of *Fagus orientalis* Lipsky. Konnert (1995) found a fast migrating allele also in Bavaria, however, she dose not provide the R_m value, so that this allele might not be identical with Vyšnỳ reported IDH-A/ A'.

Three loci control the variation of malate dehydrogenase in beech. The most fastly migrating zone is monomorphic in western European and in most central European beech woods, but Vyšnỳ (1997) and Gömöry *et al.* (1999) found a variation in this zone, however, only in *Fagus orientalis* Lipsky and in those Fagus sylvatica L. populations, which are situated close to the range of oriental beech. From four alleles, two of them are extremely rare. In

the second locus, MDH-B, three alleles (two of them are rare in the most populations) were found in our material. In the material from western Europe (Merzeau, 1991; Gömöry *et al*. 1992) three alleles (two of them, fast migrating, are rare) were found. However, substantially more alleles were found in the material originating from Germany. Konnert (1995) describes six alleles in this locus, only one of them migrating slowly than the predominating one, but Vyšnỳ (1997) found five alleles that three of them (MDH-B/A,/B,/C) are identical with the previously recorded ones, two slowly migrating alleles were found generally only in *Fagus orientalis* Lipsky and adjacent European beech populations. Two alleles were found in the locus MDH-C. This locus in contrast with European beech was monomorphic in most Iranian beech forests.

For phosphoglucose isomerase, we found two loci with 3 and 5 alleles, respectively. For interpretation of the faster zone we did not found any reference, whereas this locus in our material was monomorphic. In general, the second slower zone, PGI-B, was interpreted for phosphoglucose isomerase. In comparison with other findings, PGI-B has the highest variation in Iranian beech. Although, Vyšnỳ (1997) found the fastest allele (PGI-B/A') only in one population from eastern Caucasus, we observed it in three populations. In Western Europe, only two alleles were found (Rm=100 and 87) (Comps *et al*., 1987; Konnert 1995; Gömöry *et al*. 1992; Rossi *et al*. 1991; Larsen, 1996). Only in the southern and southeastern Europe the faster allele (Rm=113) begins to occur (Belleti & Lanteri, 1996; Comps *et al*., 1991). Although this allele is rare one in Iranian beech forests, Vyšnỳ (1997) recorded high frequencies of this allele in most oriental beech populations and in Crimea.

For phosphoglucomutase, one controlling locus was identified. Due to technical problems, we were not able to distinguish two alleles exhibiting major polymorphism, as reported by most authors (Demesure, 1991; Merzeau, 1991; Turok, 1993) and we pooled them. However, we found two further rare alleles, which were observed also by Vyšnỳ (1997) in most range of beech. Konnert (1995) and Löchelt and Franke (1995) found these alleles in Bavaria and Baden-Württemberg, respectively, as well. Turok (1996) found in Germany even a fifth slow migrating allele, although extremely rare.

For shikimate dehydrogenase, four alleles were found, similar to other authors; the second one is generally predominated (Turok, 1996; Löchelt & Franke, 1995; Merzeau *et al*. 1989; Rossi *et al*. 1991; Gömöry *et al*. 1992). Vyšnỳ (1997) found the fifth, the slowest allele, one that was extremely rare and occurring at low frequencies in some populations from Asia Minor.

For 6-phosphoglucocate dehydrogenase, we found four alleles that two of them (the slowest and fastest) were rare alleles.

Lack of cline variation patterns in allelic frequencies. Several studies suggest the relation between variation of allelic frequencies (in some loci) and environmental condition, showing possible adaptive role of those alleles, (Thiébaut *et al*., 1982; Comps *et al*., 1990, 1991; Gömöry *et al*. 1992a; Belletti & Lanteri, 1996).

Although enzyme genotypes and not alleles represent the enzymes, which catalyze the metabolism of a plant, usually allele frequencies rather than genotype frequencies are studied in relation to climatic adaptation.

Former investigations (Comps *et al*., 1990, 1991a, b) established relations between peroxidase loci (PX-A and PX-B) and geographical and climatic features of the populations. These authors quoted higher polymorphism at locus PX-A in area where climatic changes were intense. These authors suggested the possibility of a selection effect as they always observed the same allelic variations associated with the same climatic changes. Although within the regions analyzed in this study, there are extremes in climatic conditions due to the big differences between the highest and lowest distribution ranges from sea level (1000 – 1300 m), we could not find a high degree of polymorphism at locus PX-A even other loci associated with the climatic condition. At the enzyme loci GOT-B, MDH-B and SKDH-A, Paule *et al*. (1995) found some alleles, specific for one or several adjacent regions in southeastern Europe. In this study also there are several alleles identified, which occur only in some parts of the distribution range. Within 21 Italian populations of beech, Leonardi and Menozzi (1995) observed allelic frequencies related to altitude and longitude, but in Iran, we could not find the same trends in five studied vertical distribution range of beech.

Hyrcanian zone is ecophysiographically quite heterogeneous, but there are some clear and continuous ecological gradients (e.g., precipitation, edaphically conditions and etc.). From this point of view we cannot explain the lack of clear patterns of genetic differentiation observed in the presented material. A significant heterogeneity of allelic frequencies, but without unequivocal clines, probably results from random processes as well as the adaptation determined by a complex of environmental factors rather than by one predominating factor.

CONCLUSIONS

Considerable genetic diversity of beech forests in Iran (expected heterozygosity = 0.191) represents large adaptive potential of this species. These forests manage under the shelterwood system from 40 years ago. Selection for conservation of some genotypes is one of the most important processes in forest management practices that change the genetic variation of trees. During this process frequency of some genes and following that genetic variability and future adaptibility, would be strongly reduced. An approprate silvicultural system should reflect a sense of conservation, or a determined effort to provide future yields of goods and other values even while

harvesting or using those available at the moment. Therefore the rate and circumstances of utilizing resources at present would be guarantee adequate and continuous applies for future. Recent studies revealed that among silvicultural methods, the selection system and group selection are the best options for management of beech forests in a sustainable way (Sagheb-Talebi, 2000; Sagheb-Talebi & Schutz, 2002). In this research, high adaptive potential of some population (Neka at elevation 900 m.a.s.l. and Kheyrud at elevation 600 m.a.s.l. with 14 rare and specific area alleles) in comparison with limited adaptivity of others (such as Kheirud at elevation 1200 m.a.s.l. and Gorgan at elevation 2000 m.a.s.l. with two rare and specific area alleles) also emphasis on selection of close to nature silviculture base on potential in every region.

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