

# GENETIC STRUCTURE OF EUROPEAN BEECH (*Fagus sylvatica* L.) SEED STANDS FROM DIFFERENT FOREST SITES OF GORJANCI MOUNTAINS AS REVEALED BY ISOENZYMES

GENETSKA STRUKTURA SJEMENSKIH SASTOJINA OBIČNE BUKVE (*Fagus sylvatica* L.) S RAZLIČITIH ŠUMSKIH STANIŠTA NA GORJANCIMA/ŽUMBERKU UTVRĐENA METODOM IZOENZIMA

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## Summary

Two populations of registered European beech seed stands growing at altitudes of 273 m (Vrhovo) and 657 m (Kozarje) a.s.l. in the Gorjanci Mountains were genetically investigated at 16 polymorphic gene loci. A cline from minor to major allelic polymorphism was revealed at gene loci *Aco-B*, *Idh-A* and *6-Pgdh-A*. The Kozarje population of beech had a slightly higher genetic multiplicity, a slightly higher effective number of alleles per locus ( $v$ ), higher observed heterozygosity ( $H_o$ ) and a higher level of intrapopulation differentiation ( $\delta_r$ ). Statistically significant differences in the occurrence of alleles between populations were found at 5 of 16 loci. The average genetic distance of Gregorius (1974) was 6.1 %. European beech populations analyzed from different altitudes on Gorjanci Mountains appeared to be genetically differentiated.

KEY WORDS: European beech, genetic polymorphism, genetic differentiation, Slovenia

## Introduction

### Uvod

Slovenia is one of the most forested countries in Europe, with a total coverage of 1.184.369 ha or 58.4 % of total area (SFS 2011). Forests are well preserved, and sustainable, semi-natural and multifunctional management has been traditionally and legally incorporated into the forestry practice. In the 1990s, the genetic variation of beech populations in Slovenia and, in comparison, with its variation in Cen-

tral and South-Eastern Europe were studied using isoenzyme gene markers (Brus et al. 1999, Gömöry et al. 1999). Results have shown the existence of genetic differences between provenances of beech from the north-western part of the investigated area and provenances of beech from the eastern part of Balkan Peninsula. The obtained results further supported the hypothesis that during the ice ages European beech was present in microrefugia at the south-eastern periphery of Alps and on the territory of present-day Slovenia (Brus et al. 2000). Findings were confirmed by the

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study of Magri et al. (2006), which analysed large palaeobotanical and genetical data of common beech in Europe. The development of beech forests allowed a possibility that the European beech in the territory of present-day in Slovenia went through a genotypic specialisation that also resulted in locally adapted races or ecotypes. However, in its natural distribution area, very little is known about the patterns of population genetic variation in geographically smaller but heterogeneous areas. The results from beech provenance research in Slovenia represent great phenotypic and phenological differences between Slovenian provenances from various elevations, expositions, climatic conditions (Brinar 1971). Previous studies of genetic structure of beech population in Southeastern Europe have indicated a higher level of genetic diversity in this area (Paule 1995, Gömöry et al. 1999), the predominant ecotypic character of genetic differentiation of populations (Hazler et al. 1997, Gömöry et al. 2007, Ivanković et al. 2008, 2011, Jazbec et al. 2007), and the probability of a glacial refugium along the Slovenia and Croatia (Magri et al. 2006, Brus 2010). In order to look for a possible pattern of beech population genetic variation in geographically smaller and heterogeneous areas, two autochthonous *Fagus sylvatica* L. populations from different site conditions of Gorjanci Mountains in Slovenia were investigated by means of isoenzyme analyses.

### Investigation area Područje istraživanja

Gorjanci Mountains (syn. Žumberak) are 45–50 km long and 18–22 km wide mountain massive at the south-western edge of the Pannonian plains. The massive with two names (the Gorjanci and Žumberak, on the Slovenian and the Croatian sides, respectively) is characterized by a dynamic relief under influence of two major European geographical and climate units: the Pannonian basin on the north-east and the Dinaric Mountains on the west to south-west (Kutnar et al. 2002). At the bottom of Gorjanci Mountains, beech (*Fagus sylvatica* L.) forests with mixtures of different tree species, including *Quercus petraea* (Matt.) Liebl., *Carpinus betulus* L., *Picea abies* (L.) H. Karst., *Acer pseudoplatanus* L., *Acer campestre* L. and others, cover the major part of the forested area in the transition from the submontane belt to the lowlands. Due to their vicinity to human settlements, they have always sustained heavy anthropogenic impacts. In particular, there are many coppiced forests close to farms. Some of these sites were converted to coniferous monocultures, and many of them were even transformed into agricultural use. In contrast, the human impact is not so pronounced at higher zone of the Gorjanci Mountains, and more or less pure beech stands extend over a larger area. In this area, the share of the coppice and spruce monoculture forests are very low, and forest land-use is prevailing (Marinček and Čarni 2002).

Research plots were selected at different autochthonous beech sites of the Gorjanci Mountains, both belonging to Natura 2000 habitat type of 91K0 Illyrian *Fagus sylvatica* forests (Kutnar et al. 2011) in the hilly and the mountainous vegetation zone. Two populations of beech at Vrhovo and Kozarje were sampled in "selected" category of forest reproductive material in beech seed stands of provenance Ustraški boršt/Cerov Log at altitude of 273 m (ident. number GSO 5.0222) and provenance of Gorjanci/Kozarje at altitude of 657 m (ident. number GSO 5.0216), respectively (Kraigher et al. 2012). The Vrhovo population belongs to forest of beech and sessile oak with ivy (*Hedero-Fagetum* Košir 1994 var. geogr. *Epimedium alpinum* Košir 1979, syn. *Quercus petraeae-Fagetum* Košir 1962). The Kozarje population belongs to the Praedinaric mountain beech forest with dead nettles (*Lamio orvalae-Fagetum* (Horvat 1938) Borhidi 1963 var. geogr. *Dentaria polyphyllus* Košir 1962). The distance between sampled populations was 13 km with 384 m in altitude.

### Material and Methods Materijali i metode

#### Sampling – Uzorkovanje

At the research sites Vrhovo and Kozarje on Gorjanci Mountains, randomly were sampled 100 beech trees for genetic testing over an area of 3.5 ha. In the winter period of 2005/2006, we took a branch with dormant buds from each of the sampled trees, which were used for the extraction of enzymes. The buds were preserved until analysis in test tubes at a temperature of  $-20^{\circ}\text{C}$ . The sample was 50 adult trees at each site.

#### Analysis of isoenzymes – Analiza izoenzima

The genetic variability of the two sampled beech populations was analyzed by means of isoenzyme gene markers using starch electrophoresis as the separation method. Enzyme extraction from dormant buds, electrophoresis conditions and staining, and enzyme visualization was performed according to Konnert et al. (2004). Ten isozyme systems coded by sixteen gene loci were surveyed (*Aat* syn. *Got*, *Aco*, *Idh*, *Mdh*, *Mnr*, *Per*, *Pgi*, *Pgm*, *Skdh*, *6-Pgdh*). The genetic interpretation of banding patterns followed Müller-Starck et al. (2001). The laboratory part of the analyses were performed in the framework of the research tasks of the project Carbon dynamics in natural beech forests (L4-6232) in February 2006.

The results of the isoenzyme analyses were evaluated by the relative allele frequencies, calculated on different gene loci after diploid tree genotypes. Allelic structures in each gene loci were estimated by allelic profiles according to Finkeldey (1993). Genetic multiplicity was measured by the highest

**Table 1.** Survey of site characteristics of sampled beech populations**Tablica 1.** Prikaz stanišnih karakteristika istraživanih populacija

Population Populacija	Altitude (m) Nadmorska višina (m)	Latitude E Zemljopisna dužina I	Longitude N Zemljopisna širina S	Average annual precipitation prosječne godišnje oborine (mm)*	Average annual temperature prosječna godišnja temperatura (°C)*	Dominant soil types Tip tla
Vrhovo	273	45°48'25"	15°18'11"	1300–1400	10–12	District Cambisol
Kozarje	657	45°48'22"	15°27'33"	1400–1500	8–10	Eutric Cambisol, Rendzic Leposol

\* Reference period: 1971–2000 (ARSO 2010)

possible number of different alleles ( $M_{max}$ ) and the average number of alleles per polymorphic locus ( $A/L$ ). Genetic diversity was quantified using parameters of the observed level of heterozygosity ( $H_o$ ) and the conditional heterozygosity ( $H_c$ ; Gregorius et al. 1986), effective number of alleles per locus ( $v$ ; Gregorius 1978, 1987), hypothetical gametic multilocus diversity ( $v_{gam}$ ) and the level of genetic differentiation among individuals within a population ( $\delta_T$ ; Gregorius 1987) which, with larger samples, is the same as the share of expected heterozygosity, created by random mating or panmixia ( $H_e$ ; Nei 1973). For each polymorphic locus,  $\chi^2$  tests of deviation from the corresponding Hardy-Weinberg expected genotypic structures as well as Hardy-Weinberg heterozygosity at the level  $\alpha = 0.05$  were carried out to test whether the observed genotypic structure deviates from panmixia and whether there was a significant excess or deficiency of heterozygotes in a population. The degree of differentiation between populations was measured with  $\chi^2$  tests of homogeneity among allele frequencies for particular gene loci at the level  $\alpha = 0.05$  and genetic distances ( $d_0$ ) proposed by Gregorius (1974). All computation was performed with GSED software (Gillet 1998) for analyzing genetic structures from electrophoresis data.

## Results

### Rezultati

The results of genetic comparison of sampled beech populations at Vrhovo and Kozarje are shown in Tables 2 and 3 for 16 isozyme gene loci. Minor polymorphism in both populations was observed at nine loci: *Aat-A*, *Aco-A*, *Mdh-A*, *Mdh-B*, *Mnr-A*, *Pgi-B*, *Skdh-A*, *6-Pgdh-B*, *6-Pgdh-C* with the frequency of the major allele always higher than 75 %. Four loci (*Aat-B*, *Mdh-C*, *Per-B*, *Pgm-A*) showed a clear major polymorphism with the same predominant allele in both populations. A distinct transition between low and high levels of polymorphism in the test populations were found at three loci. In the Vrhovo population (from a lower altitude), minor polymorphism was expressed at loci *Aco-B* and *Idh-A* and major polymorphism at locus *6-Pgdh-A*. In the Kozarje population (from a higher altitude), a clear minor polymorphism was expressed at locus *6-Pgdh-A* and

clear major polymorphism at loci *Aco-B* and *Idh-A*. For example: alleles *Aco-B*<sub>2</sub>, *Idh-A*<sub>2</sub> and *6-Pgdh-A*<sub>4</sub> in the Vrhovo population reached 8 %, 12 %, 28 %, respectively, in the Kozarje population 20 %, 25 % and 11 %, respectively. A clear differentiation between the two populations was also observed at locus *6-Pgdh-B*, where the population of beech from higher altitude of the Gorjanci Mountains revealed much higher frequency of the second dominant allele *B*<sub>1</sub> (16 %) in comparison to the 6 % frequency at the lower altitude population.

**Table 2.** Relative allele frequencies in sampled beech populations Vrhovo and Kozarje on the Gorjanci Mountains presented per gene loci**Tablica 2.** Relativna učestalost alela u uzorkovanim populacijama Vrhovo i Kozarje na Gorjancima po genloku

Locus Lokus	Allele Alel	Vrhovo N = 50	Kozarje N = 50	Locus Lokus	Allele Alel	Vrhovo N = 50	Kozarje N = 50
Aat-A	1	0.030	0.050	Mnr-A	2	0.010	0.010
	2	0.970	0.950		3	0.930	0.900
Aat-B	2	0.350	0.320	4	–	0.030	0.030
	3	0.650	0.680	5	0.060	0.060	0.060
Aco-A	2	0.970	0.990	Per-B	1	0.260	0.260
	3	0.030	0.010		2	0.670	0.650
Aco-B	1	–	0.010	3	0.070	0.090	0.090
	2	0.080	0.200	Pgi-B	2	0.990	0.990
3	0.900	0.790	3		0.010	0.010	
Idh-A	4	0.020	–	Pgm-A	2	0.200	0.240
	2	0.120	0.250		3	0.800	0.760
Mdh-A	3	0.880	0.750	Skdh-A	3	0.930	0.960
	1	0.020	0.040		4	0.060	–
Mdh-B	3	0.980	0.960	5	0.010	0.040	0.040
	1	0.080	0.060	6-Pgdh-A	2	0.720	0.880
2	0.050	0.020	3		–	0.010	
Mdh-C	3	0.770	0.750	4	0.280	0.110	0.110
	4	0.100	0.170	6-Pgdh-B	1	0.060	0.160
1	0.310	0.270	2		0.940	0.840	
6-Pgdh-C	2	0.690	0.730	6-Pgdh-C	1	0.820	0.790
	3	0.020	0.010		3	0.020	0.010
				4	0.100	0.190	0.190
				5	0.060	0.010	0.010

Homozygous and heterozygous types that are much more frequent in the Vrhovo sample than in the Kozarje sample are: *Idh-A*<sub>33</sub> (80 % vs. 56 %), *Aco-B*<sub>33</sub> (80 % vs. 60 %), *6-Pgdh-B*<sub>22</sub> (90 % vs. 72 %), *Pghd-A*<sub>24</sub> (44 % vs. 18 %), *Pgdh-C*<sub>14</sub> (34 % vs. 20 %) and *Mdh-C*<sub>12</sub> (50 % vs. 38 %). Heterozygous or homozygous types much more frequent in the Kozarje sample than in the Vrhovo sample are: *Idh-A*<sub>23</sub> (38 % vs. 16 %), *Aco-A*<sub>23</sub> (36 % vs. 16 %), *6-Pgdh-B*<sub>12</sub> (24 % vs. 8 %), *Mdh-B*<sub>34</sub> (26 % vs. 12 %), *6-Pgdh-C*<sub>15</sub> (12 % vs. 2 %) and *6-Pghd-A*<sub>22</sub> (78 % vs. 50 %). Remarkable differences in allelic and genotypic frequencies were observed at gene locus *Skdh-A*. The allele *A*<sub>4</sub> and heterozygous genotype *A*<sub>34</sub> are stand-dependent. It was found at frequencies of 6 % and 12 % in the lower elevated Vrhovo stand, respectively, but not in the higher elevated Kozarje stand.

At 16 polymorphic enzyme gene loci, 43 allelic variants ( $M_{max}$ ) were found in two populations, which correspond to 2.69 alleles per locus (Table 2). The mean numbers of alleles per locus ( $A/L$ ) were 2.50 at Vrhovo and 2.56 at Kozarje population, which is equivalent to a ratio of 1:1.02. The average values of the effective number of alleles per locus ( $v$ ) range from 1.31 (Vrhovo) to 1.34 (Kozarje) and correspond to a ratio of 1:1.05. The numbers of genetic variants of the 16 loci gametic types that the group of 50 sample trees

in the populations ( $v_{gam}$ ) could have produced ranges from 104 (Vrhovo) to 157 (Kozarje) and corresponds to a ratio of 1:1.51. Although the calculated values ( $v_{gam}$ ) have only an indicative character, this comparison suggests greater potential of the group of beech trees at the Kozarje site than that of the group of beech trees at the Vrhovo site for the production of genetically different gametes, which will be subject to genetic variation in new generations.

The average value of observed heterozygosities ( $H_o$ ) for the 16 loci gene pool are vary between 23.5 % in the group of trees from Vrhovo to 25.1 % in the Kozarje group of trees, which corresponds to a ratio of 1:1.06. As shown in Table 3, the  $H_o$  values are not uniform. The level of observed heterozygosity explicitly differs between the test populations from Vrhovo and Kozarje at 5 loci namely at *Aco-B* (20 % vs. 38 %), *Idh-A* (16 % vs. 38 %), *Mdh-C* (50 % vs. 38 %), *6-Pgdh-A* (44 % vs. 20 %) and *6-Pgdh-B* (8 % vs. 24 %). The mean of  $H_c$  values for two populations is 84.1 %. As  $H_c$  values are independent of the underlying allele frequencies, the calculated mean indicated to a slightly lack of heterozygotes as compared to the maximum attainable value which is equal to 100 %. The average levels of genetic (allele) differentiation  $\delta_T$  in the sampled populations of beech range from 23.7 % (Vrhovo) to 25.8 % (Kozarje), which cor-

**Table 3.** Genetic variability parameters for 16 polymorphic loci in the sampled beech populations, Vrhovo "Vrh" and Kozarje "Koz" on the Gorjanci Mountains. Average number of alleles per locus ( $A/L$ ), allelic diversity ( $v$ ), intrapopulational differentiation ( $\delta_T$ ), observed and conditional heterozygosity ( $H_o$ ,  $H_c$ ), allelic genetic distance ( $d_o$ ) and value of  $\chi^2$  test of the homogeneity of genetic structures by loci and significance level  $\alpha = 0.05$  (\*),  $\alpha = 0.01$  (\*\*)

**Tablica 3.** Genetski parametri varijabilnosti za 16 polimorfnih lokusa, Vrhovo "Vrh" i Kozarje "Koz" na Gorjancima. Prosječni broj alela po lokusu ( $A/L$ ), raznolikost alela ( $v$ ), unutarpopulacijska diferencijacija ( $\delta_T$ ), promatrana i uvjetna heterozigotnost ( $H_o$ ,  $H_c$ ), alelna genetska udaljenost ( $d_o$ ) i vrijednost  $\chi^2$  testa homogenosti genetskih struktura po lokusu na nivou značajnosti  $\alpha = 0.05$  (\*),  $\alpha = 0.01$  (\*\*)

Locus Lokus	$A/L$		$v$		$\delta_T$ (%)		$H_o$ (%)		$H_c$ (%)		$d_o$ (%)	$\chi^2$ value $\chi^2$ vrijednost
	Vrh	Koz	Vrh	Koz	Vrh	Koz	Vrh	Koz	Vrh	Koz		
Aat-A	2	2	1.06	1.11	5.9	9.6	6.0	6.0	100	60.0	2.0	n.s.
Aat-B	2	2	1.84	1.77	46.0	44.0	42.0	44.0	60.0	68.8	3.0	n.s.
Aco-A	2	2	1.06	1.02	5.9	2.0	6.0	2.0	100	100	2.0	n.s.
Aco-B	3	3	1.22	1.51	18.5	33.9	20.0	38.0	100	90.5	13.0	8.859 *
Idh-A	2	2	1.27	1.60	21.3	37.9	16.0	38.0	66.7	76.0	13.0	4.775 *
Mdh-A	2	2	1.04	1.08	4.0	7.8	4.0	8.0	100	100	2.0	n.s.
Mdh-B	4	4	1.64	1.68	39.2	40.9	34.0	42.0	73.9	84.0	7.0	n.s.
Mdh-C	2	2	1.75	1.65	43.2	39.8	50.0	38.0	80.6	70.4	4.0	n.s.
Mnr-A	3	4	1.15	1.23	13.3	18.7	14.0	16.0	100	80.0	3.0	n.s.
Per-B	3	3	1.92	2.01	48.3	50.7	46.0	48.0	69.7	68.6	2.0	n.s.
Pgi-B	2	2	1.02	1.02	2.0	2.0	2.0	2.0	100	100	0.0	n.s.
Pgm-A	2	2	1.47	1.57	32.3	36.8	32.0	32.0	80.0	66.7	4.0	n.s.
Skdh-A	3	2	1.15	1.08	13.3	7.8	14.0	8.0	100	100	6.0	7.848 *
6-Pgdh-A	2	3	1.68	1.27	40.7	21.6	44.0	20.0	78.6	83.3	17.0	10.010 **
6-Pgdh-B	2	2	1.13	1.37	11.4	27.2	8.0	24.0	66.7	75.0	10.0	4.137 *
6-Pgdh-C	4	4	1.51	1.46	34.3	31.7	38.0	36.0	90.5	100	9.0	n.s.
Gene pool	2.50	2.56	1.31	1.34	23.7	25.8	23.5	25.1	85.5	82.7	6.1	

responds to a ratio of 1:1.09 and indicates larger genetic differentiation among individuals at the higher elevated site than among individuals at the lower elevated site. No significant deviations of observed genotypes from expected genotypes were found at 15 of the total of 16 analysed gene loci when applying  $\chi^2$  test ( $\alpha = 0.05$ ). The only exception was detected at the locus *6-Pgdh-B* at Vrhovo stand where no statistically significant excess of homozygotes over Hardy-Weinberg expectations was confirmed. The observed frequencies of genotypes in both populations of beech agree with Hardy-Weinberg expectations under random mating at the level of risk  $\alpha = 0.05$ .

The degree of genetic differentiation between populations was analysed with  $\chi^2$  tests of homogeneity among allele frequencies for particular gene loci at the level  $\alpha = 0.05$  and  $\alpha = 0.01$ . The results have shown significant differences in allelic (genetic) structures between sampled populations of beech at 5 out of 16 analysed gene loci (Table 3). The sampled populations of beech did not share 17 % of their alleles at locus *6-Pgdh-A* and 13 % of their alleles at loci *Aco-B* and *Idh-A* as well as 30 % of their genotypes at locus *6-Pgdh-A*, 24 % of their genotypes at locus *Idh-A* and 12 % of their genotypes at locus *Skdh-A* (genotypes values not introduced in Table 3). The mean value of Gregorius (1974) allelic and genotypic genetic distances ( $d_0$ ) amounts to 6.1 % and 11.4 %, respectively, shows that the genetic differences of two local sampled beech populations from different sites on the Gorjanci Mountains are comparatively high.

## Discussion Rasprava

Two very specific beech forests of Gorjanci Mountains on significantly different forest sites, in different ecological conditions and with different forest-management history were selected. The Vrhovo population was sampled in the hilly area and belongs to site of *Hedero-Fagetum*. The Kozarje population was sampled in the mountainous zone and belongs to site *Lamio orvalae-Fagetum*. The results of the genetic study refer to the genotypes of 100 adult trees in two populations at 16 polymorphic co-dominant inherited isoenzyme loci.

The observed genotypes were consistent with the expectations under random mating, and indicated that both sampled populations are randomly mating. At 16 polymorphic enzyme gene loci, 43 allelic variants were found in total, which correspond to 2.69 alleles per locus. Beech from the sampled local stands in Gorjanci Mountains seems to have a similar number of alleles per locus as the overall value for beech in Europe, according to Müller-Starck et al. (1992). This value also corresponds well with  $A/L = 2.8$  obtained for Slovenia (Brus et al. 1999) and was higher than was reported for north-western Italy  $A/L = 2.12$  (Belletti and Lan-

teri 1996) and Germany  $A/L = 2.51$  (Konnert 1995). The average  $A/L$  values observed in the sampled beech populations on the Gorjanci Mountains (2.50 and 2.56) show higher values than was determined for 13 beech populations in Slovenia (Brus et al. 1999) where  $A/L$  values varied in range from 2.08 to 2.42 and were close to the highest value obtained in the population of Luknja from Prečna close to Novo Mesto. Beech from Luknja originated from mainly southern-orientated steep limestone walls close to the Palaeolithic site from which beech charcoal from the late Pleistocene has been excavated (Osole 1990); it may represent a relict population that persisted in a sheltered micro-refugium where it was able to survive the last glaciations (Brus et al. 1999). Therefore it seems that no restriction in genetic multiplicity was determined in beech populations at Gorjanci Mountains.

The populations of European beech at Vrhovo and Kozarje which belong to sites of *Hedero-Fagetum* in the hilly area and *Lamio orvalae-Fagetum* in the mountainous zone, respectively are autochthonous. The higher altitude stand at Kozarje showed higher genetic diversity than the lower altitude stand at Vrhovo. Differences are evident in the frequencies of some alleles and genotypes, observed heterozygosity and particularly in the hypothetical gametic multilocus diversities. The frequency of the alleles *6-Pgdh-B<sub>1</sub>*, *Aco-B<sub>2</sub>* and *Idh-A<sub>2</sub>* at the higher elevated Kozarje stand were more than twice as high as of the lower elevated stand. The frequency of the allele *6-Pgdh-A<sub>4</sub>* was about three times higher in the (lower elevated) Vrhovo stand than in the (higher elevated) Kozarje stand. At locus *Skdh-A*, allele ( $A_4$ ) was observed only in the Vrhovo stand as a heterozygote genotype *Skdh-A<sub>34</sub>* (12 %). The frequency of the genotype *Skdh-A<sub>34</sub>* significantly changed with altitude. They might be naturally distributed in lower elevated stands but not present in the upper elevated stands and might be a confirmation of the existence of a discontinuity point between these two local populations. Further studies in forests of Gorjanci Mountains should be preformed to prove the hypothesis of the interaction between disruptive natural selection and gene flow in development of among population genetic differentiation.

Our findings confirm findings from comparable studies of beech populations in Germany, related to climatic adaptation with an altitudinal transect from the base of the mountain to its top. In an inventory study on the isozyme genetic variation of beech stands growing at elevations between 350 m and 1250 m a.s.l. in mountain Schauinsland, southwest Germany, allele frequencies of different enzyme systems at gene loci *Aco-B*, *Idh-A*, *Pgm-A* were found to be changed (Löchelt and Franke 1995). Climate adaptation and man-made selection were considered to be causes. A detailed investigation of six autochthonous beech stands growing at elevations between 150 m and 660 m a.s.l. in the same

mountain slope on Vogelsberg in central Germany showed that some genotypes are more frequent at the higher elevated stands than in lower elevated stands and vice versa (Sander et al. 2000).

Beech populations sampled from different sites on Gorjanci Mountains appeared to be genetically different. Genetic differentiation, as expressed by the proportion of alleles not shared between lower and higher elevated population was relatively high for the small geographical region studied ( $d_0 = 6.1\%$ ). For example, the genetic distances of beech populations in Germany (Bavaria) studied using the same analyzing method were reported to range between  $d_0 = 2.6\%$  and  $d_0 = 10.9\%$  (Konnert and Henkel 1997). The observed clines at gene loci *Aco-B*, *Idh-A*, *6-Pgdh-A* and the allelic distances from 13% to 17% between sampled stands in Gorjanci Mountains could indicate that gene flow between sampled stands is limited or that specific site conditions in sampled locations demonstrate a possible effect of environmental adaptation to this gene loci.

The structure of the obtained genetic information is the result of the sampled stands' high adaptation and specialization to specific homogenous environmental conditions in the investigated ecosystems and historical influences of anthropogenic impacts. Under constant environmental conditions, the higher genetic variability within populations means a higher fitness of the population. This situation remains optimal as long as the site conditions are not affected by climate changes and manmade selection to that extent that a process of adaptation to new site conditions is interrupted.

As reported Mátyás et al. (2010) for the beech forests at the xeric limit in Southeast Europe (case Hungary), the very similar pattern might be expected at Gorjanci Mountains where low-elevation beech forests (*Hedero-Fagetum* forests) might be threatened by the warming more than higher-elevation beech forests (*Lamio orvalae-Fagetum* forests). In more preserved, less degraded sites of *Hedero-Fagetum* forest, beech is well developed and has high competitive potential against other tree species. However, at more degraded sites of this forest community, where intensive human influence took place in the past, beech is not so competitive and its growth is less intensive, and the quality of beech stems is not so high. In such degraded sites of *Hedero-Fagetum*, some species of earlier forest development stages, like *Quercus petraea*, *Q. cerris* and *Carpinus betulus*, are more competitive and more frequent. In such human-degraded forms of this forest community, the coppice forest is more common. In such forest conditions, the lability of forest sites with the dominate Luvisol to moderately dystric Cambisol might have been aggravated by inappropriate forest management in the past, such as litter-raking, and intensive use without any management concept. Due to old

settlements at the bottom of Gorjanci Mountains, the forests in the hilly zone have been under human impact. Thus, the relative high share of coppice, litter-raking forest sites planted by non-native tree species and other degraded forests in this area reflect the negative human impacts in the past. The mountain beech forests of *Lamio orvalae-Fagetum* at Gorjanci Mountains, compared to more degraded sites of *Hedero-Fagetum* forest, have a stable biocenotic structure. Even after larger clearings, all development stages of the forests can be renewed by beech trees. Due to their more remote and isolated location, the sites of these forests were not transformed into agricultural land (Marinček and Čarni 2002). Beside the genetic potential of beech, the future development of these forests is related to the past and future land use, and to impact of climate changes (Kutnar and Kobler 2011) and other degradation processes (Kutnar et al. 2011). As the ability of forest ecosystems is strongly related to the existence of intrapopulation genetic diversity, even in mixed natural stands, the stability is threatened as soon as the genetic diversity of one of the involved species is threatened; there is a great need for investigations of the genetic basis of forest ecosystems in geographically smaller but heterogeneous areas.

## Conclusions

### Zaključci

The sampled populations of beech from Vrhovo and Kozarje sites are autochthonous. The observed genotypes were consistent with the expectations under random mating and no restriction in genetic multiplicity was determined. The Kozarje population, from the higher mountainous zone, showed higher genetic diversity than the Vrhovo population from the hilly area. Results of our study verify the existence of genetic differentiation between two local beech populations in Gorjanci Mountains associated with variation of significantly different forest sites, different ecological conditions and different forest-management history. Further studies should be preformed to prove the hypothesis of the interaction between disruptive natural selection and gene flow in development of among population genetic differentiation. Attention to the transfer of beech reproductive material from higher to lower elevations of the Gorjanci Mountains is recommended.

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## Sažetak

Slovenija sa svojih 58,4 % pokrovnosti teritorija šumama, odnosno s ukupno 1.184.369 ha (Vir: Zavod za gozdove Slovenije 2012) spada među šumovitije zemlje Europe. Istraživanja genetske varijabilnosti populacija obične bukve u Sloveniji i njihovo uspoređivanje s populacijama jugoistočne i srednje Europe uporabom izoenzimskih genskih markera, započela su 90-tih godina (Brus i sur. 1999, Gömöry i sur. 1999). Rezultati ukazuju na postojanje diferencijacije između sjeverozapadnih provenijencija istraživanog područja i provenijencija istočnog dijela balkanskog poluotoka.

Kako bi se istražila moguća genetska varijabilnost obične bukve sa geografski manjeg i heterogenog područja, analizom izoenzima uspoređivane su dvije populacije sa dva različita položaja planine Gorjanci (na Hrv. strani Žumberak). Za istraživano područje odabrana su dva značajno različita staništa bukve, jedno u brdskoj i drugo u planinskoj vegetacijskoj zoni. Obje autohtone sastojine i Vrhovo (*Hedero-Fagetum*) i Kozarje (*Lamio orvalae-Fagetum*) su šumski sjemenski objekti i prema kategoriji šumskog reprodukcijskog materijala sjemenske sastojine, selekcionirano sjeme, provenijencije Ustraški boršt/Cerov Log na nadmorskoj visini 273 metara i provenijencije Gorjanci/Kozarje na nadmorskoj visini od 657 metara. Udaljenost između dviju sastojina je 13 km, a razlika u nadmorskoj visini iznosi 384 m (Tablica 1).

Genetičko istraživanje varijabilnosti dviju populacija provedeno je pomoću 10 izoenzimskih sustava (*Aat*, *Aco*, *Idh*, *Mdh*, *Mnr*, *Per*, *Pgi*, *Pgm*, *Skdh*, *6-Pgdh*) koji kodiraju za 16 genskih lokusa. Rezultati analiza izoenzima ocijenjeni su prema relativnoj frekvenciji alela i genotipova. Obrada podataka napravljena je sa GSED software (programski paket) za analizu genetske strukture iz podataka dobivenih elektroforezom (Gillet 1998). Rezultati genetskog istraživanja (Tablica 2) odnose se na genotipove 100 stabala na 16 polimorfnih co-dominantnih nasljednih izoenzimskih lokusa u dvije populacije.

Populacija Kozarje, sjemenska sastojina na višoj nadmorskoj visini, iz planinskog područja, pokazuje veću genetičku varijabilnost od populacije Vrhovo, odnosno populacije sa brdskog područja. Razlikovnost je posebice vidljiva u frekvencijama pojedinih alela i genotipova, promatranom heterozigotnosti te osobito u hipotetskoj gametskoj multilokusnoj razlikovnosti.

Učestalost (frekvencija) alela *Aco-B<sub>2</sub>*, *Idh-A<sub>2</sub>* and *6-Pgdh-B<sub>1</sub>* u sastojini Kozarje dvostruko je veća od sastojine na nižoj nadmorskoj visini. Frekvencija alela *6-Pgdh-A<sub>4</sub>* je približno tri puta veća u nižoj sastojini (Vrhovo) od sastojine Kozarje. Na lokusu *Skdh*, alel (*A<sub>4</sub>*) zabilježena je samo u sastojini Vrhovo kao heterozigotan genotip *Skdh-A<sub>34</sub>* (12 %). Statistički značajna odstupanja u učestalosti alela između populacija pronađena je na 5 od 16 lokusa (Tablica 3).

Promatrana klima na genskim lokusima *Aco-B*, *Idh-A*, *6-Pgdh-A* i ukupna prosječna genetska udaljenost prema Gregorius (1974) između uzorkovanih lokalnih bukavih populacija na planini Gorjanci je relativno visoka za promatrano (istraživano) malo geografsko područje. Istraživane sjemenske sastojine obične bukve (sjemenske sastojine obične bukve na različitim lokacijama, različitim nadmorskim visinama) uzorkovane u ovim istraživanjima ukazuju na genetičku razlikovnost.

Iako planinske bukove šume *Lamio orvalae-Fagetum* imaju stabilniju biocenotičku strukturu u usporedbi s manje očuvanom i više degradiranom bukavom sastojinom *Hedero-Fagetum*, preporuča se pozornost prilikom prijenosa šumskog reprodukcijskog materijala iz sjemenskih sastojina obične bukve od viših prema nižim nadmorskim visinama u planinama Gorjanci.

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KLJUČNE RIJEČI: obična bukva, genetski polimorfizam, genetska diferencijacija, Slovenija