

Genetic Variability of two *Fagus sylvatica* (L.) Populations in the South-Western Edge of the Pannonian Plain¹

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Abstract – Two beech populations at altitudes of 273 m (Vrhovo) and 657 m (Kozarje), growing on significantly different forest site, in different ecological conditions and with different forest-management history were selected on Gorjanci Mountains and genetically investigated by means of isoenzyme analysis. A cline from minor to major allelic polymorphism was revealed at gene loci Aco-B, Idh-A and 6-Pgdh-A. At locus Skdh-A, allele A₄ was observed only at the lower altitude population Vrhovo as a heterozygote genotype Skdh-A₃₄ (12%). The Kozarje population of beech had a slightly higher genetic multiplicity, a slightly higher effective number of alleles per locus, higher observed heterozygosity and a higher level of intrapopulation differentiation. 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. The results presented are also discussed in the light of the climate change impact on forests.

European beech / isoenzyme / genetic polymorphism / genetic differentiation / forest site / Slovenia

Kivonat – Két bükk populáció genetikai változatossága a Kárpát medence délnyugati peremén. Két jelentősen eltérő termőhelyen tenyésztő bükk populációt izoenzim analízis segítségével genetikailag elemeztünk (Vrhovo, 273 m és Kozarje 657 m). A Gorjánai hegyekben tenyésztő populációk ökológiai feltételei és erdőművelési múltja különbözök. Az Aco-B, Idh-A and 6-Pgdh-A lokuszokon az allél polimorfizmus klínje olt megfigyelhető. Skdh-A lokuszon az A₄ allél csak az alacsonyabb fekvésű populációban (Vrhovo) volt kimutatható, mint Skdh-A₃₄ heterozigóta genotípus. A Kozarje-i bükkös genetikai változatossága kissé nagyobb volt, magasabb effektív lokuszonkénti allélszámmal, magasabb heterozigozissal és magasabb populáción belüli differenciálódással. Az allélek összetétele a 16 génhely közül 5 esetben szignifikáns eltérést mutatott. Az átlagos Gregorius-féle genetikai távolság 6.1% volt. A különböző tengerszint feletti magasságban tenyésztő populációk genetikailag eltérőnek bizonyultak. A tanulmány az eredményeket a klímaváltozással összefüggésben is elemzi.

közöségés bükk / izoenzimek / genetikai polimorfizmus / genetikai differenciáltság / Termőhelyi különbségek / Szlovénia

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1 INTRODUCTION

Slovenia is one of the most forested countries in Europe, with a total coverage of 1.184.369 ha or 58.45 of total area (SFS 2012). Forests are well preserved, and sustainable, semi-natural and multifunctional management has been traditionally and legally incorporated into the forestry practice. After the primary succession in the postglacial period, the larger part of the Slovenian territory was overgrown by forests, above all by beech and fir-beech forests (Šercelj 1996). According to Perko (2007), 70% of forests in Slovenia grow on potential beech (44%), fir-beech (15%) or beech-oak (11%) sites. European beech is autochthonous, dominant and the most economically and ecologically important tree species in Slovenia. It occurs on all terrain positions and slope orientations on calcareous, silicate and mixed calcareous-silicate bedrocks (Urbančič et al. 2005), from hills (150 m a.s.l.) to the subalpine belt (1650 m a.s.l.) (Daksobler 2008).

In the 1990s, the genetic variation of beech populations in Slovenia and, in comparison, with its variation in Central and Southeast Europe were studied using isoenzyme gene markers (Paule et al. 1995, Brus 1999, Brus et al. 1999, Gömöry et al. 1999). Results of these studies have shown the existence of genetic differences between beech populations from the north-western part and those 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, Brus 2010). Findings were confirmed by the study of Magri et al. (2006), which analysed large palaeobotanical and genetical data of common beech in Europe. The territory of present-day Slovenia was one of the main source areas for the post-glacial development of beech and supposedly the most important glacial refugia for its re-colonisation 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.

As genetic diversity is a fundamental and critical part of biodiversity the results of the genetic variation constituted a professional basis for the conservation of natural genetic variability *in-situ*. The objective is to maintain continuous evolution of a tree population. Natural regeneration of adult beech stands in Southeast Europe enables a sustainable conservation of gene resources. The decision for the choice of gene resources and form of their protection should be based on all available information, including that which can be obtained from genetic inventories on isoenzymes. In general it has been found that it is more worth to designate for conservation relatively large units than many smaller units within gene conservation practices (Paule 1995). Such gene reserve forests should cover at least 100 ha in order to contain sufficient genetic variability. But for small, locally adapted populations it may be better to establish a large number of smaller reserves (von Wühlisch 2008). However, in its natural distribution area, very little is known about the patterns of population genetic variation in geographically smaller but heterogeneous areas.

Genetic study by Comps et al. (1991) based on beech populations from Croatia originated from different climatic regions, soils and forest communities has shown differences between highland and lowland populations within the Mediterranean region as well as between populations belonging to the forest community *Seslerio-Fagetum* on carbonate soils and other forest communities. The results from beech provenance trails represent great phenotypic and phenological differences among Slovenian provenances from various elevations, expositions, climatic conditions (Brinar 1971, Sittler 1981, Robson et al. 2011).

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 were selected on Gorjanci Mountains (southeast Slovenia) to avoid any difference in population history and genetically analysed by isoenzymes gene markers.

2 STUDY AREA

Gorjanci Mountains are 45–50 km long and 18–22 km wide mountain massive at the south-western edge of the Panonnian plains. The massive is characterized by a dynamic relief under influence of two major European geographical and climate units: the Panonnian 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 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 – Čarni 2002).

Research plots were selected at different autochthonous beech sites of 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* var. geogr. *Epimedium alpinum*, syn. *Quercus petraeae-Fagetum*). The Kozarje population belongs to the Praedinaric mountain beech forest with dead nettles (*Lamio orvalae-Fagetum* var. geogr. *Dentaria polyphyllus*).

Table 1. Survey of site characteristics of sampled beech populations on Gorjanci Mountains

Population	Altitude	Latitude E	Longitude N	Average annual precipitation *	Average annual temperature *	Dominant soil types
	(m)			(mm)	(°C)	
Vrhovo	273	45°48'25"	15°18'11"	1300–1400	10–12	Distric Cambisol
Kozarje	657	45°48'22"	15°27'33"	1400–1500	8–10	Eutric Cambisol, Rendzic Lepsol

* Reference period: 1971–2000 (ARSO 2010)

The research site at Vrhovo is overgrown by *Fagus sylvatica* L. (84%), *Quercus petraea* (Matt.) Liebl. (9%), *Picea abies* (L.) H. Karst. (3%), *Acer pseudoplatanus* L. (3%) and *Carpinus betulus* L. (1%) with an estimated stand growing stock of 205 m³ ha and increment of 5.6 m³ ha yr, and at Kozarje by *Fagus sylvatica* L. (90%), *Acer pseudoplatanus* L. (4%), *Prunus avium* L. (4%), *Carpinus betulus* L. (1%) and other broadleaves (1%) with a stand growing stock of 310 m³ ha and increment of 9,7 m³ ha yr (Simončič et al. 2007). The distance between sampled beech populations was 13 km with 384 m in altitude.

3 MATERIAL AND METHODS

3.1 Sampling

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 $-20\text{ }^{\circ}\text{C}$. The sample size was 50 adult trees at each site.

3.2 Analysis of isoenzymes

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 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 laboratory work was done at the Bayerische Amt für forstliche Saat - und Pflanzenzucht in Teisendorf, Germany.

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 four allelic profiles according to Finkeldey (1993). Genetic multiplicity was measured by the highest 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 (δ_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, χ^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 χ^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.

4 RESULTS

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 eight loci: Aat-A, Aco-A, Mdh-A, Mnr-A, Pgi-B, Skdh-A, 6-Pgdh-B, 6-Pgdh-C with the frequency of the major allele always higher than 80%. Four loci (Aat-B, Mdh-C, Per-B, Pgm-A) showed a clear major polymorphism with the same predominant allele in both populations. On locus Mdh-B the frequencies of the major allele were from 75% to 77%,

with frequencies of the second predominant allele from 10% to 17%. 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-B₂, Idh-A₂ and 6-Pgdh-A₄ 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 from higher altitude of the mountain ridge revealed much higher frequency of the second dominant allele B₁ (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 Gorjanci Mountains presented per gene loci

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

As enzyme genotypes represent the enzymes that catalyse the metabolism of plants, the genotypes of trees in sampled populations were also studied. Homozygous and heterozygous types that are much more frequent in the Vrhovo sample than in the Kozarje sample are: Idh-A₃₃ (80% vs. 56%), Aco-B₃₃ (80% vs. 60%), 6-Pgdh-B₂₂ (90% vs. 72%), Pghd-A₂₄ (44% vs. 18%), Pgdh-C₁₄ (34% vs. 20%) and Mdh-C₁₂ (50% vs. 38%). Heterozygous or homozygous types much more frequent in the Kozarje sample than in the Vrhovo sample are: Idh-A₂₃ (38% vs. 16%), Aco-A₂₃ (36% vs. 16%), 6-Pgdh-B₁₂ (24% vs. 8%), Mdh-B₃₄ (26% vs. 12%), 6-Pgdh-C₁₅ (12% vs. 2%) and 6-Pghd-A₂₂ (78% vs. 50%). Remarkable differences in allelic and genotypic frequencies were observed at gene locus Skdh-A. The allele A₄ and

heterozygous genotype A₃₄ are stand-dependent. It was found at frequencies of 6% and 125 in the lower elevated Vrhovo population, respectively, but not in the higher elevated Kozarje population.

On the study area, we observed 43 different alleles (M_{\max}) which correspond to 2.69 alleles per polymorphic locus in both sampled populations (Table 2). The mean numbers of alleles per locus (A/L) were 2.50 at Vrhovo and 2.56 at Kozarje population (Table 3), which is equivalent to a ratio of 1:1.02. The mean 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.

Table 3. Genetic variability parameters for 16 polymorphic loci in the sampled beech populations, Vrhovo "Vrh" and Kozarje "Koz" on Gorjanci Mountains. Average number of alleles per locus (A/L), allelic diversity (v), intrapopulation differentiation (δ_T), observed and conditional heterozygosity (H_o , H_c), allelic genetic distance (d_0) and value of χ^2 test of the homogeneity of genetic structures by loci and significance level $\alpha = 0.05$ (*), $\alpha = 0.01$ (**)

Locus	A/L		v		δ_T (%)		H_o (%)		H_c (%)		d_0 (%)	χ^2 value
	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	

The average values of observed heterozygosities (H_o) for the 16 loci gene pool varied between 23.55 in Vrhovo sampled population to 25.1% in the Kozarje sampled population, 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 value 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 δ_T in the sampled populations of beech range from 23.7% (Vrhovo) to 25.8% (Kozarje), which corresponds 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 χ^2 test ($\alpha = 0.05$). The only exception was detected at the locus 6-Pgdh-B at Vrhovo population 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 χ^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 Gorjanci Mountains are comparatively high.

5 DISCUSSION AND CONCLUSIONS

Two very specific beech populations on Gorjanci Mountains, growing 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*. We have analyzed 50 trees per sample to capture the most of the genetic variation present at the different sites. If population history is corrected for (as we can guess it is here as they are from the same Mountain range), the difference in allele frequencies obtained in two sampled populations from different altitudes may better be explained by selection and adaptation rather than by population structure.

The observed genotypes of the two beech populations on Gorjanci Mountains 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 populations on 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 – Lanteri 1996) and Germany $A/L = 2.51$ (Konnert 1995). The average A/L values observed in the sampled beech populations on 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 population at Kozarje showed higher genetic diversity than the lower altitude population at Vrhovo. Differences are evident in the frequencies of some alleles and genotypes, observed heterogosity and particularly in the hypothetical gametic multilocus diversities. The frequency of the alleles 6-Pgdh-B₁, Aco-B₂ and Idh-A₂ at the higher elevated Kozarje population were more than twice as high as of the lower elevated stand. The frequency of the allele 6-Pgdh-A₄ was about three times higher in the (lower elevated) Vrhovo population than in the (higher elevated) Kozarje population. At locus Skdh-A, allele (A₄) was observed only in the Vrhovo population as a heterozygote genotype Skdh-A₃₄ (12%). The frequency of the genotype Skdh-A₃₄ significantly changed with altitude.

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 the Black Forest (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 – Franke 1995). Climate adaptation and manmade 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 – 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 populations on Gorjanci Mountains could indicate that gene flow between sampled populations is limited or that specific site conditions in sampled locations demonstrate a possible effect of environmental adaptation to this gene loci.

Although the obtained results do not allow us to infer any certain conclusions on possible adaptive role of the enzyme systems under analysis, also because of only two populations studied (one in each ecological conditions) and possible samplings mistakes on account of the large size of analysed samples, they verify the existence of genetic differentiation between two local beech populations on Gorjanci Mountains associated with variation of significantly different forest sites, different ecological conditions and different forest-management history. As the obtained results also confirm the similar patterns or correlations of the types mentioned in German studies focused in different ecological conditions in altitude gradient and in different parts of the beech distribution range (Löchelt – Franke 1995, Sander et al. 2000), the unknown selective pressure would have been considered as the causal agent for differentiation between European beech populations. However, to

prove the hypothesis of the interaction between disruptive natural selection and gene flow in development of among population genetic differentiation of beech further studies should be preformed.

The structure of the obtained genetic information may be the result of the sampled stands' high adaptations and specializations 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. Regarding the climate change impact on forests, the different studies predict the decrease of beech based on climate change scenarios in the future in Europe (Brzeziecki et al. 1995, Fotelli et al. 2002, Geßler et al. 2007, Mátyás et al. 2010, Kutnar – Kobler 2011).

For the Slovenian territory, the reduction of European beech sites was forecasted (Kutnar – Kobler 2011). In Central Europe (including Slovenia), beech forests are likely to be threatened because of beech's sensitivity to low water availability (Ellenberg 1996) and longer drought periods (Fotelli et al. 2002); and physiological performance, growth and the competitive ability of beech may be adversely affected by such changing climate conditions (Peuke et al. 2002, Geßler et al. 2007). Moreover, in Slovenia, the situation may be aggravated by the fact that the area of distribution of beech forests includes many sites with shallow limestone- and dolomite-derived soils with low water storage capacity. Beside different abiotic factors, the existence of beech is threatened by harmful pests and new tree diseases caused by different pathogen organisms (Ogris et al. 2008).

For Slovenia, the GIS models showed that under warmer conditions the shift of vegetation belts upwards could be expected (Kutnar – Kobler 2011). This means that *Fagus*-dominated communities in the submontane belt might eventually be replaced by oak-hornbeam communities, and the shift of tree-line to a higher elevation is predicted. 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 area 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 dystic 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, 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.

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. Although the results of our study have to be confirmed by further genetic studies, attention to the transfer of beech reproductive material from higher to lower elevations of Gorjanci Mountains is recommended.

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REFERENCES

- ARSO (2010): Environmental Atlas of Slovenia. Ministry of Agriculture and Environment RS, Slovenian Environment Agency. Ljubljana, Slovenia.
URL: http://gis.arso.gov.si/atlasokolja/profile.aspx?id=Atlas_Okolja_AXL@Arso
- BELLETTI, P. – LANTERI, S. (1996): Allozyme variation among European beech (*Fagus sylvatica* L.) stands in Piedmont, North-Western Italy. *Silvae Genetica*, 45: 33–37.
- BRINAR, M. (1971): O ekološki in dedni pogojenosti razhajanja nekaterih morfoloških, fenoloških in anatomskih značilnosti naše bukve. [The ecological and genetic divergences of some morphological, phenological and anatomical characteristics of our beech]. *Zbornik gozdarstva in lesarstva*, 10: 5–64 (in Slovenian with German abstract)
- BRUS, R. (1999): Genetska variabilnost bukve (*Fagus sylvatica* L.) v Sloveniji in primerjava z njeno variabilnostjo v srednji in jugovzhodni Evropi. [Genetic variation of the beech (*Fagus sylvatica* L.) in Slovenia and comparison with its variation in central and southeastern Europe]. Dissertation, Biotehniška fakulteta, Ljubljana, 130 p. (in Slovenian with English abstract)
- BRUS, R. (2010): Growing evidence for the existence of glacial refugia of European beech (*Fagus sylvatica* L.) in the south-eastern Alps and north-western Dinaric Alps. *Periodicum Biologorum*, 112, 3: 239–246.
- BRUS, R. – HORVAT-MAROLT, S. – PAULE, L. – GÖMÖRY, D. (1999): Genetska variabilnost bukve (*Fagus sylvatica* L.) v Sloveniji. [Genetic variability of European beech (*Fagus sylvatica* L.) in Slovenia]. *Zbornik gozdarstva in lesarstva*, 60: 85–106. (in Slovenian with English abstract)
- BRUS, R. – HORVAT-MAROLT, S. – PAULE, L. (2000): Nova spoznanja o obstoju ledenodobnih zatočišč bukve (*Fagus sylvatica* L.) na ozemlju današnje Slovenije. [New recognitions of the existence of the beech (*Fagus sylvatica* L.) glacial refugia on the present Slovenian territory]. In: POTOČNIK, I. (ed.), *Nova znanja v gozdarstvu - prispevek visokega šolstva : zbornik referatov študijskih dni, Kranjska gora, 11-12. 5. 2000*. Biotehniška fakulteta, Oddelek za gozdarstvo in obnovljive gozdne vire, 77–88, Ljubljana. (in Slovenian with English abstract)
- BRZEZIECKI, B. – KIENAST, F. – WILDI, O. (1995): Modelling potential impacts of climate change on the spatial distribution of zonal forest communities in Switzerland. *Journal of Vegetation Science*, 6: 257–268.
- COMPS, B. – THIEBAUT, B. – SUGAR, I. – TRINAJSTIĆ, I. – PLAZIBAT, I. (1991): Genetic variation of the Croatian beech stands (*Fagus sylvatica* L.): spatial differentiation in connection with the environment. *Annales des Sciences Forestières*, 48: 15–28.

- DAKSKOBLER, I. (2008): Pregled bukovich rastišč v Sloveniji. [A Review of beech sites in Slovenia]. Zbornik gozdarstva in lesarstva, 87: 3–14. (in Slovenian with English abstract)
- ELLENBERG, H. (1996): Vegetation Mitteleuropas mit den Alpen, 5th edn.- Ulmer, Stuttgart, Germany.
- FINKELDEY, R. (1993): Die Bedeutung allelischer Profile für die Konservierung genetischer Ressourcen bei Waldbäumen. Gött. Forstgenet. Bericht, 14, 176 p.
- FOTELLI, N.M – RENNENBERG, H. – GEßLER, A. (2002): Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ¹⁵N uptake and partitioning, responses of amino acids and other N compounds. Plant Biol., 4: 311–320.
- GEßLER, A. – KEITEL, C. – KREUZWIESER, J. – MATYSSEK, R. – SEILER, W. – RENNENBERG, H. (2007): Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. Trees, 21: 1–11.
- GILLET, E.M. (1998): GSED – Geneti Structure from Electrophoresis Data, Version 1.1e. Institut für Forstgenetik und Forstpflanzenzüchtung Universität Gettingen.
- GÖMÖRY, D. – PAULE, L. – BRUS, R. – ZHELEV, P. – TOMOVIĆ, Z. – GRAČAN, J. (1999): Genetic differentiation and phylogeny of beech on the Balkan Peninsula. J. Evol. Biol, 12: 746–754.
- GREGORIUS, H.R. (1974): Genetischer Abstand zwischen Populationen. I. Zur Konzeption der genetischen Abstandsmessung. Silvae Genetica, 23: 22–27.
- GREGORIUS, H.R. (1978): The concept of genetic diversity and differentiation. Theor. Appl. Genet. 74: 397–401.
- GREGORIUS, H.R. – KRAUHAUSEN, J. – MÜLLER-STARCK, G. (1986): Spatial and temporal genetic differentiation among the seed in stand of *Fagus sylvatica* L. Heredity, 57: 255–262.
- GREGORIUS, H.R. (1987): The relationship between the concepts of genetic diversity and differentiation. Theor. Appl. Genet., 74: 397–401.
- KONNERT, M. (1995): Investigations on the genetic variation of beech (*Fagus sylvatica* L.) in Bavaria. Silvae Genetica, 44: 346–351.
- KONNERT, M. – HENKEL, W. (1997): Untersuchungen zur genetischen Variation der Buche (*Fagus sylvatica* L.) in Thüringen. Allgemeine Forst-und Jagdzeitung, 168, 10: 128–190.
- KONNERT, M. – HUSSENDOERFER, E. – DOUNAVI, A. (2004): Handbücher für Isoenzymanalyse. Anleitung für Isoenzymuntersuchung bei Buche (*Fagus sylvatica*), URL: (<http://www.genre.de/fgrdeu/blag/iso-handbuecher>).
- KRAIGHER, H. – BOŽIČ, G. – VERLIČ, A. (2012): Seznam gozdnih semenskih objektov – stanje na dan 1. 1. 2012. [List of forest seed objects – State of art at the day 1. 1. 2012]. Urad. list Republike Slovenije, 13.1.2012, 3: 206–212.
- KUTNAR, L. – ZUPANČIČ, M. – ROBIČ, D. – ZUPANČIČ, N. – ŽITNIK, S. – KRALJ, T. – TAVČAR, I. – DOLINAR, M. – ZRNEC, C. – KRAIGHER, H. (2002): Razmejitev provenienčnih območij gozdnih drevesnih vrst v Sloveniji na osnovi ekoloških regij. [The delimitation of the regions of provenance of forest tree species in Slovenia based on ecological regions]. Zbornik gozdarstva in lesarstva, 67: 73–117. (in Slovenian with English abstract)
- KUTNAR, L. – KOBLER, A. (2011): Prediction of forest vegetation shift due to different climate-change scenarios in Slovenia. Šumarski list 135 (3–4): 113–126.
- KUTNAR, L. – MATIJAŠIČ, D. – PIŠEK, R. (2011): Conservation Status and Potential Threats to Natura 2000 Forest Habitats in Slovenia. Šumarski list, 135 (5–6): 215–231.
- LÖCHELT, S. – FRANKE, A. (1995): Bestimmung der genetischen Konstitution von Buchen - Beständen (*Fagus sylvatica* L.) entlang eines Höhentransekten von Frieburg auf den Schauinsland. Silvae Genetica, 44, 312–318.
- MAGRI, D. – VENDRAMIN, G.G. – COMPS, B. – DUPANLOUP, I. – GEBUREK, T. – GÖMÖRY, D. – LATAŁOWA, M. – LITT, T. – PAULE, L. – ROURE, J.M. – TANTAU, I. – KNAAP, W.O. – PETIT, R.J. – BEAULIEU, J.L. (2006): A new scenario for the Quaternary history of European beech populations: Palaeobotanical evidence and genetic consequences. New Phytologist, 171, 1: 199–221.
- MARINČEK, L. – ČARNI, A. (2002): Commentary to the vegetation map of forest communities of Slovenia in a scale 1:400.000. Založba ZRC, ZRC SAZU, Biološki inštitut Jovana Hadžija, Ljubljana, 158 p.
- MÁTYÁS, CS. – BERKI, I. – CZÚCZ, B. – GÁLOS, B. – MÓRICZ, N. – RASZTOVITS, E. (2010): Future of Beech in Southeast Europe from the Perspective of Evolutionary Ecology. Acta Silv. Lign. Hung. 6: 91–110.

- MÜLLER-STARCK, G. – BARADAT, PH. – BERGMANN, F. (1992): Genetic variation within European tree species. *New Forests*, 6: 23–47.
- MÜLLER-STARK, G. – WÜHLISCH, G. VON – KONNERT, M. (2001): Labormanual zur Durchfuehrung von Isoenzymanalysen bei Buche (*Fagus sylvatica* L.), URL: (<http://www.forst.tumuenchen.de/EXT/LST/GENET/index.htm>).
- NEI, M. (1973): Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA*, 70, 3321–3323.
- OGRIS, N. – JURC, M. – JURC, D. (2008): Varstvo bukovih gozdov - danes in jutri. [Protection of beech forests – today and tomorrow]. In: BONČINA, A. (ed.). *Bukovi gozdovi-ekologija in gospodarjenje: zbornik razširjenih povzetkov predavanj*. Biotehniška fakulteta, Oddelek za gozdarstvo in obnovljive gozdne vire, Ljubljana, 36–39. (in Slovenian with English abstract)
- OSOLE, F. (1990): Lukenjska jama pri Prečni : Novo mesto. [The Lukanja cave at Prečno : Novo mesto]. *Arheo* (Ljubljana), special number, 7–10. (in Slovenian)
- PAULE, L. (1995): Gene conservation in European beech (*Fagus sylvatica* L.). *Forest Genetics* 2 (3): 161–170.
- PAULE, L. – GÖMÖRY, D. – VYŠNY, J. (1995): Genetic diversity and differentiation of beech populations in Eastern Europe. In: MADSEN, S. (ed.). *Genetics and silviculture of beech*. Forskningsserien (Copenhagen) 11: 159–167.
- PERKO, F. (2007): Slovenian forests and forestry. Ljubljana, Zveza gozdarskih društev, Ministrstvo za kmetijstvo, gozdarstvo in prehrano RS, Zavod za gozdove Slovenije, Ljubljana, 39 p.
- PEUKE, A.D. – SCHRAML, C. – HARTUNG, W. – RENNENBERG, H. (2002): Identification of drought-sensitive beech ecotypes by physiological parameters. *New Phytol.*, 154: 373–387.
- ROBSON, T.M. – ALIÁ, R. – BOŽIČ, G. – CLARK, J. – FORSTREUTER, M. – GÖMÖRY, D. – LISEBACH, M. – MERTENS, P. – RASZTOVITS, E. – ZITOVÁ, M. – WÜHLISCH, G. VON (2011): The timing of leaf flush in European beech (*Fagus sylvatica* L.) saplings. In: *Proceedings of the COST E52 Final Meeting "Evaluation of beech genetic resources for sustainable forestry"*, Spain, May 2010, Monografías INIA Forestal, 22: 61–79.
- SANDER, T. – KÖNIG, S. – ROTHE, G.M. – JANSSEN, A. – WEISGERBER, H. (2000): Genetic variation of European beech (*Fagus sylvatica* L.) along an altitudinal transect at mount Vogelsberg in Hesse, Germany. *Molecular Ecology*, 9: 1349–1361.
- SFS (2012): Forest Report for the year 2011. Slovenia Forest Service, Ljubljana, 133 p. (in Slovenian)
- SIMONČIČ, P. – URBANČIČ, M. – BRIŠNIK, T. – FERLAN, M. – KOBAL, M. – VERLIČ, A. (2007): Introduction into project Carbon dynamics in natural beech forest. In: FERLAN, M. (ed.). *Carbon dynamics in natural beech forests*, Ljubljana: Slovenian Forestry Institute, 23 p.
- SITTLER, B. (1981): Experimentelle ökologische Untersuchungen an 15 slowenischen Buchenprovenienzen zur Beurteilung ihrer Anbaufähigkeit in der Bundesrepublik Deutschland. [Experimental ecological studies on the origin of 15 Slovenian beech provenances to assess their growth in the Federal Republic of Germany]. Dissertation. Albert-Ludwig-University of Freiburg, 205 p.
- ŠERCELJ, A. (1996): The origins and development of forest in Slovenia. *Slovenska akademija znanosti in umetnosti. Razred za naravoslovne vede, Dela (Opera)*, Ljubljana, 35: 142 p.
- URBANČIČ, M. – SIMONČIČ, P. – PRUS, T. – KUTNAR, L. (2005): Atlas gozdnih tal Slovenije. [Atlas of forest soils in Slovenia]. Ljubljana: Zveza gozdarskih društev Slovenije, Gozdarski vestnik, Gozdarski inštitut Slovenije, Ljubljana, 100 p. (in Slovenian with English abstract)
- WÜHLISCH, G. VON (2008): EUFORGEN Technical Guidelines for genetic conservation and use for European beech (*Fagus sylvatica*). *Bioversity International*, Rome, 6 p.