GENETIC VARIABILITY AND SUITABILITY OF UNDER-PLANTED BEECH IN SELECTED NORWAY SPRUCE MONOCULTURES ON THE POHORJE MASSIF

GENETSKA VARIABILNOST IN PRIMERNOST PODSAJENE BUKVE V IZBRANIH SMREKOVIH MONOKULTURAH NA POHORJU

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ABSTRACT   UDC 630*23:582.632.2(497.4Pohorje)
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Genetic variability and suitability of under-planted beech in selected Norway spruce monocultures on the Pohorje massif

In order to investigate the genetic variability of under-planted young beech in Norway spruce monocultures, 100 randomly chosen individuals on the research plots Brička and Kladje were analysed by means of isozyme gene markers at 17 polymorphic gene loci. The single locus mean value of the Gregorius (1974) allelic distance between the sample populations was relatively high (d_0 = 9.6 %). The results do not support the hypothesis of a common origin of the planting material from a single ancestral population. A proposal is made for an extension of the network of forest beech seed stands for future melioration of spruce monocultures on Pohorje.

Key words: conversion of spruce monoculture, *Fagus sylvatica* (L.), artificial regeneration, isoenzyme, genetic variation, forest site, seed stand, Slovenia

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Genetska variabilnost in primernost podsajene bukve v izbranih smrekovih monokulturah na Pohorju

Za raziskavo genetske variabilnosti podsajenih mladih bukev v smrekovih monokulturah na Pohorju smo analizirali 100 naključno izbranih osebkov na raziskovanih ploskvah Brička in Kladje. Uporabili smo metodo analize izoencimskih genskih označevalcev. Genetsko variabilnost smo ocenili na 17 polimorfnih genskih lokusih. Genetska razdalja (d_0) med vzorčenima populacijama izračunana po Gregorius (1974) je sorazmerno velika in znaša 9,6 %. Rezultati raziskave ne podpirajo hipoteze o skupnem izvoru saditvenega materiala iz enotne predniške populacije. Podan je predlog za razširitev mreže gozdnih semenskih objektov bukve za potrebe premene smrekovih monokultur na Pohorju.

Ključne besede: premena smrekove monokulture, *Fagus sylvatica* (L.), umetna obnova, izoencim, genetska variabilnost, gozdno rastišče, semenski sestoj, Slovenija

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1. INTRODUCTION

At the beginning of the nineties, storm damage and a gradual increase in bark beetles reduced the stability of Norway spruce stands in Europe and caused a perceptible reduction in vitality, which was observed until the end of the last decade. The forestry profession endeavoured to find a method of increasing the long term stability and preserving the permanence of these stands, which led to the choice of habitats of the most suitable tree species. Because of dubious management methods and public pressure and the demand for increased sustainability, the insertion of under-planted deciduous trees in coniferous forests became a widely established silvicultural method in Europe. The silvicultural grounds for such a transformation were relatively poorly defined.

In the second half of the last century, the conversion of pure spruce stands (*Picea abies* L. Karst.) was one of the main forest-silviculture challenges in Europe (Zerbe 2002; Teuffel, Heinrich & M. Baumgarten 2004). It is thought that there are at least 6 to 7 million hectares of pure spruce stands outside their natural habitats in Europe, of which 4-5 million hectares are established on sites on which deciduous or mixed deciduous-coniferous forests should predominate. The current state is the result of forest management decisions in the past, when mixed stands were exposed to agricultural exploitation, littering, grazing and obtaining firewood (Johann et al. 2004). The great need for timber brought about by industrialisation led to an acceleration of monocultures of fast growing trees. In the last 200 years, spruce was most often chosen as the tree species for artificial renewal of stands because of its fast growth, the simple establishment of stands, low requirements in relation to thinning and because it was not excessively burdened by grazing game.

The introduction of spruce outside its natural area of distribution is linked to numerous, for the most part short-term benefits. The species is economically very interesting, having the potential to produce high quality timber in a short time span. European deciduous forests in the past covered larger areas than they do today, so the general opinion is held in Europe that planning deciduous stands is a step towards sustainable forestry (Hannah, Carr & Lankerani 1995; Stanturf & Madsen 2002). One of the ways of establishing them is to change spruce into deciduous or mixed forest. The transformation of the widespread homogeneous spruce into natural deciduous forests has been used in forestry practice in Europe for the last fifty years.

A number of factors argue in favour of the transformation. Spruce has been shown to be unstable in many cases, it is exposed to a reduction of vitality, storm damage, attacks of bark beetles, drought and it has a negative impact on the soil (Larsen 1995). Appreciable changes in the environment and landscape, with consequential changes or reduction of biotic diversity (Grabherr & Koch 1993) have made public opinion less favourable. Climate change, and especially the increasing likelihood of the occurrence of climatic extremes, such as storms, have exposed the vulnerability of spruce monocultures and stress the need for reducing the risk by including other tree species in spruce stands (Bradshaw et al. 2001). The increase in the price of deciduous timber has reduced the economic justification of planning anthropogenic coniferous stands (Abildtrup, Riis & Jellesmark-Thorsen 1997; Spiecker 2000).

For successful melioration, under-planting with beech (*Fagus sylvatica* L.), either below the shade of the parent stand or in gaps is a possible alternative to a clearcut method of management, which is still used in Europe. Under-planting is an old silvicultural technique, which is receiving renewed attention today (Otto 1986; Spellmann & Wagner 1993). The advantages of under-planting are fewer weeds, a more favourable micro-climate, protection from winter frosts (Löf 2000), as well as a more favourable attitude of the public, in contrast to clearcut (Mattsson & Li 1994).

In Slovenia, conversion of pure spruce into mixed stands began in the mid-20th century. Although forests in Slovenia are relatively well-preserved in comparison to Central Europe, because of the heritage of the past, the present tree composition of forests deviates from what is considered natural (Diaci & Grecs 2003). The share of spruce in timber stocks is 32 %, while the estimated natural share is approximately 8 %. Slovenia is a country of deciduous, mainly beech forests (Šercelj 1996, Ž. Košir et al. 1974, Dakskobler 2008). Seventy percent of forests in Slovenia are classified into forest communities in which beech is the leading species.

The period of active melioration of spruce stands started in the early 50s with an instruction from the Ministry of Forestry that a campaign should be started for the planting of noble deciduous trees in spruce plantations, mainly sycamore maple (*Acer pseudoplatanus*) (Diaci 2006). Efforts were not for the most part successful because of too high a density of large herbivores (Diaci 2006, summarised from oral sources Mlinšek 2004). The success of realising the first regulatory plans after the Second World War were essentially more successful. In 1954, Mlinšek elaborated an extensive regulatory plan for the Mislinja forest-management unit,

which mainly embraced spruce plantations (Mlinšek 1955). It began with gradual conversion on the basis of a control method, with detailed analysis of increment, selective thinning was introduced and increased inspection. He introduced an overall methodology of under-planting spruce plantations with seedings and pullings of beech (seedlings pulled from natural forest regeneration in a forest stand) and individual and group protection against grazing, ensured an adapted approach to sites. Various methods of individual protection were tried and considerable success was achieved with cement mortar. Because of a lack of seed trees of beech and seedings, pullings of beech were transferred from more or less suitable sites on the north side of Pohorje (Diaci 2006).

In order to evaluate the genetic variability of the under-planted beech in pure spruce stands on Pohorje, we performed genotype analysis in the juvenile development stage of beech of test populations, using isozymes. The purpose of the study was to assess the level of genetic relationship of populations of under-planted beech by the method of isozymes gene markers. We wished to test the hypothesis that the sampled populations of beech have a similar genetic structure and do not show major deviation from average value parameters. The study is intended to contribute to a more detailed understanding of genetic structures, genetic diversity and genetic differentiation of beech populations that have been introduced into spruce monocultures on Pohorje.

2. STUDY AREA

Pohorje is a distinctive mountain plateau, lying at an altitude of 1200-1500 m and is an extension of the Central Alps. The massif is constructed of metamorphic and magmatic rocks and sediments (Budnar-Tregubov 1958). The climate is medium mountain. In the last 4000 to 5000 years, various forms of fir beech forests with a modest share of spruce have predominated in primary forests (Culiberg & Šercelj 2000). The original forests on Pohorje consisted mainly of beech, fir and spruce, which larch interspersed in places (Breznikar et al. 2006). Natural spruce forests are edaphically conditioned and thus limited to swampy level areas, bowl-shaped dips and gently inclined slopes on the Pohorje plateau. In the last two centuries, the tree composition of Pohorje forests has changed. Today, anthropogenically altered spruce stands predominate. Because of the negative influences of the tree composition, the sustainable yield from the forests is also threatened (Breznikar et al. 2006). In the past, in the period from 1800 to the end of the Second World War, because of the influence of the German school of forest management and its agricultural principles, spruce was intensively introduced in the Pohorje forests and the renewal of forests was directed towards establishing pure spruce stands. After the Second World War, this silvicultural principle was gradually abandoned. Anthropogenic spruce stands on Pohorje, among which belong stand constructions with a predominant share of spruce on beech and fir habitats, today cover 27.000 ha or 45 % of the total forest area on Pohorje (Breznikar et al. 2006).

The negative effects of the altered tree composition dictate a search for forest management-actions by which

a more natural intermixture of tree species in forest stands will be introduced. Because of the negative impact of encouraging spruce on beech and fir sites, planning measures in spruce monocultures requires an understanding of the processes of degradation of sites. With a predominant share of spruce in stands, raw humus begins to accumulate in the soil and the lability of the soil also contributes to its damaging action. Spruce, because of its shallow roots, over-exploits the upper soil horizons, and the lower horizons begin to compact. A worsening of the physical properties of the soil occurs (reduction of aeration) and the acidity of the soil increases, the availability of nutrients is reduced and the intensity of humification and mineralisation of organic substances in the soil is reduced, initiating the accumulation of unfavourable forms of humus. With the gradual compaction of the soil profile and increasing of the impermeable horizon the biologically active depth of the soil is reduced and, because of the worsened physical properties of the soil, the soil water capacity is reduced. Moist forms of raw humus and mosses increase the surface accumulation of rainwater and condition the process of pseudogleyification of the soil (Breznikar et al. 2006).

The development of more stable forest structures and retarding degradation processes in forest soils are the main strategic aims in planning the development of forests on Pohorje. Planned interventions for melioration of the altered stand constructions and targeted silviculture measures began in the Mislinje part of Pohorje in the fifties of the last century and have gradually been extended to the entire region of Pohorje. The main melioration measure is the introduction of broadleaf trees,

mainly beech, in spruce stands and eliminating the impact of over-numerous game on young forest trees.

We chose research sites on the southern side of Pohorje, in research plots of the Slovenian Forestry Institute, at the locations Brička above Mislinjski jarek (SFS District unit Slovenj Gradec) and Kladje near Osankarica (SFS District unit Maribor) (Table 1). Both sites are presumed to have been covered with beech or fir-beech forests, with interspersed spruce, before the more intensive interventions and degradation processes. The cited locations are potential natural sites of acidophilous beech with white wood-rush (*Luzula luzuloides*), a geographic variant with *Cardamine trifolia* (*Luzulo albidae-Fagetum* Meusel 1937 var. geogr. *Cardamine trifolia* (Marinček 1983) Marinček & Zupančič 1995). Relatively well preserved acidophilous beech forest still grows on a smallish area on the edge of the Brička site. The wider region of the Kladja site is considerably changed but, on the basis of individual remains of such forest, it be concluded that it is a potential site of this association. However, in the wider area of this part of Pohorje, the association of beech with *Cardamine waldsteinii* has been charted. This is a zonal association of Pohorje high mountain beech forest, which grows on the upper part of the montane belt of the Pohorje massif, i.e., at an altitude from 1000 to 1300 m. In addition to the characteristic tree species of beech and sycamore maple, fir and spruce also appear with larger shares in relatively well preserved stands of this association. On this basis, a special geographic variant of this association with fir was defined (*Cardamini savensi-Fagetum* Ž. Košir 1962 var. geogr. *Abies alba* Ž. Košir 1979).

Because of the promotion of spruce in the past, the area of the sites is overgrown with spruce monocultures. The starting point for the selection of research plots, in addition to the requirement for spruce covered potential beech sites, also included the presence of a larger number of vital under-planted trees of beech within individual areas, a similar age of the planted beech in comparable locations and possibilities of including the research plots within the framework of other investigations.

Table 1: Survey of research site characteristics according to ČATER (2011)
Preglednica 1: Značilnosti izbranih raziskovalnih objektov (ČATER 2011)

Plot (Ploskev)	Altitude (m) (Nadmorska višina)	Latitude (Severna zemljepisna širina)	Longitude (Vzhodna zemljepisna dolžina)	Annual precipitation (mm) (Letne padavine)	Annual average air T (°C) (Povprečna letna temperatura zraka)	Dominant soil type (Prevladujoči talni tip)
Brička (BRI)	1093	46°28'40"	15°15'40"	1190	9.1	Dystric Cambisol (Distrična rjava tla)
Kladje (KLA)	1308	46°28'48"	15°23'24"	1066	9.2	Dystric Cambisol (Distrična rjava tla)

The research sites are overgrown by mature trees of spruce and under-planted beech in the juvenile development stage. At Brička above Mislinjski jarek, the area is covered by 173 spruce / ha, with an estimated growing stock of spruce stands of 477 m^3 / ha, and at Kladje near Osankarica, 126 trees/ha with a growing stock of 302 m^3 / ha (ČATER 2011). In 2006, the age of the sampled under-planted beech was estimated at around 10-15 years (ČATER 2011).

3. MATERIAL AND METHODS

3.1 Sampling

At the research plots Brička and Kladje on Pohorje, we randomly sampled 100 beech trees for genetic testing. On each area of a size 100 m x 100 m, we uniformly covered vital trees of under-planted beech. In the winter period of 2005 / 2006, we took a branch with dormant buds from each of the sampled trees, which we used for the extraction of enzymes. The buds were preserved until analysis in test tubes at a temperature of -20° C. The size of the sample was 50 trees at each site.

3.2 Analysis of isoenzymes

We investigated the genetic characteristics of the beech by the method of isozyme analysis. The research material consisted of dormant buds. The genotype of each tree was established by means of horizontal electrophoresis on starch gels. Enzyme extraction from the dormant buds, electrophoresis, staining the gels and reading the electrophoregrams was performed by standard methodological procedures for the analysis of beech samples (KONNERT, HUSSENDOERFER & DOUNAVI 2004). The lab-

oratory part of the analysis was performed in the framework of the research tasks of project "Carbon dynamics in natural beech forests" (L4-6232) in February 2006.

The laboratory work was done in the Bayerische Amt für forstliche Saat - und Pflanzenzucht in Teisendorf, Germany, under the leadership of Dr. Monika Konnert.

Table 2: Gene loci analysed
Preglednica 2: Analizirani genski lokusi

Enzyme system (Encimski system)	E.C. Code (E.C. koda)	Analysed gene loci (Analizirani genski lokusi)	Number of alleles (Število alelov)
Aspartate aminotransferase (Aat) syn. Glutamate oksaloacetate transaminase (Got) (Aspartat aminotransferaza syn. Glutamat oksalacetat transaminaza)	2.6.1.1	Aat-A, Aat-B	2, 2
Aconitase (Aco) (Akonitaza)	4.2.1.3	Aco-A, Aco-B	2, 3
Isocitrate dehydrogenase (Idh) (Izocitrat dehidrogenaza)	1.1.1.42	Idh-A	2
Malate dehydrogenase (Mdh) (Malat dehidrogenaza)	1.1.1.37	Mdh- A, Mdh-B, Mdh-C	2, 4, 2
Menionreduktase (Mnr) (Menionreduktaza)	1.6.99.2	Mnr-A	3
Peroxidases (Per) (Peroksidaze)	1.11.1.7	Per-A, Per-B	2, 3
Phosphoglucose isomerase (Pgi) (Fosfoglukoza izomeraza)	5.3.1.9	Pgi-B	2
Phosphoglucomutase (Pgm) (Fosfoglukomutaza)	2.7.5.1	Pgm-A	2
Shikimate dehydrogenase (Skdh) (Šikimat dehidrogenaza)	1.1.1.25	Skdh-A	3
6-Phosphogluconate dehydrogenase (6-Pgdh) (6-Fosfoglukonat dehidrogenaza)	1.1.1.44	6-Pgdh-A, 6-Pgdh-B, 6-Pgdh-C	3, 2, 4
Total (Skupaj)	10	17	43

The results of isozyme analysis were evaluated by the relative frequency of alleles and genotypes, by individual gene loci. The genetic structure of the population was described on the basis of the frequency of individual alleles at polymorphic loci. Any gene locus at which we found at least one allele was considered to be a polymorphic locus, irrespective of its relative frequency in the population (without criteria).

The frequencies of distribution of alleles at loci were described according to individual populations by 4 allele profiles, which we defined according to FINKELDEY (1993), as: i) fixation, if there was only 1 allele (allele frequency 100 %) at an individual gene locus, ii) low level of polymorphism if there were frequent alleles at an individual gene locus (allele frequency > 80 %) and one or more rare alleles, iii) high level of polymorphism if there were at least two alleles as predominant at an individual gene locus (allele frequency > 20 %), iv) untypical profile if the allele structure at an individual gene locus could not be classified into the first three profiles.

We interpreted the genetic variability within individual populations by the parameters of genetic multiplicity and genetic diversity. With the parameter of genetic multiplicity, which takes into account only the

number of different gene types in individual populations, we calculated three parameters: (1) share of polymorphicity of the analysed gene loci (P %); (2) the highest possible number of different alleles (M_{max}); (3) the average number of alleles per polymorphic locus (A/L). With the parameter of genetic diversity, which, in contrast to the parameter of genetic multiplicity takes into account in addition to the number of different gene types also their frequency of appearance in a population, we calculated observed heterozygosity (H_a) and conditional heterozygosity (H_c; GREGORIUS, KRAUHAUSEN & MUELLER-STARCK 1986), effective allele diversity (ν; GREGORIUS 1978, GREGORIUS 1987), multilocus hypothetical gametic diversity ($ν_{gam}$) 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). We used the χ2 test to check how the actual heterozygosity shares in under-planted young beech differed from expected heterozygosity shares created by random mating (Hardy-Wienberg equilibrium), which we carried out by loci separately for each sample population individually, at a level of risk α = 0.05.

We gave genetic differentiation between the sampled populations of under-planted beech at the sites Brička and Kladje by the criteria of average genetic distance (d_0; Gregorius 1974). Statistical comparison of the genetic structures of the beech was carried out on the basis of the χ2 test of the homogeneity of allele frequencies by loci. The null hypothesis of homogeneity of allele distributions between populations was rejected at the level of risk α = 0.05. For calculating individual parameters, we used the GSED programme for evaluating isozyme analysis data (Gillet 1998).

4. RESULTS

The research was primarily intended to shed light from a genetic point of view on monocultures of spruce on Pohorje meliorated with under-planted beech. The aim was to evaluate the genetic structure of under-planted beech at the Brička and Kladje sites, i.e., on degraded potential natural sites of acidophilous beech forest with white wood-rush (*Luzula luzuloides*), the geographic variant with *Cardamine* (*Luzulo albidae-Fagetum* Meusel 1937 var. geogr. *Cardamine trifolia* Marinček (1983) Marinček & Zupančič 1995). We wished to evaluate whether there are differences in the genetic structures among groups of trees of under-planted beech. The results of genetic comparison are shown in Tables 3 and 4 for 17 polymorphic gene loci.

4.1 Allele and genotype frequencies

It is clear from the relative allele frequencies of the analysed loci (Table 3) that there is polymorphism at all 17 analysed gene loci in the populations (P = 100 %). In both populations, we discovered a low level of polymorphism at 3 loci (*Aat-A, Aco-A, Skdh-A*), a high level of polymorphism at 2 loci (*Aat-B, Per-A*). Locus *Idh-A* also shows a similar polymorphism. We found untypical profiles in both populations at 6 loci (*Aco-B, Mdh-A, Mnr-A, Pgi-B, 6-Pgdh-A, -B*). We found a distinct transition between low and high levels of polymorphism in the test populations at 5 loci. Loci *Per-B* and *6-Pgdh-C* reflect low polymorphism in the sample from Brička and high polymorphism in the sample from Kladje, while loci *Mdh-B, -C* and *Pgm-A* have low polymorphism in the sample from Kladje and high in the sample from Brička. The same predominant allele is always present in both sampled populations. The frequency of the main allele was found in a range of 54 % to 99 %. Frequencies above 10 % could additionally be achieved by alleles $Aat\text{-}B_2$, $Aco\text{-}B_2$, $Idh\text{-}A_2$, $Mdh\text{-}A_1$, $Mdh\text{-}B_4$, $Mdh\text{-}C_1$, $Per\text{-}A_1$, $Per\text{-}B_1$, $Pgm\text{-}A_2$, $6\text{-}Pgdh\text{-}A_4$, $6\text{-}Pgdh\text{-}B_1$ and $6\text{-}Pgdh\text{-}C_4$, while other alleles appeared with low frequencies.

The allele frequencies, for example, change greatly with alleles $Mdh\text{-}B_4$, $Per\text{-}B_1$, $Pgm\text{-}A_2$ and $6\text{-}Pgdh\text{-}C_4$. The frequencies of alleles $Per\text{-}B_1$ and $6\text{-}Pgdh\text{-}C_4$ are lower in the sample from Brička than in the sample from Kladje (11 % vs. 37 % and 8 % vs. 23 %), while the allele frequencies of $Mdh\text{-}B_4$ and $Pgm\text{-}A_2$ are higher in the sample from Brička than in that from Kladje (16 % vs. 5 % and 40 % vs. 9 %). The allele variants $Aat\text{-}A_1$, $Mdh\text{-}B_2$, $Pgi\text{-}B_3$, $6\text{-}Pgdh\text{-}A_4$, $6\text{-}Pgdh\text{-}C_5$ are also interesting, which are only rarely present in the Kladje population (a frequency of less than 5 %) but more frequently in the Brička population (up to 11 %) or $Mdh\text{-}A_1$, which is rare in the Brička population (1 %) and more frequent in the population from Kladje (10 %).

Comparison of the genotype frequencies indicates the existence of differences between the compared sample populations at individual gene loci, namely at 7 of the total of 17 analysed gene loci. Homozygous and heterozygous types that are much more frequent in the Brička sample than in the Kladje are: $Per\text{-}B_{22}$ (70 % vs. 32 %), $Pgm\text{-}A_{23}$ (44 % vs. 18 %), $Pgm\text{-}A_{22}$ (18 % vs. 0 %), $Mdh\text{-}A_{33}$ (98 % vs. 80 %), $Mnr\text{-}A_{33}$ (90 % vs. 74 %), $6\text{-}Pgdh\text{-}C_{15}$ (20 % vs. 4 %) and $Pgi\text{-}B_{23}$ (16 % vs. 2 %). Heterozygous or homozygous types much more frequent in the Kladje sample than in the Brička sample are: $Pgm\text{-}A_{33}$ (82 % vs. 38 %), $Mdh\text{-}A_{13}$ (20 % vs. 2 %), $Per\text{-}B_{12}$ (30 % vs. 14 %), $Per\text{-}B_{11}$ (20 % vs. 4 %), $Mnr\text{-}A_{35}$ (20 % vs. 6 %) and $Mdh\text{-}B_{33}$ (72 % vs. 54 %).

Table 3: Relative allele frequencies of under-planted beech populations from the Brička (BRI) and Kladje (KLA) locations on Pohorje presented per gene loci
Preglednica 3: Relativne alelne frekvence populacij podsajene bukve z območja Brička (BRI) in Kladje (KLA) na Pohorju s prikazom po lokusih

Locus (Lokus)	Alelle (Alel)	BRI N = 50	KLA N = 50	Locus (Lokus)	Alelle (Alel)	BRI N = 50	KLA N = 50
Aat-A	1	0.060	0.030	Per-A	1	0.280	0.240
	2	0.940	0.970		2	0.720	0.760
Aat-B	2	0.330	0.290	Per-B	1	0.110	0.370
	3	0.670	0.710		2	0.830	0.540
					3	0.060	0.090
Aco-A	2	0.970	0.990	Pgi-B	2	0.920	0.990
	3	0.030	0.010		3	0.080	0.010
Aco-B	2	0.170	0.090	Pgm-A	2	0.400	0.090
	3	0.810	0.910		3	0.600	0.910
	4	0.020	-				
Idh-A	2	0.180	0.160	Skdh-A	2	0.010	-
	3	0.820	0.840		3	0.990	0.960
					5	-	0.040
Mdh-A	1	0.010	0.100	6-Pgdh-A	2	0.890	0.950
	3	0.990	0.900		3	-	0.010
Mdh-B	1	0.050	0.080		4	0.110	0.040
	2	0.090	0.010				
	3	0.700	0.860	6-Pgdh-B	1	0.120	0.090
	4	0.160	0.050		2	0.880	0.910
Mdh-C	1	0.250	0.180	6-Pgdh-C	1	0.810	0.720
	2	0.750	0.820		3	0.010	0.030
					4	0.080	0.230
Mnr-A	3	0.930	0.870		5	0.100	0.020
	4	-	0.030				
	5	0.070	0.100				

4.2 Intra-population genetic variation

4.2.1 Genetic multiplicity

In terms of meaning, the expression genetic multiplicity refers exclusively to the number of genetic categories (alleles, genotypes) of a population or parts of it. On the entire study area, we discovered 43 different alleles (M_{max}) at 17 polymorphic loci in the sample populations. We did not find all possible allele variants in either population of under-planted beech. Total allele multiplicity is represented in rejuvenated groups of beech from 93.0 % (Brička) to 95.3 % (Kladje). Both populations on average have the same number of alleles per locus present (A/L = 2.40).

4.2.2 Genetic diversity

Genetic diversity is the variability of individuals within a population, which is expressed by the frequency of dif-ferent genetic categories within the population. The criterion of genetic (allele) diversity in a population is the actual available or effective number of alleles at an individual locus. Because the contribution of rare alleles (i.e., alleles with a frequency < 5 %) to the total amount is small, this criterion also expresses the actual level of equilibrium of frequent alleles. Comparison of the avarage values of the effective number of alleles per locus (v) indicates the diversity of the sampled populations. Values range from 1.28 (Kladje) to 1.34 (Brička), which corresponds to a ratio of 1:1.05. The corresponding smaller value of effective number of alleles per locus in the Kladje sampled population, with a value of A/L = 2.40, indicates the presence of a larger share of rare alleles. Values of allele diversity (v) by individual loci are shown in Table 4. Analysis of the hypothetical multi-loci gametic diversity (v_{gam}) of the test trees showed that the number of genetic variants of the 17 loci gametic types that the group of 50 sample trees in the populations could have produced ranges from 99.8 (Kladje) to 203.1 (Brička)

and corresponds to a ratio of 1:2.04. Although the calculated values have only an indicative character, this comparison suggests greater potential of the group of under-planted beech trees at the Brička site than that of the group of under-planted beech trees at the Kladje site for the production of genetically different gametes, which will be subject to genetic variation in new generations. The average value of actual (observed) heterozygosity (H_a) for the 17 loci gene pool are in a range from 21.1 % in the group of trees from Kladje to 23.5 % in the Brička group of trees, which corresponds to a ratio of 1:1.11. The level of heterozygosity explicitly differs between the test populations from Brička and Kladje at 5 loci, namely at *Mdh-A* (2 % vs. 20 %), *Mnr-A* (6 % vs. 26 %), *Per-B* (26 % vs. 48 %), *Pgi-B* (16 % vs. 2 %) and *Pgm-A* (44 % vs. 18 %).

Table 4: Comparison of indicators of genetic variability for under-planted populations of beech at Brička (BRI) and Kladje (KLA): number of alleles per locus (A/L), allelic diversity (ν), average heterozygosity (H_a,

H_c), intrapopulational genetic differentiation (δ_T), genetic distance (d_0) and value of the χ2 test of homogeneity of genetic structures by loci, with significance level α = 0.05 (*), α = 0.01 (**), α = 0.001 (***) for 17 polymorphic loci. Bold type indicates significant deviation (p < 005) of genotype frequencies from Hardy-Weinberg equilibrium, underlined values indicate a significant heterozygote deficiency.

Preglednica 4: Primerjava kazalnikov genetske variabilnosti pri podsajenih populacijah bukve Brička (BRI) in Kladje (KLA): število alelov na lokus (A/L), alelna raznolikost (ν), povprečna heterozigotnost (H_a, H_c), genetska diferenciacija med osebki znotraj populacije (δ_T), genetska razdalja (d_0) in vrednost χ2 testa homogenosti genetskih struktur po lokusih, z nivojem značilnosti α = 0,05 (*), α = 0,01 (**), α = 0,001 (***) za 17 polimorfnih lokusov. Krepki tisk označuje značilno odstopanje (p < 0,05) frekvenc genotipov od Hardy-Weinbergovega ravnotežja, podčrtane vrednosti označujejo značilni deficit heterozigotov.

Locus (Lokus)	A/L		ν		δ_T (%)		H_a (%)		H_c (%)		d_0 (%)	χ² value (χ² vrednost)
	BRI	KLA	BRI	KLA	BRI	KLA	BRI	KLA	BRI	KLA		
Aat-A	2	2	1.13	1.06	11.4	5.9	12.0	6.0	100	100	3.0	n.s.
Aat-B	2	2	1.79	1.70	44.7	41.6	36.0	**<u>22.0</u>**	51.5	37.9	4.0	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	2	1.46	1.20	31.8	16.5	28.0	18.0	73.7	100	10.0	n.s.
Idh-A	2	2	1.42	1.37	29.8	27.2	24.0	24.0	66.7	75.0	2.0	n.s.
Mdh-A	2	2	1.02	1.22	2.0	18.2	2.0	20.0	100	100	9.0	7.792**
Mdh-B	4	4	1.90	1.34	47.9	25.4	40.0	28.0	66.7	100	19.0	14.495**
Mdh-C	2	2	1.60	1.42	37.9	29.8	42.0	32.0	84.0	88.9	7.0	n.s.
Mnr-A	2	3	1.15	1.30	13.2	23.5	**6.0**	26.0	42.9	100	6.0	n.s.
Per-A	2	2	1.68	1.57	40.7	36.8	**52.0**	**48.0**	92.9	100	4.0	n.s.
Per-B	3	3	1.42	2.29	29.8	56.9	26.0	48.0	76.5	52.2	29.0	20.822***
Pgi-B	2	2	1.17	1.02	14.9	2.0	16.0	2.0	100	100	7.0	5.701*
Pgm-A	2	2	1.92	1.20	48.5	16.5	44.0	18.0	55.0	100	31.0	25.976***
Skdh-A	2	2	1.02	1.08	2.0	7.8	2.0	**4.0**	100	50.0	4.0	n.s.
6-Pgdh-A	2	3	1.24	1.11	19.8	9.7	14.0	6.0	63.6	60.0	7.0	n.s.
6-Pgdh-B	2	2	1.27	1.20	21.3	16.5	**12.0**	18.0	50.0	100	3.0	n.s.
6-Pgdh-C	4	4	1.49	1.75	33.1	43.2	38.0	36.0	100	64.3	17.0	n.s.
Gene pool (Genski sklad)	2.40	2.40	1.34	1.28	25.6	22.3	23.5	21.1	77.9	84.0	9.6	

Genetic variation among individuals within an individual population, independent of the population size or the number of studied individuals, is defined by the level of genetic differentiation (δ_T). The average levels of genetic (allele) differentiation δ_T in the sampled populations of under-planted beech range from 22.3 % (Kladje) to 25.6 % (Brička), which corresponds to a ratio of 1:1.15. Observed frequencies of genotypes at the majority of loci correspond to the frequencies of genotypes expected according to Hardy-Weinberg equilibrium. We found significant deviation from a Hardy-Weinberg structure at the Brička site at the loci *Mnr-A*, *Per-A* and *6-Pgdh-B* and at the Kladje site at loci *Aat-B*, *Per-A* and *Skdh-A*. In the Kladje sample population, a significant deficiency of heterozygotes also appeared at locus *Aat-B*. According to the Hardy-Weinberg law, deviation of expected genotype frequencies from the Hardy-Weinberg structure suggest that random mating does not exist, that there is gene flow into the population or that natural selection is in operation. The highest realised level of heterozygosity (H_c) in the sampled populations is from 77.9 % (Brička) to 84.0 % (Kladje).

The calculated value (H_c) for both sample populations together, though, indicates a deficiency of heterozygotes in comparison with the highest achievable values, which equals 100 %. However, because of the lack of data on the origin of the parental stands of beech and past development phases of the groups of trees under-planted in the spruce monocultures on Pohorje included in the analysis, we cannot establish whether the reproductive system, the method of obtaining them and the use of forest reproduction material and/or various forms of (survival) selection influenced this phenomenon.

4.3 Genetic differentiation between the sampled populations

The frequency of individual alleles at some loci (e.g., $Pgm\text{-}A_2$ (9 % vs. 40 %), $Per\text{-}B_1$ (11 % vs. 37 %), $Mdh\text{-}B_4$ (5 % vs. 16 %), $Mdh\text{-}A_1$ (1 % vs. 10 %), $6\text{-}Pgdh\text{-}C_4$ (8 % vs. 23 %)) partially indicates great diversity of the stands. If we compare the allele distribution of the sample populations of under-planted beech, the probability of deviations from homogeneous allele structures is statistically significant at 5 of the 17 analysed loci (Table 4). The calculated values show that deviations from the homogeneity of allele distributions are highly significant at loci $Per\text{-}B$ and $Pgm\text{-}A$ ($\alpha = 0.001$), $Mdh\text{-}A$ ($\alpha = 0.01$) and, at a level of risk $\alpha = 0.05$, also at $Mdh\text{-}B$ and $Pgi\text{-}B$. The share of alleles that do not differentiate the test populations (d_0), for the genetic fund of 17 polymorphic loci, amounts on average to 9.6 %. In a comparison of individual loci, we also find high values of genetic distance. The sample groups of beech, which at locus $Pgm\text{-}A$ are separated into 31 % allele and 44 % genotype shares, and at loci $Per\text{-}B$ and $Mdh\text{-}B$ into 29 % or 19 % allele and 38 % or 26 % type shares, respectively, already allow the finding of major genetic differentiation between them. The hypothesis that the sample groups of beech under-planted in the two spruce monocultures on Pohorje (Brička in Kladje) have a similar genetic structure, cannot therefore be confirmed.

5. DISCUSSION

In the selected spruce monocultures on Pohorje, the population of under-planted beech at the Brička site has greater effective allele diversity, hypothetical multi-locus gametic diversity, observed heterozygosity and greater differentiation among individuals within the population in the gene pool than the population of under-planted beech at the Kladje site. The reasons for the different comparative values of the individual sample populations are not known. The established differences in the level of genetic variability may reflect different sources of provenance, historical differences in interventions in the area of the parental stands, differences in the method of obtaining and using the forest reproductive material and/or different forms of survival selection of juvenile beech, both in the parental and in the new, changed conditions of their living environment. These are only theoretical suppositions, of course, which have not in this case been verified. Additional research of the genetic structure of beech stands with natural regeneration that have survived the period of spruce monocultures in the area of Pohorje is recommended, in order to know whether the analysed genetic structure of the sampled beech in our research was representative or not.

The first evidence of the existence of genetic differences between the studied groups of under-planted beech at the Brička and Kladje sites is provided by the result of a statistical test of homogeneity of allele structure at polymorphic gene loci. Among the allele distributions of sampled populations of beech, we obtained significant deviations at 5 of 17 loci. The level of analysed genetic differentiation between the sampled populations is relatively large. Between the groups of under-planted beech in selected spruce monocultures in the southern part of Pohorje, the genetic differentiation (d_0) is expressed by the share of alleles that do not distinguish the two populations, 9.6 %. This finding is similar to the established values of allele (genetic) distance (d_0; GREGORIUS 1974) among the most differentiated populations of beech in Bavaria (Germany), which differed in a range from 2.6 % to 10.9 % (KONNERT & HENKEL 1997). Similarly high values of genetic differentiation, established among the four populations with the most different habitats, is also cited by research of twenty autochthonous populations of beech from the area of western Germany (TUROK 1994).

The observed deviation between the sampled populations from Brička and Kladje on Pohorje, in the event of representativeness of the genetic structures of the studied populations, may indicate that the gene flow between the parental stands of beech was limited or that the greater genetic differentiation may also be a consequence of isolation and specific selection processes that the beech at these locations experienced. This could also mean that the planting material that was under-planted

in Brička and Kladje does not derive from the same parental origin of beech. All these links can certainly not be understood in greater detail and their impact evaluated without new, in-depth research. The results of our investigation thus point to the conclusion that the group of beech trees in the spruce monoculture at Brička is genetically slightly different from the group of beech trees in the spruce monoculture of Kladje.

With under-planting of beech in spruce monocultures (without including naturally regeneration of beech), there is a constant danger of the loss of alleles (or genetic information) because of genetic drift, which can occur in small, isolated populations because of a reduction of the original size of the population during development and growth of the future beech stand. This danger is potentially greater in our case for the Kladje beech population, which shows a lower level of intra-population genetic variability and has a larger share of rare alleles (with frequencies < 5 %) than the Brička beech population.

Silviculture treatment is a crucial step in the development of forest. The use of natural regeneration in stands is therefore recommended for the renewal of forests. In cases in which this is not possible, forest reproductive material (seeds and pullings) obtained from neighbouring seed stands should be used. Collection and use of forest reproductive material is regulated by the Act on Forest Reproductive Material (ULRS, no. 58/02, 85/02, 45/04) and the Regulation for approval of basic material for production of forest reproductive material (FRM) of the categories "source indentified" and "selected" and the national list of basic material (URLS, no. 91/03). »For the majority tree species (primarily beech, pedunculate oak, sessile oak, fir and spruce), the use of seeds and seedlings within the altitudinal belt and provenance region from which it comes or from a neighbouring region is recommended. The use of seeds and seedlings from other regions of provenance is less recommended, and only exceptionally does either the forester or silviculture expert prescribe also the use of seeds and seedlings from neighbouring altitudinal zones. For minority tree species, the whole of Slovenia is considered a uniform region of provenance, divided into 4 altitudinal belts. Nevertheless, even for these species, the use of seed in the region from which it originates is recommended« (MEDVED et al. 2011, p. 137). Forest seed is genetic material, so the selection of forest seed sources has far-reaching consequences. There are not a large number of high-quality seed sources or stands and, because of the general changeability of forests and because of the anthropogenic burdening of forests to date, there are ever fewer. In order to spread the risk and as a condition of the ecological adaptability of forests, forest seed sources must embrace a sufficiently large biological diversity, i.e., all the more important tree species with their local races and their genetic variability. This is especially important given the present fast climate change and other anthropogenically caused uncertainties. Biological diversity and its adaptation to local ecological conditions can still be expected in well-preserved natural and autochthonous forests.

Only one seed stand is approved for obtaining forest reproductive material in the Pohorje region of provenance, namely provenance Osankarica (ident. no. GSO: 2.0119) at an altitude of 1240 m, »selected« category (KRAIGHER, BOŽIČ & VERLIČ 2011). This seed stand is also proposed as a forest genetic reserve in Slovenia and dynamic gene conservation unit on the European level (WESTERGREN, BOŽIČ & KRAIGHER 2010). Pullings are also used for conversion spruce monocultures on Pohorje, collected in a "selected" category seed stand (ident. number GSO: 4.0175, provenance Temenjak) in vicinity (KRAIGHER, BOŽIČ & VERLIČ 2011). An approved seed stand at an altitude from 650 to 700 m is classified in the Savinjska-Šaleška ecological sub-region (code 4.3) of the Prealpine provenance region (KUTNAR et al. 2002).

There are relatively few preserved beech forests on Pohorje. The majority of these have been transformed into spruce monocultures. In addition, Pohorje is overgrown with natural spruce forests, especially at higher altitudes, i.e., in the altimontane and lower sub-alpine zone. The montane zone is covered with fir forests. The natural possibilities of the preserved genetic heritage of beech are limited in area and the structure of stands in these areas is relatively poor. Seed beech trees are rare, the majority are stump-grown or mixed stands of stump-grown and seed trees. Regardless of the stand form, it will be necessary to a certain extent additionally to select for seed stands, even though the area may be very limited. Additional seed stands could be chosen in the Pohorje provenance region or in similar ecological conditions in neighbouring provenance regions (alpine and subalpine). In all cases, these are stands that are placed in the associations (syntaxa) *Luzulo-Fagetum* Meusel 1937, *Hieracio rotundati-Fagetum* Ž. Košir 1994 and *Cardamine savensi-Fagetum* Ž. Košir 1962 var. geogr. *Abies alba* Ž. Košir 1994 in the montane/altimontane zone and the associations (syntaxa) *Castaneo-Fagetum sylvaticae* Marinček & Zupančič (1979) 1995 and *Hedero-Fagetum* Ž. Košir (1962) 1994 var. geogr. *Polystichum setiferum* Ž. Košir 1994 in the colinar zone.

We propose an enlargement of the network of seed stands on Pohorje (including Rdeči breg) and Kobansko, with the aim of exploiting existing potentials of beech that has survived the period of spruce monocultures for the needs of renewal of these complexes with the use of habitats of adapted planting material. The importance of this measure is in preparation of a high-quality base

of planting material suitable for under-planting beech in spruce monocultures on Pohorje, with simultaneous preservation of the adaptation potential for growth and development of beech in suitable areas of degraded forests in the light of possible climate change. Economically interesting conifers, such as spruce and fir, have a relatively large share in the timber stock of very varied forest associations in Slovenia. However, the surface share of potential forest communities in which conifers predominate is relatively small. Model-based forecasts indicate that this share will probably be further reduced. As has been established for Western and Central Europe (KIENAST, BRZEZIECKI & WILDI 1998; LEXER et al. 2002, MARACCHI, SIROTENKO & BINDI 2005; KOCA, SMITH & SYKES 2006), it can also be expected in Slovenia that there will be a pronounced replacement of coniferous with deciduous forests (KUTNAR, KOBLER & BERGANT 2009; KUTNAR & KOBLER 2011). Simulations of climatic effects on spruce indicate an explicit fall in the share and

worsened prospects of this species with the realisation of generally applicable climate scenarios, which envisage further atmospheric warming in the future (OGRIS & JURC 2010, KOBLER & KUTNAR 2010).

The problem of seed sources of beech on Pohorje therefore deserves particular attention. The work of preserving seed sources and the biological diversity of forests should include both the protection of seed sources with the aid of legislation and regulations and the protection of seed sources by means of the status of special purpose forests and other forestry nature conservation efforts for preserving the natural genetic heritage by protecting and supplementing the network of forest gene reservoirs and other areas with important seed sources. It is also sensible to continue directly applicable research and development work for the needs of the forest seed and sapling trade, including in depth research into the genetic characteristics of populations of forest tree species in Slovenia.

6. CONCLUSIONS

On the basis of the results of analysis of the genetic structure of under-planted juvenile beech in selected spruce monocultures on Pohorje with isozyme gene markers, we conclude:

- Under-planted beech at the Brička site are genetically slightly different from the under-planted beech at the Kladje site.
- The genetic variability of under-planted beech within individual populations, according to indicators of genetic diversity (H_a, v, v_{gam}, δ_T) is greater at the Brička site that at the Kladje site.
- There is greater potential danger of the loss of ge-

netic information (alleles) because of genetic drift at the Kladje site, which can occur in smaller, isolated populations in the development of future stands (than in the population at the Brička site).
- The allele (genetic) distance among under-planted beech at the Brička and Kladje sites, which we calculated according to GREGORIUS (1974), is relative large for the studied gene pool ($d_0 = 9.6\%$).
- The problem of seed sources of beech on Pohorje deserves special attention. There is a need to select relatively well-preserved beech stands on the most varied potential forest sites on Pohorje and Kobansko, which could be selected as seed stands.

POVZETEK

Uvod

V začetku devetdesetih let so vetrolomi in gradacije podlubnikov zmanjšali stabilnosti smrekovih nasadov v Evropi in povzročili opazno zmanjševanje vitalnosti, ki smo mu bili priča do konca prejšnjega desetletja. Gozdarska stroka si je prizadevala najti načine za povečanje dolgoročne stabilnosti in ohranitev trajnosti teh nasadov, kar je vodilo v izbor rastišču primernejših drevesnih vrst. Zaradi vprašljivega golosečnega načina gospodarjenja in pritiska s strani javnosti ter zahtev po povečanju

trajnosti je postal v Evropi vnos s podsadnjo listavcev v iglastih gozdovih široko uveljavljen gozdnogojitven ukrep. Gozdnogojitvena izhodišča so bila za tovrstne premene razmeroma slabo opredeljena.

V zadnji polovici prejšnjega stoletja je bila premena čistih smrekovih sestojev (*Picea abies* L. Karst.) eden poglavitnih gojitvenih izzivov v Evropi (ZERBE 2002; TEU-FFEL, HEINRICH & M. BAUMGARTEN 2004). Ocenjujejo, da je v Evropi najmanj 6 do 7 milijonov hektarov čistih smrekovih sestojev zunaj svojih naravnih rastišč, od katerih je 4-5 milijonov hektarov osnovanih na rastiščih,

kjer bi sicer prevladovali listnati ali mešani iglasto-listnati gozdovi. Sedanje stanje je posledica gozdnogospodarskih odločitev v preteklosti, ko so bili mešani sestoji izpostavljeni kmetijskemu izkoriščanju, steljarjenju, paši in pridobivanju lesa za kurjavo (Johann s sod. 2004). Velika potrebe po lesu zaradi industrializacije je vodila v pospeševanje monokultur hitrorastočega drevja. V zadnjih 200 letih je bila smreka najpogosteje izbrana kot drevesna vrsta za umetno obnovo sestojev zaradi hitre rasti, preprostega osnovanja sestojev, majhnih zahtev glede redčenj in zaradi ne pretirano obremenjajočega objedanja divjadi.

Vnos smreke zunaj njenega naravnega areala je povezan s številnimi, v glavnem kratkoročnimi koristmi. Vrsta je ekonomsko zelo zanimiva, ima potencial za proizvodnjo visokokakovostnega lesa v kratkih časovnih obdobjih. Evropski listnati gozdovi so v preteklosti pokrivali občutno večje površine, kot jih pokrivajo danes, zato velja v Evropi splošno mnenje, da je snovanje listnatih sestojev korak k trajnostnemu gozdarstvu (Hannah, Carr & Lankerani 1995; Stanturf & Madsen 2002). Eden od načinov osnovanja je premena smrekovij v listnate ali v mešane gozdove. Premena v Evropi široko razširjenih homogenih smrekovij v naravne listnate gozdove se uporablja v gozdarski praksi zadnjih 50 let.

V prid premeni govori več dejavnikov. Smreka se je v velikih primerih pokazala kot nestabilna, izpostavljena je zmanjšanju vitalnosti, vetrolomom, napadom podlubnikov, suši in negativno vpliva na tla (Larsen 1995). Občutne spremembe v okolju in krajini s posledicami na spremembe ali zmanjševanje biotske raznolikosti (Grabherr & Koch 1993) so zmanjšale naklonjenost javnega mnenja. Klimatske spremembe in posebno naraščajoča verjetnost pojavljanja klimatskih ekstremov kot npr. neurij so izpostavile ranljivost smrekovih monokultur in poudarjajo potrebo po zmanjševanju tveganja z vključevanjem ostalih drevesnih vrst v smrekove sestoje (Bradshaw s sod. 2001). Dvig cen lesa listavcev je zmanjšal ekonomsko upravičenost snovanja antropogenih iglastih sestojev (Abildtrup, Riis & Jellesmark-Thorsen 1997; Spiecker 2000).

Za uspešno premeno je podsadnja z bukvijo (*Fagus sylvatica* L.) bodisi pod zastorom matičnega sestoja ali v svetlobnih jaških možna alternativa golosečnemu načinu gospodarjenja, ki je v Evropi še vedno prisotno. Podsadnja je star gojitveni postopek, ki je danes deležen nove pozornosti (Otto 1986; Spellmann & Wagner 1993). Prednosti podsadnje so manjše zapleveljevanje, ugodnejša mikroklima, varstvo pred poznimi pozebami (Löf 2000), kot tudi večja stopnja naklonjenosti javnega mnenja v nasprotju z golosečnjami (Mattsson & Li 1994).

V Sloveniji so s premenami začeli spreminjati čiste smrekove sestoje v mešane sredi 20. stoletja. Čeprav so gozdovi v Sloveniji v primerjavi s Srednjo Evropo razmeroma ohranjeni, pa zaradi dediščine preteklosti trenutna drevesna sestava gozdov odstopa od ocenjene naravne (Diaci & Grecs 2003). Delež smreke v lesni zalogi je 32 %, ocena naravnega deleža je približno 8 %. Slovenija je dežela listnatih, predvsem bukovih gozdov (Šercelj 1996, Ž. Košir s sod. 1974, Dakskobler 2008). 70 % gozdov Slovenije uvrščamo v združbe, v katerih je bukev vodilna vrsta.

Obdobje aktivne premene nasadov smreke ima začetke v zgodnjih 50. letih z navodili Ministrstva za gozdarstvo za pričetek kampanje sadnje plemenitih listavcev v nasade smreke, predvsem gorskega javorja (Diaci 2006). Prizadevanja večinoma niso bila uspešna zaradi previsokih gostot velikih rastlinojedov (Diaci 2006 povzeto po ustnem viru Mlinšek 2004). Bistveno boljši je bil uspeh uresničevanja prvih ureditvenih načrtov po drugi svetovni vojni. Mlinšek je leta 1954 izdelal obsežen ureditveni načrt za enoto Mislinja, ki je zajemal predvsem nasade smreke (Mlinšek 1955). Pričel je s postopno premeno na osnovi kontrolne metode s podrobnimi analizami prirastka, uvedel izbiralna redčenja in podaljšal obhodnje, vpeljal celovito metodologijo podsadnje nasadov smreke s sadikami in puljenkami bukve ter posamično in skupinsko zaščito proti objedanju, zagovarjal je rastiščem prilagojen pristop. Preizkušali so različne metode posamične zaščite in dosegli precejšnje uspehe s cementno malto. Zaradi pomanjkanja semenskih dreves bukve in sadik, so puljenke bukve prenašali iz bolj ali manj primerljivih rastišč na severni strani Pohorja (Diaci 2006).

Da bi ocenili genetsko variabilnost podsajene bukve v čistih smrekovih sestojih na Pohorju, smo v juvenilni fazi razvoja bukve opravili genotipsko analizo testnih populacij z uporabo biokemijskih genskih označevalcev. Namen študije je z metodo analize izoencimov oceniti stopnjo genetske sorodnosti populacij podsajene bukve. Preizkusiti želimo hipotezo, da imajo testne populacije bukve podobno populacijsko genetsko strukturo in ne nakazujejo večjih odstopanj v povprečnih vrednostih parametrov. Z raziskavo želimo prispevati k podrobnejšemu poznavanju genetskih struktur, genetske raznolikosti in genetske diferenciranosti bukovih populacij, ki so jih vnesli v smrekove monokulture na Pohorju.

Študijsko območje

Pohorje je izrazita gorska planota, leži v višini 1200-1500 m ter je podaljšek Centralnih Alp. Masiv je zgrajen iz

metamorfnih in magmatskih kamenin ter sedimentov (Budnar-Tregubov 1958). Klima je sredogorska. V zadnjih 4000 do 5000 letih so v prvotnih gozdovih prevladovale različne oblike jelovo bukovih gozdov ob skromni udeležbi smreke (Culiberg & Šercelj 2000). Prvobitne gozdove na Pohorju so sestavljale predvsem bukev, jelka in smreka, ponekod pa je bil primešan tudi macesen (Breznikar s sod. 2006). Naravni smrekovi gozdovi so edafsko pogojeni in s tem omejeni na zamočvirjene zaravnice, skledaste uleknine in na blago nagnjena pobočja na pohorskem platoju. V zadnjih dveh stoletjih je bila drevesna sestava pohorskih gozdov spremenjena. Danes tu prevladujejo antropogeno spremenjeni smrekovi sestoji. Zaradi negativnih vplivov spremenjene drevesne sestave je ogrožena tudi trajnost donosov iz gozda (Breznikar s sod. 2006). V preteklosti se je v obdobju od leta 1800 do konca druge svetovne vojne zaradi vpliva nemške šole gospodarjenja z gozdovi in njenih poljedelskih načel v pohorske gozdove intenzivno vnašala smreka, obnova gozda pa je bila usmerjena v osnovanje čistih smrekovih sestojev. Po drugi svetovni vojni so se ta gozdnogojitvena načela postopoma opuščala. Antropogeni smrekovi sestoji na Pohorju, med katere sodijo sestojne zgradbe s prevladujočim deležem smreke na bukovih in jelovih rastiščih, pokrivajo danes 27 000 ha oziroma 45 % skupne gozdne površine na Pohorju (Breznikar s sod. 2006).

Negativne posledice spremenjene drevesne sestave narekujejo iskanje ustreznih ukrepov pri gospodarjenju z gozdovi, s katerimi bi postopoma vzpostavili bolj naravno zmes drevesnih vrst v gozdnih sestojih. Načrtovanje ukrepov v smrekovih monokulturah zahteva poznavanje procesov degradacije rastišča zaradi negativnega vpliva pospeševanja smreke na bukovih in jelovih rastiščih. Pri prevladujočem deležu smreke v sestoju se začne na tleh kopičiti surov humus, k njegovemu škodljivemu delovanju pa dodatno prispeva labilnost tal. Smreka zaradi plitvega koreninjenja premočno izkorišča zgornje talne horizonte, spodnji talni horizonti pa se začno zgoščevati. Pride do poslabšanja fizikalnih lastnosti tal (zmanjševanje zračnosti), povečuje se kislost tal, zmanjšuje se dostopnost hranilnih snovi, zmanjša se intenzivnost humifikacije in mineralizacije organskih snovi v tleh, prične se kopičenje neugodnih oblik humusa. S postopnim zbijanjem talnega profila in dviganjem nepropustnega horizonta se zmanjšuje biološko aktivna globina tal, zaradi slabših fizikalnih lastnosti tal pa se zmanjša kapaciteta tal za vodo. Vlažne oblike surovega humusa in mahovi povečujejo površinsko akumulacijo padavinske vode in pogojujejo procese pseudooglejevanja tal (Breznikar s sod. 2006).

Razvoj stabilnejših gozdnih struktur in zaviranje degradacijskih procesov v gozdnih tleh so glavni strateški cilji pri načrtovanju razvoja gozdov na Pohorju. Načrtni posegi za sanacijo spremenjenih sestojnih zgradb in usmerjeno gozdnogojitveno ukrepanje so se začeli na mislinjskem delu Pohorja v petdesetih letih prejšnjega stoletja in se postopoma širili na celotno območje Pohorja. Glavni ukrep sanacije je vnos listavcev, predvsem bukve, v smrekove sestoje in izločitev vpliva preštevilne divjadi na gozdno mladje.

Raziskovane objekte na Pohorju smo izbrali na južni strani Pohorja na raziskovanih ploskvah Gozdarskega inštituta Slovenije in sicer na lokaciji Brička nad Mislinjskim jarkom (ZGS OE Slovenj Gradec) in Kladje pri Osankarici (ZGS OE Maribor) (Preglednica 1). Oba objekta je pred intenzivnejšimi posegi in degradacijskimi procesi domnevno poraščal bukov ali jelovo-bukov gozd s primesjo smreke. Navedeni lokaciji veljata za potencialno naravno rastišča acidofilnega bukovja z belkasto bekico, geografska varianta s trilistno penušo (*Luzulo albidae-Fagetum* Meusel 1937 var. geogr. *Cardamine trifolia* (Marinček 1983) Marinček & Zupančič 1995). Na robu objekta Brička še danes na manjši površini raste razmeroma dobro ohranjen acidofilni bukov gozd. Širše območje objekta Kladje je precej spremenjeno, vendar na osnovi posameznih ostankov tovrstnega gozda lahko sklepamo na potencialna rastišča asociacije. Vendar pa je na širšem območju tega dela Pohorja bila kartirana združba bukve z zasavsko konopnico. To je conalna združba pohorskega visokogorskega bukovega gozda, ki naseljuje zgornji del montanskega pasu masiva Pohorje, to je v nadmorskih višinah od 1000 do 1300 m. Poleg značilnih drevesnih vrst bukve in gorskega javorja se v razmeroma dobro ohranjenih sestojih te združbe pojavljata z večjim deležem tudi jelka in smreka. Na osnovi tega je bila opredeljena posebna geografska varianta te združbe z jelko (*Cardamini savensi-Fagetum* Ž. Košir 1962 var. geogr. *Abies alba* Ž. Košir 1979).

Zaradi pospeševanja smreke v preteklosti območje objektov porašča smrekova monokultura. Izhodišča za izbiro raziskovalnih objektov so poleg zahteve po zasmrečenih potencialnih bukovih rastiščih zajemala še prisotnost večjega števila vitalnih podsajenih dreves bukve znotraj posamezne ploskve, podobno starost vnesene bukve na primerjalnih lokacijah ter možnosti za vpetost raziskovalnih objektov v okvire drugih preučevanj. Raziskovalna objekta gradijo zrela drevesa smreke in podsajene bukve v juvenilni fazi razvoja. Značilnosti izbranih raziskovalnih ploskev so navedene v preglednici št. 1. Na ploskvi Brička nad Mislinjskim jarkom je ploskev poraščalo 173 smrek / ha z ocenjeno lesno zalogo smrekovega sestoja na 477 m^3 / ha, na ploskvi Kladje pri Osankarici pa 126 dreves / ha z lesno zalogo 302 m^3 / ha (Čater 2011). V letu 2006 je bila starost vzorčene podsajene bukve ocenjena na okoli 10 do 15 let (Čater 2011).

Material in metode

Nabiranje vzorcev

Na raziskovalnih objektih Brička in Kladje na Pohorju smo za genetske analize vzeli vzorec stotih naključno izbranih dreves bukve. V vzorec na vsaki ploskvi v velikosti 100 m x 100 m smo enakomerno zajeli vitalna drevesa posajene bukve. Z vsakega od poskusnih dreves smo v zimskem obdobju leta 2005 / 2006 odvzeli vejo s spečimi popki, ki smo jih uporabili za ekstrakcijo encimov. Popke smo do analize hranili v epruvetah pri temperaturi -20° C. Velikost vzorca je bila 50 dreves na posamezno ploskev.

Analiza izoencimov

Raziskave genetskih značilnosti bukve smo izvedli z metodo analize izoencimov na škrobnem gelu. Raziskovani material so speči popki. Encimske izvlečke smo ekstrahirali iz bukovih popkov po že preizkušenih metodah. Genotip vsakega drevesa smo ugotavljali s pomočjo horizontalne elektroforeze na škrobnem gelu. Ekstrakcijo encimov iz spečih popkov, elektroforezo, barvanje gelov in odčitavanja elektroforegramov smo izvajali po standardiziranih metodoloških postopkih za analizo bukovih vzorcev (KONNERT, HUSSENDOERFER & DOUNAVI 2004). Laboratorijski del analiz smo izvedli februarja 2006 v okviru nalog raziskovalnega projekta Dinamika ogljika v naravnem bukovem gozdu (L4-6232). Analize smo izvedli v genetskem laboratoriju Bayerische Amt für forstliche Saat- und Pflanzenzucht v Teisendorfu, Nemčija, pod vodstvom dr. Monike Konnert.

V analize smo zajeli 10 encimskih sistemov, ki jih kodira 17 genskih lokusov (preglednica št. 2). Rezultate izoencimskih analiz smo vrednotili z relativnimi frekvencami alelov in genotipov po posameznih genskih lokusih Genetsko strukturo populacije smo opisali na osnovi pogostosti pojavljanja posameznih alelov na polimorfnih lokusih. Kot polimorfen lokus smo upoštevali vsak genski lokus, na katerem smo ugotovili vsaj še en alel, ne glede na njegovo relativno pogostost v populaciji (brez kriterija).

Frekvenčne porazdelitve alelov na lokusih smo po posamezih populacijah opisali s 4 alelnimi profili, ki smo jih določili po FINKELDEYU (1993), in sicer kot: i) fiksacijo, če je na posameznem genskem lokusu samo 1 alel (alelna frekvenca = 100 %), ii) nizko stopnjo polimorfizma, če je na posameznem genskem lokusu je prisoten pogosti alel (alelna frekvenca > 80 %) in eden ali več redkih alelov, iii) visoko stopnjo polimorfizma, če sta na posameznem genskem lokusu najmanj dva alela

kot prevladujoča (alelna frekvenca > 20 %), iiii) netipični profil, če alelne strukture na posameznem genskem lokusu ne moremo uvrstiti v 1., 2. ali 3. profil.

Genetsko variabilnost znotraj posameznih populacij smo interpretirali s parametri genetske pestrosti in genetske raznolikosti. Pri parametru genetske pestrosti, ki v posamezni populaciji upošteva samo številčnost različnih genetskih tipov, smo izračunali tri parametre: (1) delež polimorfnosti analiziranih genskih lokusov (P %); (2) največje možno število različnih alelov (M_{max}); (3) povprečno število alelov na polimorfni lokus (A/L). Pri parametru genetske raznolikosti, ki za razliko od parametra genetske pestrosti poleg števila različnih genetskih tipov upošteva tudi njihovo pogostnost pojavljanja v populaciji, smo izračunali opaženo heterozigotnost (H_a) in pogojeno hetrozigotnost (H_c; GREGORIUS, KRAUHAUSEN & MUELLER-STARCK 1986), efektivno alelno raznolikost (v; GREGORIUS 1978, GREGORIUS 1987), večlokusno hipotetično gametska raznolikost (v_{gam}) ter stopnjo genetske diferenciacije med osebki znotraj populacije (δ_T; GREGORIUS 1987), ki je pri večjih vzorcih enaka stopnji pričakovanih hetrozigotnih deležev, ki nastanejo pri panmiksični oplodnji (H_e; NEI 1973). Za preverjanje, kako se dejanski heterozigotni deleži v podsajenem mladju bukve razlikujejo od pričakovanih hetrozigotnih deležev, ki nastanejo pri panmiksični oplodnji (Hardy-Wienbergovo ravnotežje) smo uporabili χ2 test, ki ga izvajamo po lokusih ločeno za vsako vzorčeno populacijo posebej, pri stopnji tveganja α = 0,05.

Genetsko diferenciranost med vzorčenima populacijama podsajene bukve na lokacijah Brička ter Kladje smo podali z merilom povprečne genetske razdalje (d_0; GREGORIUS 1974). Statistično primerjavo genetskih struktur bukve smo izvedli na osnovi χ2 testa homogenosti alelnih frekvenc po lokusih. Ničelno hipotezo homogenosti alelnih porazdelitev med populacijami zavračamo na nivoju tveganja α = 0,05. Za izračun posameznih parametrov genetske variabilnosti in statističnih primerjav smo uporabili program za ovrednotenje podatkov izoencimskih analiz GSED (GILLET 1998).

Rezultati

V tej raziskavi želimo osvetliti predvsem genetski vidik premene monokultur smreke na Pohorju s podsadnjo bukve. Cilj raziskave je ugotoviti genetsko strukturo podsajene bukve na lokacijah Brička in Kladje, to je na degradiranih potencialno naravnih rastiščih acidofilnega bukovja z belkasto bekico, geografska varianta s trilistno penušo (*Luzulo albidae-Fagetum* Meusel 1937 var.

geogr. *Cardamine trifolia* Marinček (1983) Marinček & Zupančič 1995). Ugotoviti želimo ali so razlike v genetskih strukturah med skupinami dreves podsajene bukve. Rezultate genetskih primerjav prikazujemo v preglednicah št. 3 in 4 za 17 polimorfnih genskih lokusov.

Frekvence alelov in genotipov

Iz relativnih alelnih frekvenc analiziranih lokusov je razvidno (preglednica št. 3), da je v populacijah polimorfnih vseh 17 analiziranih genskih lokusov (P = 100 %). V obeh populacijah smo odkrili nizko stopnjo polimorfnosti na 3 lokusih (*Aat-A, Aco-A, Skdh-A*), visoko stopnjo polimorfnosti na 2 lokusih (*Aat-B, Per-A*). Podobno polimorfnost nakazuje tudi lokus *Idh-A*. Netipičen profil smo v obeh populacijah odkrili na 6 lokusih (*Aco-B, Mdh-A, Mnr-A, Pgi-B, 6-Pgdh-A, -B*). Razločen prehod med nizko in visoko stopnjo polimorfizma pri testnih populacijah smo ugotovili pri 5 lokusih. Lokusa *Per-B* in *6-Pgdh-C* odražata nizek polimorfizem v vzorcu Brička in visok polimorfizem v vzorcu Kladje, medtem ko imajo lokusi *Mdh-B, -C* in *Pgm-A* nizek polimorfizem v vzorcu Kladje ter visok v vzorcu Brička. V obeh vzorčenih populacijah je vedno prisoten isti prevladajoči alel. Frekvenco glavnega alela smo odkrili v razponu od 54 % do 99 %. Frekvence nad 10 % lahko dosegajo še aleli $Aat-B_2$, $Aco-B_2$, $Idh-A_2$, $Mdh-A_1$, $Mdh-B_4$, $Mdh-C_1$, $Per-A_1$, $Per-B_1$, $Pgm-A_2$, $6-Pgdh-A_4$, $6-Pgdh-B_1$, $6-Pgdh-C_4$, medtem ko se drugi aleli pojavljajo v nizkih frekvencah.

Alelne frekvence se na primer močno spremenijo pri alelih $Mdh-B_4$, $Per-B_1$, $Pgm-A_2$ in $6-Pgdh-C_4$. Frekvenci alelov $Per-B_1$ in $6-Pgdh-C_4$ sta v vzorcu Brička nižji kot v vzorcu Kladje (11 % vs. 37 % in 8 % vs. 23 %), medtem ko sta alelni frekvenci $Mdh-B_4$ in $Pgm-A_2$ večji kot v vzorcu Kladje (16 % vs. 5 % in 40 % vs. 9 %). Zanimive so tudi alelne variante $Aat-A_1$, $Mdh-B_2$, $Pgi-B_3$, $6-Pgdh-A_4$, $6-Pgdh-C_5$, ki so v populaciji Kladje le redko prisotne (to je s frekvenco manj kot 5 %) ter pogostnejše v populaciji Brička (do 11 %) oziroma alel $Mdh-A_1$, ki je redek v populaciji Brička (1 %) ter pogostnejši v populaciji Kladje (10 %).

Primerjava genotipskih frekvenc nakazuje na obstoj razlik med primerjanima vzorčenima populacijam na posameznih genskih lokusih in sicer na 7 od skupaj 17 analiziranih genskih lokusov. Homozigotni in heterozigotni tipi močneje ferkventni v vzorcu Brička v primerjavi z vzorcem Kladje so: $Per-B_{22}$ (70 % vs. 32 %), $Pgm-A_{23}$ (44 % vs. 18 %), $Pgm-A_{22}$ (18 % vs. 0 %), $Mdh-A_{33}$ (98 % vs. 80 %), $Mnr-A_{33}$ (90 % vs. 74 %), $6-Pgdh-C_{15}$ (20 % vs. 4 %), $Pgi-B_{23}$ (16 % vs. 2 %). Heterozigotni ali homozigotni tipi močneje ferkventni v vzorcu Kladje v

primerjavi z vzorcem Brička so: $Pgm-A_{33}$ (82 % vs. 38 %), $Mdh-A_{13}$ (20 % vs. 2 %), $Per-B_{12}$ (30 % vs. 14 %), $Per-B_{11}$ (20 % vs. 4 %), $Mnr-A_{35}$ (20 % vs. 6 %) in $Mdh-B_{33}$ (72 % vs. 54 %).

Genetska variabilnost znotraj posameznih vzorčenih populacij

Genetska pestrost

Izraz genetska pestrost se pomensko nanaša izključno na število genetskih kategorij (alelov, genotipov) populacije ali njenih delov. Na celotnem območju preučevanja smo v vzorčenih populacijah podsajene bukve na 17 polimorfnih lokusih odkrili skupaj 43 različnih alelov (M_{max}). V nobeni populaciji nismo odkrili vseh možnih alelnih variant. Celotna alelna pestrost je v pomladitvenih skupinah bukve zastopana od 93,0 % (Brička) do 95,3 % (Kladje). Obe populaciji imata v povprečju enako število prisotnih alelov na lokus (A/L = 2,40).

Genetska raznolikost

Genetska raznolikost je variabilnost osebkov v populaciji, ki jo izražajo frekvence različnih genetskih kategorij znotraj populacije. Merilo genske (alelne) raznolikosti v populaciji je dejansko razpoložljivo ali efektivno število alelov na posameznem lokusu. Ker je prispevek redkih alelov (to je alelov s frekvenco < 5 %) k skupni vsoti majhen, ta mera izraža tudi dejansko stopnjo uravnoteženosti pogostih alelov. Primerjava srednjih vrednosti efektivnega števila alelov na lokus (v) nakazuje na različnost vzorčenih populacij. Vrednosti se gibljejo v razponu od 1,28 (Kladje) do 1,34 (Brička) kar ustreza razmerju 1:1,05. Ustrezno manjša vrednost efektivnega števila alelov na lokus pri vzorčeni populaciji Kladje z vrednostjo A/L = 2,40 nakazuje prisotnost večjega deleža redkih alelov. Vrednosti alelne raznolikosti (v) po posameznih lokusih so navedene v preglednici št. 4. Analiza hipotetične večlokusne gametske raznolikosti (v_{gam}) poskusnih dreves bukve je pokazala, da se število genetsko različnih 17 lokusnih gametskih tipov, ki bi jih lahko proizvedle skupine 50 vzorčnih dreves v populacijah, giblje v razponu vrednosti od 99,8 (Kladje) do 203,1 (Brička) in ustreza razmerju 1:2,04. Čeprav imajo izračunane vrednosti samo nakazovalni značaj, ta primerjava nakazuje na večji potencial skupine podsajenih dreves bukve na lokaciji Brička kot pri skupini podsajenih dreves bukve na lokaciji Kladje za proizvodnjo genetsko različnih gamet, ki bodo v novi generaciji podvržene genetski variaciji. Povprečni vre-

dnosti dejanske (opažene) heterozigotnosti (H_a) za 17 lokusni genski sklad sta v razponu od 21,1 % v skupini dreves Kladje do 23,5 % v skupini dreves Brička, kar ustreza razmerju 1:1,11. Stopnje heterozigotnosti se med testnima populacijama Brička in Kladje izrazito spreminjajo na 5 lokusih in sicer na *Mdh-A* (2 % vs. 20 %), *Mnr-A* (6 % vs. 26 %), *Per-B* (26 % vs. 48 %), *Pgi-B* (16 % vs. 2 %), *Pgm-A* (44 % vs. 18 %).

Genetsko variabilnost med osebki znotraj posamezne populacije, neodvisno od populacijske velikosti oziroma števila proučevanih osebkov, opredeljujemo s stopnjo genetske diferenciacije (δ_T). V vzorčenih populacijah podsajene bukve se povprečne stopnje genetske (alelne) diferenciacije δ_T gibljejo v razponu od 22,3 % (Kladje) do 25,6 % (Brička), kar ustreza razmerju 1:1,15. Opažene frekvence genotipov se pri večini lokusov ujemajo s frekvencami genotipov, pričakovanimi po Hardy-Weinbergovem ravnotežju. Značilna odstopanja od Hardy-Weinbergove strukture smo na lokaciji Brička ugotovili na lokusih *Mnr-A*, *Per-A*, *6-Pgdh-B*, na lokaciji Kladje pa na lokusih *Aat-B*, *Per-A* in *Skdh-A*. V vzorčeni populaciji Kladje je na lokusu *Aat-B* prišlo tudi do pojava značilnega primanjkljaja heterozigotov. Odstopanja pričakovanih genotipskih frekvenc od Hardy-Weinbergove strukture v skladu s Hardy-Weinbergovem zakonom nakazujejo, da je v populacijah lahko prišlo do drugačnega prenosa genov kot pri panmiksični oplodnji ali pa so na testne populacije delovale različne oblike genetske selekcije. Največja možna stopnja hetrozigotnosti (H_c) je v vzorčenih populacijah realizirana od 77,9 % (Brička) do 84,0 % (Kladje). Izračunana vrednost (H_c) za obe vzorčeni populaciji skupaj sicer nakazuje pomanjkanje heterozigotov v primerjavi z največjo dosegljivo vrednostjo, ki je enaka 100 %. Vendar zaradi pomanjkanja podatkov o izvoru matičnih sestojev bukve in preteklih razvojnih fazah v analizi zajetih skupin dreves, podsajenih v monokulture smreke na Pohorju, ne moremo ugotoviti, ali je na ta pojav vplival reproduktivni sistem, način pridobivanja in rabe gozdnega reprodukcijskega materiala in / ali tudi različne oblike (preživetvene) selekcije.

Genetska diferenciacija med vzorčenima populacijama

Pogostosti posameznih alelov na nekaterih lokusih (npr. *Pgm-A*$_2$ (9 % vs. 40 %), *Per-B*$_1$ (11 % vs. 37 %), *Mdh-B*$_4$ (5 % vs. 16 %), *Mdh-A*$_1$ (1 % vs. 10 %), *6-Pgdh-C*$_4$ (8 % vs. 23 %)) deloma nakazujejo veliko različnost sestojev. Če primerjamo alelne porazdelitve vzorčeni populacij podsajene bukve je verjetnost odklonov od homogenih alelnih struktur statistično značilna pri 5 od 17 analiziranih lokusov (preglednica 4). Izračunane vrednosti kažejo, da

so odkloni od homogenosti alelnih porazdelitev visoko značilni na lokusih *Per-B* in *Pgm-A* ($\alpha = 0,001$), *Mdh-A* ($\alpha = 0,01$), s stopnjo tveganja $\alpha = 0,05$ pa tudi na *Mdh-B* in *Pgi-B*. Delež alelov, ki si jih testni populaciji med seboj ne delita (d_0), za genski sklad 17 polimorfnih lokusov, v povprečju znaša 9,6 %. Na primeru posameznih lokusov ugotavljamo tudi visoke vrednosti genetskih razdalj. Vzorčeni skupini bukve, ki se na lokusu *Pgm-A* ločita v 31 % alelnem in 44 % genotipskem deležu, na lokusih *Per-B* in *Mdh-B* pa v 29 % oz. 19 % alelnem ter v 38 % oz. 26 % genotipskem deležu, že dovoljujeta ugotovitev o večji genetski diferenciranosti med njima. Hipoteze, da imajo vzorčene skupine dreves bukve podsajene v dveh smrekovih monokulturah na Pohorju (Brička in Kladje), podobno populacijsko genetsko strukturo, v našem primeru ne moremo potrditi.

Razprava

V izbranih smrekovih monokulturah na Pohorju ima populacija podsajene bukve na lokaciji Brička v genskem skladu večjo efektivno alelno raznolikost, hipotetično večlokusno gametsko raznolikost, opaženo heterozigotnost ter večjo diferenciranost med osebki znotraj populacije kot populacija podsajene bukve na lokaciji Kladje. Razlogi za različne primerjalne vrednosti pri posameznih vzorčenih populacijah niso poznani. Ugotovljene razlike v stopnji genetske variabilnosti so lahko odraz razlik v izvoru provenienc, zgodovinskih razlik v posegih v prostor na območju matičnih sestojev, razlik v načinih pridobivanja in rabe gozdnega reprodukcijskega materiala in / ali različne oblike preživitvene selekcije mladic bukve tako v matičnih kakor tudi v novih, spremenjenih razmerah njihovega življenjskega okolja. Seveda so to le teoretične predpostavke, ki v našem primeru niso bile preverjene. Priporočljivo bi bilo še dodatno raziskati genetske strukture sestojev bukve z naravnim mladjem, ki so preživela obdobje smrekovih monokultur na območju Pohorja, da bi lahko spoznali, ali je bila v naši raziskavi analizirana genetska struktura vzorčene bukve sploh reprezentativna ali ne.

Prvi dokaz o obstoječih genetskih razlikah med preučevanima skupinama podsajene bukve na lokaciji Brička in Kladje podaja rezultat statističnega testa homogenosti alelnih struktur na polimorfnih genskih lokusih. Med alelnima porazdelitvama vzorčenih populacij bukve smo dobili značilno različne odklone kar pri 5 od 17 genskih lokusov. Stopnja analize genetske diferenciranosti bukve je med vzorčenima populacijama sorazmerno velika. Med skupinama podsajene bukve v izbranih smrekovih monokulturah na južnem delu Pohorja je genetska diferenciacija (d_0) izražena z deležem alelov, ki

si jih populaciji med seboj ne delita, 9,6 %. Ta vrednost je podobna ugotovljeni vrednosti alelne (genske) razdalje (d_0; Gregorius 1974) med najbolj diferenciranimi populacijami bukve na Bavarskem (Nemčija), ki se med seboj sicer razlikujejo od 2,6 % do 10,9 % (Konnert & Henkel 1997). Podobne visoke vrednosti genetske diferenciacije, ki so jih ugotovili med štirimi populacijami z najbolj različnih rastišč, navaja tudi raziskava dvajsetih domnevno avtohtonih populacij bukve iz območja zahodne Nemčije (Turok 1994).

Opažena odstopanja med vzorčenima populacijama Brička in Kladje na Pohorju bi lahko v primeru reprezentativnosti genetskih struktur preučevanih populacij nakazovala, da je bil pretok genov med matičnimi sestoji bukve omejen ali pa, da je večja genetska diferenciranost lahko tudi posledica izolacije in specifičnih selekcijskih procesov, ki jih je doživela bukev na teh lokacijah. Kar nadalje lahko pomeni tudi, da saditveni material, ki je bil podsajen v smrekovih monokulturah Brička in Kladje, ne prihaja iz istega matičnega izvora bukve. Dejstvo pa je, da vseh teh povezav ni mogoče podrobneje spoznati ter oceniti njihovih vplivov brez novih poglobljenih raziskav. Rezultati naših preučevanj zato nakazujejo na sklep, da je skupina dreves bukve v smrekovi monokulturi Brička genetsko nekoliko različna od skupine dreves bukve v smrekovi monokulturi Kladje.

Pri podsadnjah bukve v smrekovih monokulturah (brez vključevanja naravnega pomlajevanja bukve) obstaja stalna nevarnost za izgubo alelov (oz. genetske informacije) zaradi genetskega zdrsa, ki bi lahko nastal v majhnih izoliranih populacijah zaradi zmanjševanja prvotne velikosti populacij pri razvoju in rasti bodočega sestoja bukve. Ta nevarnost je v našem primeru potencialno večja za populacijo bukve Kladje, ki nakazuje nižjo stopnjo genetske variabilnosti znotraj populacije in ima večji delež redkih alelov (s frekvencami < 5 %) kot populacija bukve Brička.

Zasnova bodočega gozda je kritična faza v življenju gozda. Za obnovo gozdov je zato priporočljivo uporabiti naravno pomlajevanje sestojev. V primerih, kjer to ni mogoče, pa uporabo semenskega materiala in puljenk pridobljenih iz odobrenih semenskih sestojev. To je v tistih semenskih objektih, ki jih skladno z določbami Zakona o gozdnem reprodukcijskem materialu ULRS, št. 58/02, 85/02, 45/04) odobri Gozdarski inštitut Slovenije in so vpisani v Register gozdnih semenskih objektov (ULRS, št. 91/03). »Za večinske drevesne vrste (predvsem za bukev, dob, graden, jelko in smreko) se priporoča uporaba semena in sadik v okviru višinskega pasu in provenienčnega območja, iz katerega izhaja, ali iz sosednjega območja. Manj priporočljiva je uporaba semena in sadik iz drugih provenienčnih območij, le izjemoma gozdar oz. gojitelj predpiše tudi uporabo semena in sadik iz so-

sednjih višinskih pasov. Za manjšinske drevesne vrste velja, da je vsa Slovenija enotno provenienčno območje, razdeljeno na 4 višinske pasove. Kljub temu je tudi za te vrste priporočljiva uporaba semena v območju, iz katerega izvira« (Medved s sod. 2011, str. 137). Gozdno seme je genetski material, zato ima izbor gozdnih semenskih virov zelo daljnosežne posledice. Kakovostnih semenskih virov oziroma sestojev zaradi splošne spremenjenosti gozdov ni veliko in jih je zaradi sedanje antropogene obremenjenosti gozdov vedno manj. Kot porazdelitev tveganja in kot pogoj za ekološko prilagodljivost gozda morajo gozdni semenski viri zajemati dovolj veliko biološko raznovrstnost, to je vse pomembnejše drevesne vrste z njihovimi krajevnimi rasami in njihovo genetsko variabilnostjo. To je še posebej pomembno ob sedanjem naglem spreminjanju podnebja in drugih antropogeno povzročenih nepredvidljivostih. Biološko raznovrstnost in njeno prilagojenost krajevnim ekološkim razmeram še najprej lahko pričakujemo v gozdovih z dobro ohranjeno naravnostjo in avtohtonostjo.

Za pridobivanje gozdnega reprodukcijskega materiala je v pohorskem provenienčnem območju odobren le en semenski sestoj in sicer provenienca Osankarica (ident. številka GSO: 2.0119) na nadmorski višini 1240 m, kategorija »izbran« (Kraigher, Božič & Verlič 2011). Ta semenski sestoj je predlagan tudi za gozdni genski rezervat v Sloveniji in za enoto dinamičnega varstva genov na ravni Evrope (Westergren, Božič & Kraigher 2010). Za potrebe premene smrekovih monokultur na Pohorju se uporabljajo tudi puljenke, nabrane v semenskem sestoju kategorije »izbran« (ident. številka GSO: 4.0175, provenienca Temenjak) v sosedstvu (Kraigher, Božič & Verlič 2011). Odobreni semenski sestoj na nadmorski legi od 650 m do 700 m uvrščamo v Savinjsko-Šaleško ekološko podregijo (koda 4.3) Predalpskega provenienčnega območja (Kutnar s sod. 2002).

Na Pohorju je razmeroma malo ohranjenih bukovih gozdov. Večina le teh je spremenjena v smrekove monokulture. Poleg tega je Pohorje poraščeno z naravnimi smrekovimi gozdovi, zlasti v višjih nadmorskih legah, to je v altimontanskem in nižjem subalpinskem pasu. Montanski pas pa poraščajo jelovi gozdovi. Naravne možnosti ohranjene genetske dediščine bukve so površinsko omejene, na teh površinah pa je struktura sestojev razmeroma slaba. Bukovi semenovci so redki, več je panjevcev ali mešanih sestojev panjevca in semenovca. Ne glede na sestojno obliko bi bilo potrebno kolikor toliko ustrezne sestoje dodatno izbrati za semenske sestoje, čeprav morda površinsko zelo omejene. Dodatne semenske sestoje lahko izberemo v pohorskem provenienčnem območju ali v podobnih ekoloških razmerah v sosednjih provenienčnih območjih (alpskem in predalpskem). V vseh primerih gre za sestoje, ki jih uvrščamo v

asociacije (sintaksone) *Luzulo-Fagetum* Meusel 1937, *Hieracio rotundati-Fagetum* Ž. Košir 1994 in *Cardamine savensi-Fagetum* Ž. Košir 1962 var. geogr. *Abies alba* Ž. Košir 1994 v montansko / altimontanskem pasu ter v asociacije (sintaksone) *Castaneo-Fagetum sylvaticae* Marinček & Zupančič (1979) 1995 in *Hedero-Fagetum* Ž. Košir (1962) 1994 var. geogr. *Polystichum setiferum* Ž. Košir 1994 v kolinskem pasu.

Predlagamo razširitev mreže semenskih objektov na Pohorju (vključno z Rdečim bregom) in Kobanskem s ciljem izkoriščanja obstoječih potencialov bukve, ki je preživela obdobje smrekovih monokultur za potrebe obnove teh kompleksov z uporabo rastišču prilagojenega sadilnega materiala. Pomembnost tega ukrepa je v pripravi kvalitetne baze saditvenega materiala primernega za podsadnjo bukve v smrekove monokulture na Pohorju, ob hkratnem ohranjanju prilagoditvenega potenciala za rast in razvoj bukve na ustreznih površinah degradiranih gozdov v luči možnih podnebnih sprememb. Gospodarsko zanimivi iglavci, kot sta smreka in jelka, imajo razmeroma velik delež v lesni zalogi zelo različnih gozdnih združb v Sloveniji. Vendar pa je površinski delež potencialnih združb, v katerih so iglavci prevladujoči, razmeroma majhen. Kot kažejo napovedi modela, se bo verjetno ta delež še dodatno zmanjšal. Tako kot ugotavljajo za zahodno in srednjo Evropo (Kienast, Brzeziecki & Wildi 1998; Lexer s sod. 2002, Maracchi, Sirotenko & Bindi 2005; Koca, Smith & Sykes 2006), lahko pričakujemo tudi pri nas, da bo prišlo do izrazite zamenjave gozdov iglavcev z gozdovi listavcev (Kutnar, Kobler & Bergant 2009; Kutnar & Kobler 2011). Simulacije podnebnih učinkov na smreko nakazujejo izrazit upad deleža in slabše perspektive te vrste ob uresničitvi splošno veljavnih podnebnih scenarijev, ki predvidevajo nadaljnje segrevanje ozračja v prihodnosti (Ogris & Jurc 2010, Kobler & Kutnar 2010).

Problematika semenskih virov bukve na Pohorju zato zasluži še posebno pozornost. Delo pri ohranjanju semenskih virov in biološke raznovrstnosti gozda naj vključuje tako zavarovanje semenskih virov s pomočjo zakonodaje in predpisov, kot zavarovanje semenskih virov s statusom gozda s posebnim namenom in druga gozdarska naravovarstvena prizadevanja za ohranjanje naravne genetske dediščine z zavarovanjem in dopolnjevanjem mreže gozdnih genskih rezervatov in drugih površin s pomembnimi semenskimi viri. Smiselno je tudi nadaljevati z neposredno uporabnimi raziskavami in razvojnim delom za potrebe gozdnega semenarstva in drevesničarstva vključno s poglobljenimi raziskavami genetskih značilnosti populacij gozdnih drevesnih vrst v Sloveniji.

Zaključki

Na osnovi rezultatov analiz genetske strukture podsajene mlade bukve v izbranih smrekovih monokulturah na Pohorju z izoencimskimi genskimi označevalci sklepamo:

- Podsajene bukve na lokaciji Brička so genetsko nekoliko različne od podsajenih bukev na lokaciji Kladje.
- Genetska variabilnost podsajenih bukev znotraj posamezne populacije je po kazalcih genetske raznolikosti (H_a, v, v_{gam}, δ_T) večja na lokaciji Brička kot pa na lokaciji Kladje.
- V populaciji bukve na lokaciji Kladje obstaja večja potencialna nevarnost za izgubo genetske informacije (alelov) zaradi genetskega zdrsa, ki bi lahko nastal v manjših izoliranih populacijah pri razvoju bodočega sestoja (kot v populaciji na lokaciji Brička).
- Alelna (genska) razdalja med podsajeno bukvijo na lokaciji Brička in Kladje, ki smo jo izračunali po Gregorius (1974) je za preučevani genski sklad sorazmerno velika ($d_0 = 9,6$ %).
- Problematika semenskih virov bukve na Pohorju zasluži posebno pozornost. Potrebno je poiskati razmeroma ohranjene bukove sestoje na čim bolj različnih rastiščih na Pohorju in Kobanskem, ki bi potencialno lahko bili izbrani kot semenski sestoji.

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